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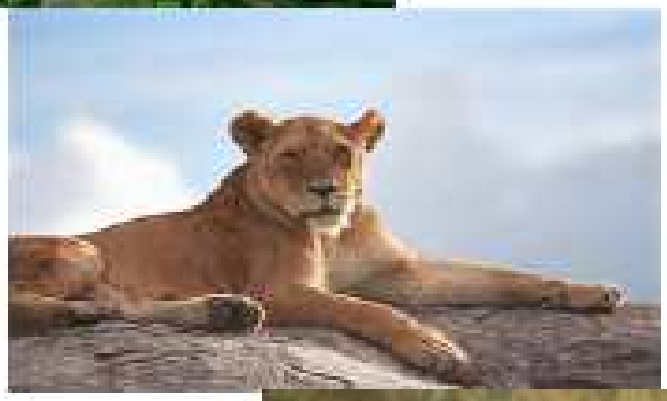

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SURVEY OF FACTORS AFFECTING THE SUSTENANCE OF A TROPICAL STREAM IN NIGERIA

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ABSTRACT

Man is at the center of natural resources development and destruction. This study assessed the productivity level of Gada stream and examined the factors militating against its sustenance. A total of 150 respondents, consisting of local fishers, fresh and dry fish traders, fish consumers and those living along the stream were randomly sampled. Data collection was by personal observation, oral interview and semi-structured questionnaires. The data was analyzed using descriptive statistics. Results showed that there was decreased fish production in the stream. Factors militating against fish production in Gada stream were continuous excavation of sand (81.33%), destruction of stream banks due to farming activities (60.0%), water pollution (48.67%), seldom flood (42.67%), over exploitation of fish (28.0%) and the types of fishing gears and techniques involved (21.33%). Management techniques such as avoidance of sand excavation (85.33%), use of nets of 1 cm^2 mesh size (78.0%), controlled fishing activity (70.67%), use of buffer zones (68.67%) and planting of horticultural crops along the stream banks may be adopted for the stream's revitalization, management and sustenance.

Keywords: Gada stream, Productivity, Factors affecting sustenance, Revitalization, Management, Conservation

INTRODUCTION

Fishery is one of the most important constituent of wildlife resources globally. It is therefore important to conserve our water bodies such that the fish productivity level can be sustain and maintain at the level capable of providing the need of the growing human population across the globe. African Wildlife Foundation, AWF (2007), reported that sustenance of fish productivity in the African water bodies for economic growth (streams, rivers, dams, lakes etc) can only be attained through effective management of aquatic ecosystems, of which Gada stream falls within the area of concern.

According to Hunter (1990) fish species should be maintain even for its scientific and posterity value, this is why water bodies are considered as field laboratories for fishery experts. It is therefore necessary to conserve the Gada stream so as to maintain its fish productivity level. Gabon (1993) reported that stream conservation and management are affected by human activities such as unguided fishing, overuse of water for other purposes, farming along the river banks and dumping of refuse among others.

Consequently, this research considered the status of the Gada stream as it affects the human populace of Mubi in terms of social and economic role because Amos (2002), reported

that our water bodies have immense role in maintaining a healthy environment, as these ecosystems provide protein for man and other animals. In addition, they provide the water required by wild and domestic animals and also sustain the flora and fauna hence it is necessary to effectively manage the Gada stream as most of the fresh fishes consumed in Mubi yearly from the months of August to October comes from the Gada Stream. This situation can only be overcome based on Brown (1987) who reported, that the management of a given resource for its revitalization and sustenance can only be achieved through the co-operation of the resource users and the government arm charged with its management. Brown (1987) further stated that the future economic stand of a particular resource depends on the level of its utilization and management. Correspondingly, Gabon (1993) supported this observation that people catch fishes, excavate sand, destroy the stream banks through farming practices, use the water for roads and house constructions as well as pollute the water through various means including the use of Agro-chemicals for fishing, thereby altering the physical and chemical factors as well as destroying the biological components of the stream. Consequently, following these ugly acts of man, Amos (2002) suggested that there is need for effective management of our natural and man-made water bodies for increased fish production across Sub-Saharan Africa as this sector plays a vital role in the economic growth of any nation.

This study examined the pattern of fishing, nature of fishing equipment, management techniques in practice and how they can be effectively improved for the revitalization and sustenance of the fish productivity level of Gada stream through the following objectives: (i) identification of fish species usually caught, (ii) examined the fishing pattern, (iii) identification of the fishing equipment in use, (iv) identification of the management techniques in practice and their effectiveness, (v) identification and ascertaining the factors militating against the management techniques in use (vi) estimation of the fish productivity level of the stream from 2005 – 2007 and (vii) develop techniques that could be used in the management of the Gada stream.

MATERIALS AND METHODS

The study was carried out on Gada Stream of Mubi – North Local Government Area of Adamawa State, Nigeria covering its major fishing sites that include; Yawa, Madanya, Gipalma, Gada, Blue House and Vimtim. The study covered a period of 3 years from 2005 to 2007. The climatic condition is typical that of tropical regions of the world, with mean daily temperature ranging between 28 – 34^{o}C. During harsh periods, usually from March to May, the temperature may rise up to 38 – 39^{o}C. The relative humidity is variable with the peak of it during rainy season especially from late July to September (Toyo, 1996).

The mean annual rainfall ranging from 700 – 900mm and the rainy season lasts for about 3 – 4 months, usually June to September (Akosim *et al.*, 1999). The inhabitants of the study area are primarily subsistence farmers although few of the population are made up of civil servants and petty traders. A total of 26km length of the stream was covered.

A total of 150 respondents were randomly sampled using a close-ended questionnaire and semi-structured oral interviews, using simple random sampling technique (Jen, 2002). The sampled population consisted of local fishers, fresh and dry fish traders, fish consumers as well as those living along the stream. The fish productivity was assessed physically and also using information gotten from the sampled population. The estimate of each fish species caught was done using basket quantification as a measure.

For easy administration of the questionnaires, participatory rural appraisal technique was employed (Dunn, 1994). The contents of the questionnaire include; Name of respondent, fish species usually caught, types of fishing pattern, fishing equipment in use, management techniques in practice, factors militating against the management techniques, estimated fish productivity and management techniques that could be used in the sustenance

of fish productivity. The data collected was analysed using descriptive statistics.

RESULTS AND DISCUSSIONS

The fish species usually caught included; *Mormyrus rume*, *Mormyrus macrophthalmus*, *Petrocephalus bane*, *Marcusenius psithacus*, *Alestes macrolepidotics*, *Alestes nurse*, *clarias lazera*, *Schilbe mystus, Synodontis clarias*, *Hyperopisus bebe*, *Malapterurus electricus*, *Protopterus annectens*, *Tilapia galilaea*, *Tilapia nilotica*, *Tilapia* aurea, *Tilapia zilli* and *Hemichromis fasciatus*. Reports from respondents showed that the fish species listed above were caught along the entire stream length of the study area. Main fishing activity in the study area starts from August and end in June, but fishing is done in only few selected points from March to May because a large portion of the stream dries up within this period.

The fishing equipment were mostly the locally constructed fishing nets e.g. clap nets (*Koma*), cast nets (*birge*) foul – hook longlines (*mari-mari*) and trap nets (*gussa*) (Table 1). The listed fishing gears were being complemented by free hand swimming, total draining of selected points (usually from March to May), use of funnel traps (a gear locally constructed using forbs and grasses) and construction of blockage fence across the tributaries of the stream using reeds of sticks assisted by nets. Clap nets, long lines, cast nets and total draining technique were widely accepted by fishers as the most preferred fishing methods as reported by 68.0%, 56.0%, 50.67% and 40.0% of the respondents, respectively (Table 1). The least accepted fishing methods include; use of fence, hand fishing and trap nets as reported by 6.67%, 7.33% and 10.0%, respectively (Table 1).

For the sustenance of Gada stream three management techniques were in practice as reported by the respondents (Table 2), of which controlled use of chemicals (134, 89.33%) and use of agronomical technique through planting of horticultural crops along the stream banks (97, 64.67%) were considered most effective. The sampled respondents (43.33%) also reported that awareness campaign mounted towards reduction in sand excavation also helped in the management of the stream. Although the management techniques were effective as reported by the respondents but the activities of man coupled with natural factors militated grossly against the management techniques, thereby making the techniques ineffective in achieving the desired target.

Management techniques in practice were grossly affected by some factors such as excavation of sand, (81.33%), destruction of stream bank due to farming activities (60.0%), water pollution (48.67%) and seldom flood (42.67%). These factors constituted serious threat to the management techniques in practice. Correspondingly, factors such as types of fishing gears and techniques (21.33%), followed by over-exploitation of fish (28.0%) were relatively less significant (Table 3).

The yearly quantity of each species caught indicated that there was general decrease in quantity of fish caught from 2005 to 2007 (Table 4). This may be as a result of some factors such as water pollution, continuous excavation of sand, and destruction of stream banks due to farming activities among others that were reported to be the main attributes to decreased fish production of the stream.

The study also revealed that fish species such as *Tilapia* species, *Clarias* species and *Hemichromis fasciatus* tend to be less affected by the listed factors as observed based on the estimated quantities caught. For effective management of Gada Stream, the following management techniques were suggested (Table 5).

The suggested management techniques were in line with those of Amos (2002), that buffer zones created between the stream and sites of farming activity can prevent the possibility of sand deposition into the water body, which usually account for reduced volume of water in the stream.

It is obvious that when sand excavation is stopped, then there will be reformation in the stream bed and banks, leading to a relatively natural condition that will provide the

Table 1: Fishing gears and techniques in use in Gada stream, Mubi North Local Government Area, Adamawa State, Nigeria

S/No	Fishing gears/methods	Number	Percentage respondent
1.	**Clap nets**	102	68.00
2.	**Cast nets**	76	50.67
3.	**Long lines**	84	56.00
4.	**Trap nets**	15	10.00
5.	**Funnel traps**	19	12.67
6.	**Fence**	10	6.67
8.	**Hand fishing**	11	7.33

Table 2: Management techniques in practice for sustenance of Gada stream, Mubi North Local Government Area, Adamawa State, Nigeria

S/No	Management options	Number	Percentage respondent
1.	**Controlled use of chemicals**	134	89.33
2.	**Reduced sand Excavation**	65	43.33
3.	**Use of agronomical technique through planting of horticultural crops along the stream banks**	97	64.67

Table 3: Factors militating against the management techniques in practice in Gada stream, Mubi North Local Government Area, Adamawa State, Nigeria

S/No	Management Techniques	Number	Percentage respondent
1.	**Types of Fishing gears and techniques**	32	21.33
2.	**Continuous excavation of sand**	122	81.33
3.	**Destruction of stream banks due to farming activities**	90	60.00
4.	**Over-exploitation of fish**	42	28.00
5.	**Water pollution**	73	48.67
6.	**Seldom flood (Natural factor)**	64	42.67

Table 4: Fish species and yearly quantity caught from Gada stream, Mubi North Local Government Area, Adamawa State, Nigeria

S/No	Fish Species	Yearly quantity (Kg)		
		2005	**2006**	**2007**
1.	***Mormyrus* species**	2,265.0	1,862.5	1,209.00
2.	***Petrocephalus bane***	106.0	87.3	40.60
3.	***Marcusenius psithacus***	2,034.0	1,418.0	1,136.70
4.	***Alestes* species**	2,815.0	2,908.4	2,041.00
5.	***Clarias* species**	4,774.8	3,055.0	3,825.00
6.	***Schilbe mystus***	2,336.0	1,410.0	812.62
7.	***Synodontis clarias***	403.0	98.5	109.20
8.	***Hyperopisus bebe***	85.0	22.1	30.40
9.	***Malapterurus electricus***	3.0	0.85	-
10.	***Protopterus annectens***	35.0	12.0	15.60
11.	***Tilapia* species**	4,986.3	3,232.0	3,042.
12.	***Hemichromis fasciatus***	168.0	202.6	82.50
Total		20,038.1	14,309.25	12,344.12

Table 5: Management techniques for revitalization and sustenance of Gada stream in Mubi North Local Government Area, Adamawa State, Nigeria

S/No	Management Techniques	Number	Percentage Respondent
1.	**Use of Buffer zones**	103	68.67
2.	**Avoidance of sand excavation**	128	85.33
3.	**Planting of horticultural crops along the stream banks**	87	59.33
4.	**Controlled fishing activities**	106	70.67
5.	**Use of nets of $1cm^2$ mesh size**	117	78.00

environment needed for the survival of aquatic life forms, of which fish is not an exception. This technique will also help in preventing destruction of spawning sites and nests. Generally, crops such as mango, lemon, orange, guava, and banana etc. can be planted along the stream banks in other to provide a semi-natural environment for growth and development of fishes. This also helps in consolidating the stream banks thereby making it to withstand erosion due to increase in water volume hence control of flood.

Controlled Fishing Activity can help in revitalizing the stream through effective utilization without causing any damage to the productive base. Here, period for fishing activity should be spelt out clearly for users of the stream and also the act of total draining of some selected points of the stream be avoided completely.

The comparatively small to medium size fingerlings will not be affected, if fishing nets of $1cm^2$ mesh size are used. This technique will go a long way in sustaining the productive base of the stream as it applies to fish resource.

The poor economic state of most of the inhabitants of the study area made them to over-exploit the resources of the stream, a level that grossly affected the productive base of the stream. The collective practice of increased fishing activity, high demand for building components (sand, gravel, water, clay etc), and farming practices done close to river banks made this ecosystem suffer due to human threat.

The re-cognizance survey study 2007 revealed that the cumulative effect of the above listed human activities has gone beyond the stream's resistance and now the consequence is drastic reduction in water volume and diminish in fish production. This problem of resource mismanagement had led some species of fish close to extinction point, as they are rarely sighted. For instance, the electric fish *(Malapterurus_electricus)*, which have been the pride of the stream is now rarely caught. These human acts observed in Gada stream is inline with Olofin (2000), who reported that mankind is capable of destroying the delicate balances that exist between his manipulation of the environment and the range of adjustment the environment can undergo. Bwala (2000) also stressed that man's ignorance of the consequences of uncontrolled use of natural resources had led him to destroy even the productive base as observed in Gada stream without taking into cognizance the consequences of his action.

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GENETIC VARIABILITIES OF BODY TEMPERATURE AND RESTING BEHAVIOUR IN THREE STRAINS OF EGG-TYPE CHICKEN

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ABSTRACT

The study was conducted to ascertain the body temperature and resting behaviours of three strains of egg-type pullet chickens (exotic [ISA brown], improved native [Alpha] and Native chickens). A total of 90 egg-type pullets aged 12 months and their day old, non-inbred progeny pullets numbering 90 were used for the study. Both the parents and their progeny were housed at 10 per pen and 3 replicates per genotype and their progeny. The parents were fed and watered ad-libitum between 52 – 63 weeks period of the experiment. The progeny of each genotype were kept for 0 – 12 weeks, during which time, chicks were fed and watered ad-libitum as in the parents and data were collected. During this period, body temperature and resting behaviour were collected with respect to the birds and their progeny. The result showed no significant (P > 0.05) genotype effects in the body temperature of both the parents and their progeny. This implies that neither progeny nor generation had effect on body temperature. The Alpha strain exhibited more resting behaviour than did the exotic and the pure native types. Majority of the birds rested in the afternoon at 2.00 pm. This could be attributed to the fact that at 2.00 pm the weather is hot and birds search for a quiet and cool place to reduce thermal stress.

Keywords: Genetic variabilities, Body temperature, Resting behaviour, Strains, Egg-type chicken

INTRODUCTION

Poultry, though a significant contributor to the nutritional and economic well being of many Nigerians, little attention is paid to understand its behaviour and general welfare in relation to environmental, feeding and management changes (Nwankwo and Omeje, 2008). To boost the production of this group of livestock, it is imperative that all factors that contribute to low performance should be studied and avoided. External behaviour of animals is usually an index of the internal state of such animals (kilgour and Dalton, 1983). Increase in conditions that sustain normal behaviour improves meat and egg production capacities and *vice-versa.* One of these observable behavioural characteristics that have effect on productivity is resting behaviour. Hockings *et al.* (1993), Keer-Keer (1996) and Akani *et al.* (2008) reported that broiler breeds spend more time resting and less time standing than the layer when fed *ad-libitum.* Udeh (2003) also observed that heavier strains of birds have higher resting tendencies than native light weight chickens. A similar trend was reported by Omeje *et al.* (1997) and Omeje *et al.* (2001). According to them broiler birds

rested more at night than during the day. The Nigerian environment is characterized by high temperature and relative humidity typical of a tropical climate (Yakubu and Omeni, 2007) as such, animals are therefore highly susceptible to heat stress. Day temperature averaging 35 ± 5 ^{0}C in the Southern Nigeria and 42 ± 8 ^{0}C in the Northern Nigeria during the months of December to March have been reported (Longe, 2000). Poultry birds in Nigeria, therefore encounter thermal stress in addition to other stressors which affect their physiological and productive activities. The adverse effect of stressors are additive and every attempt should be made to lessen the number and intensity of such stressors (Butcher and Miles, 2004). The body temperature of domestic chicken is 38.9^{o}C ± 5^{o}C, though Oluyemi and Roberts (2007) reported a range 38.9 – 43.6^{o}C. Most birds maintain their body temperature at 40 ± 2^{o}C (Eekeren, 1995; Campbell *et al.* 2001). Domestic fowls may pant when kept under high environmental temperature (Orusiebo, 2004). Low temperature makes the birds to reduce surface area, fluff out their feathers and huddle to increase insulation, tuck their heads under wings, increase feed intake and increase activities (Oluyemi and Roberts, 2007). These physiological changes in one way or the other affect production of layers. Like many other traits, behavioural characteristics may be associated with genetic composition of animals. This study therefore investigated the effect of genotype and generation on the resting behaviour and body temperature of exotic, improved and native chickens and their F1 progeny.

MATERIALS AND METHODS

The study was carried out at the Poultry Breeding and Genetics Research Centre of the Department of Animal Science, Delta State University Asaba Campus. A total of 90 exotic (ISA Brown), Alpha (Crossbred) and Native layer birds aged 12 months and their 90 progeny pullets (at day old) were used for the study. These comprised of 30 parent birds from each genotype and 30 day old progeny from the birds. Ten parent birds from each genotype were randomly selected and assigned into three pens to ensure three replicates of the experimental unit for each genotype. The progeny birds were similarly allotted into ten chicks per pen and three replicates per experimental unit. A floor space of 1.45 square meter per bird was allowed as recommended by Adejoro (2000). Appropriate feed for the parents (Layers Marsh, Top Feed, Nigeria Limited) and chicks (Starter Marsh, Top Feed, Nigeria Limited) as well as water was provided *ad-libitum* throughout the 12 week period of the experiment. All birds were subjected to similar managerial and sanitary conditions throughout the experiment. All necessary vaccinations were also administered at appropriate ages. In order to minimize errors in the experiment, stress factors such as diseases, harsh weather, poor ventilation, poor lighting were adequately monitored and controlled in line with the guidelines of Duncan (1981).

During the 12 week period of the experiment, resting behaviour of birds was observed for 30 minutes at 9.00 am, 12.00 noon and 2.00 pm each day. The number of birds resting was recorded each time. Resting was described as birds observed lying down, standing but immobile, squatting, sleeping provided such birds were not displaying any motion (Omeje *et al.* 2001). Percentage resting was calculated as: number of birds resting divided by total number of birds x 100. The body temperature was determined using clinical thermometer as the mouth, cloaca and armpit temperatures of the birds on weekly basis and the mean temperature was recorded for an individual bird.

The experimental design adopted was a completely randomized block design (CRBD) (SAS, 2001). In accordance with Steel *et al.* (1997), age, genotype and generation were the main sources of variation, respectively. All data collected were subjected to analysis of variance (ANOVA) in one way classification. Duncan's New Multiple Range Test was used to separate means where ANOVA showed significant effects. The effects of genotype, generation and age of birds on the parameters

Table 1: Genetic variability in body temperature of three strains of egg-type chicken

Genetic Variability		Temperature °C		
		Mouth	Cloaca	Armpit
Exotic	**Parent**	40.51 ± 0.07^{ns}	40.80 ± 0.40 ^{ns}	40.48 ± 0.22 ^{ns}
	Progeny	40.00 ± 1.00 ^{ns}	40.62 ± 0.23^{ns}	40.14 ± 0.80 ^{ns}
Alpha	**Parent**	40.50 ± 0.05 ^{ns}	40.90 ± 0.10 ^{ns}	40.36 ± 0.23 ^{ns}
	Progeny	40.96 ± 0.20 ^{ns}	40.74 ± 0.16 ^{ns}	40.38 ± 0.14 ^{ns}
Native	**Parent**	40.26 ± 0.11 ^{ns}	40.28 ± 0.70 ^{ns}	40.31 ± 0.45 ^{ns}
	Progeny	40.20 ± 0.60 ^{ns}	40.20 ± 0.40 ^{ns}	40.70 ± 0.30 ^{ns}

ns = Not significant (p > 0.05)

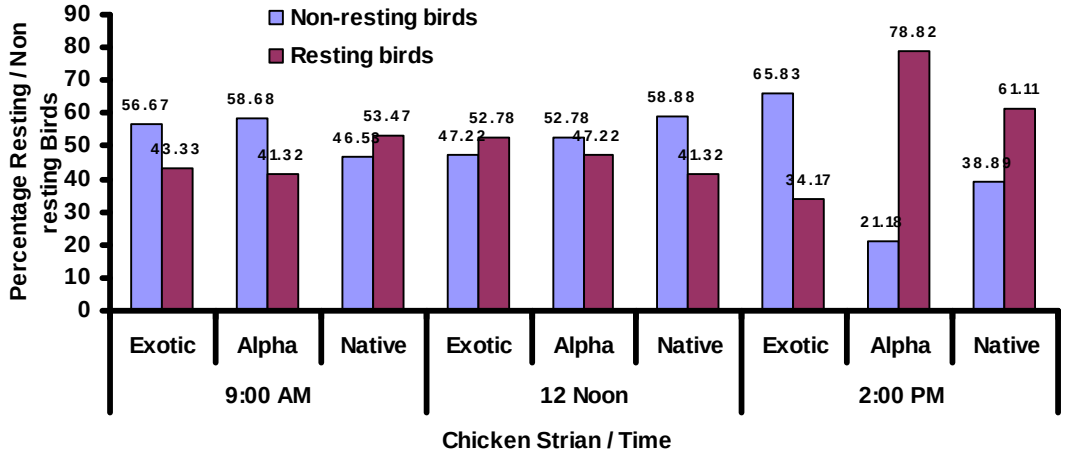

Figure 1: Genetic variability in resting behaviour of three strains of egg-type chicken parents

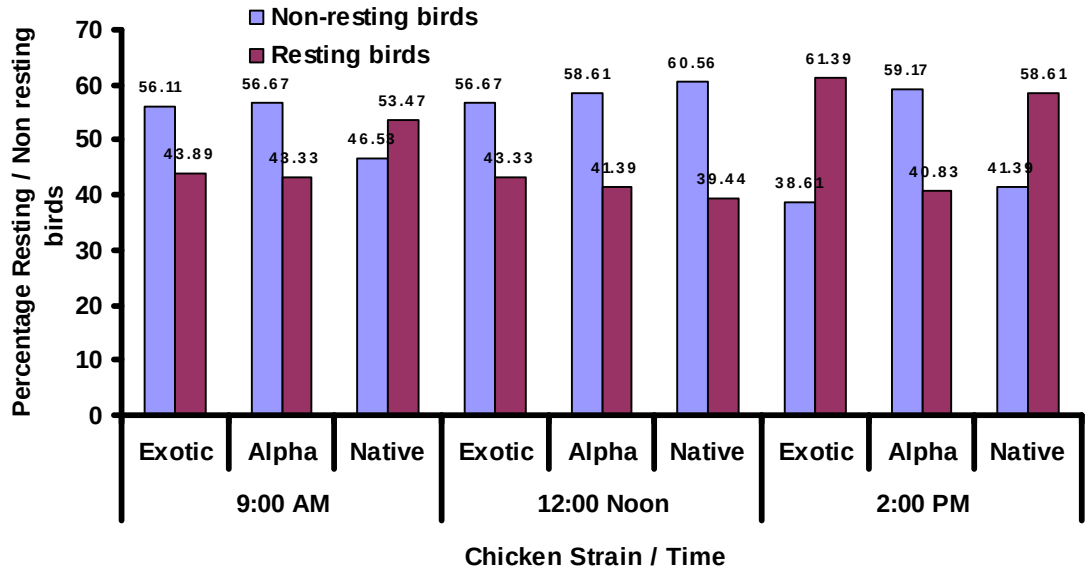

Figure 2: Genetic variability in resting behaviour of three strains of egg-type chicken progeny

monitored were estimated using linear model: Xijk = μ + Ti + Bj + Eijk, where Xijk = An observation made on the kth bird belonging to the ith genotype during the jth age period, μ = the overall population mean common to all observation, Ti = the effect of the ith genotype (1 = 1,2), Bj = the effect of the jth week of age (j = 1, 2, 3, ….. 12) and Eijk = random error associated with the experiment.

RESULTS AND DISCUSSION

There was no significant genotype effect (P > 0.05) on body temperature of both parents and progeny birds (Tables 1). The mean body temperature ranges of 40.2 – 41.0 ^{o}C reported by Oluyemi and Roberts (2007) was similar to our finding. Our data also confirmed the findings of Campbell *et al.* (2001) that birds maintain their body temperature at 40.0 ± 2 ^{o}-C. Our findings were also in support of the findings of Eekeren (1995) that constant body temperature for birds ranged between 40.5 – 42.5 ^{o}C. The mean body temperature of the parents and their progeny were similar (Figures 1 and 2) (P > 0.05). Our findings contradicted earlier report of Campbell *et al.* (2001), who reported that smaller birds may have higher temperature than bigger ones. In the 95% confidence interval, among the parents, the native varied more, followed by the Alpha and the exotic, while in the progeny, the natives varied more followed by the exotic and the alpha. It implied that the more varied the interval, the better the genetic improvement in the body temperature.

The resting behaviour of birds in each genotype and their progeny indicated that at 9.00 am and 12.00 noon among the parents, the exotic breed rested more than the alpha and the native while at 2.00 pm the alpha rested more than the exotic and the native, respectively. Among the progeny groups at 9.00 am, 12 noon and 2.00 pm, the exotic rested more followed by the alpha and lastly the native chickens. Majority of birds rested at 2.00 pm with the following values 78.47%, 61.39%, 78.82%, 59.17%, 61.11% and 58.61% for exotic, alpha and native parents and their progeny, respectively (Figures 1 and 2). This implied that heavier strains of birds have more tendencies for resting than the lighter strains. A similar observation was made by Omeje *et al.* (2001) and Udeh (2003). Hockings *et al.* (1993) and Keer-Keer *et al.* (1996) reported that broilers spend more time resting and less time standing than layers when fed *ad-libitum*. The tendency of the birds to rest at 2.00 pm may be attributed to the fact that at 2.00 pm, the weather is hotter and as a result they search for a quiet and cool place to rest.

Conclusion: Non-significant (p > 0.05) differences were recorded in body temperature among parents and their progeny genotypes. It implied that neither genotype nor generation had effect on body temperature. In the resting behavior, observations were made that most of the birds rested at 2.00 pm and a lesser percentage at 9.00 am and 12.00 pm respectively. The exotic and alpha strains rested more than their native counterparts.

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SYMPTOMS ASSOCIATED IN THE DIAGNOSIS AND MANAGEMENT OF MALARIA IN A SEMI URBAN TROPICAL COMMUNITY

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ABSTRACT

A study of symptoms associated in the diagnosis and management of malaria in a semi urban community of Enugu State, South-Eastern Nigeria was conducted. Structured qualitative and quantitative questionnaires were administered along an in-depth interview to assess the knowledge, attitude, behaviour and practice (KABP) among school children and pregnant women attending ante-natal clinic hospitals and to ascertain their health-seeking behaviour. Symptomatic diagnosis of the 352 pregnant women and children, showed that 260 (79%) did not manifest any of the malarial symptoms within the past three months as at the time of the study, 20 (5.7%) patients had fever alone, chill and blisters (0.9%), headache, diarrhoea and joint pain (0.3%), and weakness and anorexia (0.6%), respectively. Recent fever combined with chill was highest 2.8%, followed by anorexia and weakness 2.0%, fever with headache, and weakness 1.7%, fever with anorexia and or vomiting 1.4%, fever with chill and headache and/ or amber urine or joint pain or anorexia or frequent sleeping 1.1%, headache with weakness 1.1%. Forty six (46) antenatal care patients out of the 352 population sampled for symptomatic diagnosis to predict malaria infection in symptomatic patients with recent history of treatment showed low sensitivity (41.6%), but highly specific (80%), low positive value (45.7%), high negative predictive value (77.3%), low false positive rate (20%), moderately high false negative rate (58.4%) and a high J-index (78.7%). The axillary temperature was poorly predictive for negative samples with normal axillary temperatures. The mean axillary temperature among children with positive malaria test was 37.0°C and negative malaria test was 36.4°C. Antenatal patients with positive test were 36.5°C and negative malaria test was 35.1°C. Self-diagnosis and presumptive treatment are evident based in the prevention and prompt treatment of malaria disease and this method is prevalent in the rural communities and should be an adjunct to routine microscopy in clinical diagnosis.

Keywords: Malarial symptoms, Anorexia, Diagnostic, Axillary, Sensitivity and predictive value

INTRODUCTION

Malaria morbidity and mortality is a global challenge upon the health and socio-economic development in the tropics. The fight against malaria is not achieving expected results due to lack of comprehensive knowledge and skills on malaria prevention, curative and management strategies across the general populations,

especially in the poor and remote areas with stable malaria transmission.

Malaria kills an estimated global population of 3 million people annually, and weaken 300 – 500 million more, mostly in Sub-Saharan Africa, Children under five years and pregnant women are the most vulnerable (WHO, 2000 a). The burden of malaria in Africa is particularly dangerous, causing 900,000 deaths, and every 30 seconds an African Child dies (WHO, 2000 b). Everyday, at least 1000 Africans dies of malaria and at least 20 % of all are children under five.

The disease causes widespread premature death and suffering, imposes financial hardship on poor households, retards economic growth and undermines living standards. Malaria is implicated for additional illnesses such as, cerebral malaria, respiratory infections, diarrhoeal diseases, iron-deficiency anaemia and malnutrition. The economic burden of malaria for households can be extremely high. It is responsible for the majority of poor academic performances and absenteeism among students and teachers. Treatment with anti-malarial and insecticidal are very expensive and is currently posing great challenge to interventions, considering the increasing reported cases of malaria relapses and drug resistances.

Prompt and effective treatment of all children with malaria is a critical element of malaria control. People who become ill with the disease need prompt and effective treatment to prevent the development of severe manifestations and death (WHO, 2003). Early treatment depends upon prompt recognition of symptoms and signs of malaria in the household, mainly by women. The success of this strategy depends on the behaviour of patients and caretakers of young children and it has been documented that treatment seeking behaviour is related to cultural beliefs about the cause and cure of illness (Bledsoe and Goubaud, 1985). In some cases, illnesses are seen as amenable to treatment by modern practitioners, while others are considered best treated by traditional healers (Press, 1980). Illness ideas and behaviours may enhance or interfere with the effectiveness of control measures (Klein *et al.,* 1995). An understanding of communities' beliefs and behaviours is therefore crucial to the success of a specific control measure. This study aims to ascertain the current perception of cause and treatment seeking practices of caretakers in a rural area in order to identify probable areas of intervention for the control of malaria in the under-fives.

Malaria risk and disease burden is owed to the following factors including poor housing, lack of education, increased exposure to mosquito bites, high household medical costs, reduced ability to pay for treatment, and unequal access to healthcare services so on (Bates *et al.,* 2004; Chuma *et al.,* 2006; Ekpeyong and Eyo, 2006). Decisions for prevention or treatment are made depending on economic ability of the household, perceived susceptibility and assessment of consequences. Furthermore, malaria transmission is often facilitated because environmental degradation, poor drainage and clearing of vegetation readily promote the proliferation of mosquito species such as *Anopheles gambiae* which propagates itself in sunlit, transient water bodies, notably artificial habitats associated with human activities (Fillinger *et al.,* 2004; Minakawa *et al.,* 2005; Munga *et al.,* 2006; Mushinzimana, *et al.,* 2006; Mutuku *et al.,* 2006; Ekpeyong and Eyo, 2008). Malaria, poverty and environmental change are inextricably linked and remain closely associated across most of Africa (Lindsay and Birley, 2004).

Community leaders, public health workers and representatives of various organizations working on health-related issues in the area have identified a great need for training and access to up-to-date information and technical support (Mukabana *et al.,* 2006). Rural areas have always been a major challenge for disease control worldwide, but the involvement and active participation of communities has been identified as a key factor for success in these environments (Kitron and Spielman, 1989; Brieger, 1996; Townson *et al.,* 2005; Van den and Knols, 2006; Mukabana *et al*., 2006). Malaria remains robustly endemic in most rural communities of Sub Sahara Africa, thus the central aim of the Roll Back Malaria Partnership (RBMP) is to strengthen the local

capacities of communities to identify malaria as one of their main health problems and then take the lead in developing and implementing solutions to these problems in partnership with different actors such as non-governmental organizations providing organizational support and research institutions acting as technical consultants (Manderson, 1992; Brieger, 1996; Williams and Jones, 2004; RBM, 2005; Mukabana *et al.,* 2006). In the past, malaria was predominantly viewed only as a biomedical problem; however, successful disease control at the community level needs to take the human behaviour, socio-cultural and economic context into account in order to successfully impact the disease through active participation and changing of risk behaviours (Sornmani, 1992; Williams and Jones 2004). These factors, together with the experienced obstacles of earlier vertical, top-down malaria eradication programs have contributed to the current emphasis on community-based malaria management strategies (Kroeger *et al.,* 1996; Williams and Jones, 2004). Although, considerable difficulties have been reported in conducting community-based disease control (Kidson, 1992), there is a large evidence base where such horizontal approaches have been successful because of a true partnership between the community and programme staff. Key elements of these programmes are the generation of a feeling of empowerment, local ownership and responsibility (Brieger, 1996; Kay and Vu, 2005) and the application of action-oriented and participatory approaches (Manderson, 1992; Onyango-Ouma, 2005). In the light of the above, a study of symptoms associated in the diagnosis and management of malaria in a semi urban community of Enugu State, South-Eastern Nigeria, was conducted as a means of providing base-line data require for the management of malaria globally.

MATERIALS AND METHODS

Study Area: The area of study was Affa, a semi urban community in Udi Local Government Area of Enugu State. The economy of the Affa people is based on semi-subsistence agriculture. It has one of the largest population and land mass with 58,000 people living in scattered small farms and villages. A total of 352 subjects were sampled comprising of primary school children aged between (6 and 17) years and pregnant women attending ante-natal clinic.

The study was conducted between August and December, 2007. The study sites were mapped using the Ach-GIS global positioning system (GPS) instrument as follows: Amofia-Agu Dispensary, Affa (Longitude 7.25117 East and Latitude 6.56483 North), Affa Health Centre, Affa (Longitude 7.32474 East and Latitude 6.60107 North) and Cottage Hospital, Affa. A cross-sectional study of the target populations was done using a systematic random sampling technique (Peterson index).

Ethical Approval: Ethical approval for the study was obtained from the Ministry of Health, Enugu State. At each study site, participation was voluntary and verbal informed consent sought from the subjects and from the Parent/Guardians of the children to be investigated, before an individual was recruited into the study. All sampled data and the research documents were guided with utmost confidentiality.

Diagnosis: The symptomatic diagnosis of malaria was based on the presence of fever (axillary temperature > 37.5°C) at the time of presentation to the health facilities or within the previous 48 hours, coupled with interview of the recent malarial history of the subjects. Symptoms associated with malaria such as fever (F), chill (C), amber urine (U) anorexia (A), frequent sleeping (S), vomiting (V), blisters, weakness (W) headache (H) and joint pain (J) were checked and recorded.

RESULTS AND DISCUSSION

Symptomatic diagnosis of the 352 pregnant women and children, showed that 260 (79%) did not manifest any of the above symptoms within the past three months as at the time of the study, 20 patients had recent fever alone highest at(5.7%), chill and blisters 0.9% each, headache, diarrhoea and joint pain 0.3% each, weakness and anorexia 0.6% each.

Table 1: Symptoms associated in the diagnosis and management of malaria in a semi urban tropical community in Enugu State, Nigeria

Symptoms	Frequency	Percentage (%)
No symptom	260	73.9
Fever	20	5.7
Chill	3	0.9
Headache	1	0.3
Anorexia	2	0.6
joint pain	1	0.3
Weakness	2	0.6
Diarrhoea	1	0.3
Blisters on the lips of the mouth	3	0.9
Fever + Chill	10	2.8
Fever + Headache	2	0.6
Fever + Headache + Chill	3	0.9
Fever + Headache + Chill + Amber Urine	4	1.1
Headache + Fever + Weakness + Amber Urine	2	0.6
Fever + Anorexia + Vomiting	5	1.4
Joint pain + Weakness	3	0.9
Headache + Weakness	4	1.1
Fever + Chill + Headache + Blister + Anorexia + Vomiting	1	0.3
Fever + Chill + Joint pain + Frequent sleeping	4	1.1
Fever + Joint pain	4	1.1
Fever+ Chill + Headache + Anorexia + Joint pain	4	1.1
Fever + Headache + Blister + Weakness	6	1.7
Anorexia + Weakness	7	2.0
Total	352	100.0

Table 2: Sensitivity, specificities and negative predictive values and positive likelihood ratio recorded for the symptomatic malaria diagnostic test in the health facilities studied

Type	Subject No	Sensitivity (%)	Specificity (%)	Predictive Value (%)		FPR (%)	FNR (%)	J-index (%)
				Positive	Negative			
Symptoms	46	41.6	80.0	45.7	77.3	20.0	58.4	78.7

FPR = false positive rate, FNR = false negative rate, J-index = the overall measure of reliability of a diagnostic test, which summarizes the sensitivity and specificity.

Fever combined with chill was highest (2.8%), followed by anorexia and weakness (2.0%), fever with headache and weakness (1.7%), fever with anorexia and or vomiting (1.4%), fever with chill and headache and or amber urine or joint pain or anorexia or frequent sleeping 1.1%, headache with weakness 1.1% (Table 1). Forty six (46) antenatal care patients out of the 352 population sampled for symptomatic diagnosis to predict malaria infection in symptomatic patients with recent history of treatment showed low sensitivity (41.6%), but highly specific (80%), low positive value (45.7%), high negative predictive value

(77.3%), low false positive rate (20%), moderately high false negative rate (58.4%) and a high J-index (78.7%) (Table 2). The axillary temperature was poorly predictive for negative samples with normal axillary temperatures. The mean axillary temperature among children with Acon-Pf positive at (37.0°C) and Acon-*Pf* negative at (36.4°C), antenatal patients with Acon-Pf positive at (36.5°C) and antenatal patients with Acon-Pf negative at (35.1°C).

The asymptomatic stage does not automatically indicate that one has no malaria infection. In this same vein, blisters on the lips of the mouth alone can not indicate malaria, all the major symptoms – fever, chill, headache, anorexia and joint pain are indicators of malaria infection (Manderson, 1992). However, blood test using rapid malaria diagnostic test kits and routine microscopy can only indicate infection proper. This is because other infections present similar symptoms like malaria infection (Fillinger *et al.,* 2004; Minakawa *et al.,* 2005; Munga *et al.,* 2006; Mushinzimana, *et al.,* 2006; Mutuku *et al.,* 2006; Ekpeyong and Eyo, 2008). In the remote and endemic areas of the study, the accuracy of symptom-based diagnosis was poor, as it has been reported by other researchers although specificity in this self-referring sick population should be higher than in the communities as a whole. Local transmission rates, and therefore immunity, were low, and this should have increased the symptomatic malaria sensitivity. The observation of fever alone, and/or fever in combination with chills and/or headache, achieved quite high sensitivities, but both criteria resulted in high rate of over-treatment in symptomatic patients. Any narrower combination of symptoms resulted in sensitivities unacceptable in relation to the detection of a life-threatening illness (Kroeger *et al.,* 1996; Williams and Jones, 2004). The measurement of axillary temperature failed to achieve sufficient sensitivity or specificity to be useful. The data on symptomatic malaria diagnosis shows that children may have higher axillary temperature than adult antenatal patients; reason may be because adults have developed asymptomatic immunity to malaria due to repeated exposure to malaria infection (WHO, 2000 a b). This study suggested that the symptoms identified can be used as an algorithm for the future identification of symptomatic malaria diagnosis for presumptive treatment in remote and malaria endemic regions.

The attitude of the antenatal patients to diagnosis during the study demonstrated the importance of providing patients with a reliable explanation for their illness. The responses of the rural community members and antenatal women suggested that there is an improved treatment-seeking behaviour and drug compliance. A rapid blood-based diagnosis at some cost was preferred by rural community members and antenatal patients to both the delayed free slide diagnosis and symptom-based diagnosis, despite the cost to the patient.

Conclusion: Culturally sensitive but evidence-based education interventions, utilizing participatory tools, are urgently required which consider traditional beliefs and enable understanding of causal connections between mosquito ecology, parasite transmission and the diagnosis, treatment and prevention of disease. Community-based organizations and schools need to be equipped with knowledge through partnerships with national and international research and tertiary education institutions so that evidence-based research can be applied at the grassroots level.

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TISSUE ANALYSIS OF *Clarias gariepinus* JUVENILES INJECTED WITH DIFFERENT CONCENTRATIONS OF CRUDE OIL

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ABSTRACT

Aspects of tissue analysis of Clarias gariepinus juveniles (90.60 ± 0.24g) were studied following injection of different concentrations of Bonny light crude oil (BLCO). Experimental fish were injected at concentrations: 0.00, 25.00, 50.00 and 75.00 µlg^{-1} to test fish responses during 4 days toxicity and 42 days recovery periods. A control experiment was left with fish not injected with BLCO. A 38% crude protein diet was fed to the fish at 3% body weight per day (p.w.d^{-1}) during the toxicity and 5% during the recovery period. The protein value (PV) increased as the BLCO concentration increased up to 75 µlg^{-1} at recovery phase and vis-versa at toxicity phase. This could be due to immune response mechanism which eventually dropped at recovery phase. The starch value (SV) and unsaponification values (USPV) were not affected, the saponification value (SPV) only increased between 25.00 and 0.0 µlg^{-1} and declined at 75 µlg^{-1}. The decrease in values of SPV in treated fish tissues has implication on energy metabolism. Of the four tissues values (PV, SV, SPV and USPV) of fish tested, only the USPV was not significantly different (P > 0.05) among the fish injected with the different concentrations of BLCO. The 0 and 25 µlg^{-1} has the same effect on mortality rate. Similar mortality values of the fish recorded with the 25 µlg^{-1} BLCO and the control suggests that this oil dosage was probably inadequate to alter the mortality rates of C. gariepinus juveniles. The survival rates increased with decreasing concentration.

Keywords: Tissue Analysis, Bonny Light Crude Oil, Mortality, Survival

INTRODUCTION

Oil spills constitute one of the most important sources of environmental problems in Nigeria's petroleum industry. Most of the Nigerian aquatic environments have witnessed a number of oil spillages in recent time. Nearly 3000 cases of oil spill accidents that occurred between 1976 and 1990 caused the release of about 2.40 million barrels of crude oil, resulting in various forms of environmental degradation, deprivation and spoilage (Akingbade, 1991). Oil producing communities in Nigeria have suffered most due to the oil spill menace. Akingbade (1991) recorded varying levels of petroleum hydrocarbons in the body organs of fishes, frogs and snails in areas where oil spills are prevalent. Working on rivers, lakes and reservoirs with continuous input of oil pollutants, Brown *et al*. (1991) recorded the presence of aromatic hydrocarbons including benzene in both water and fish tissues.

The degree of exposure of marine organisms to oil is often assessed by measuring their body burden of petroleum related aromatic compounds (ACs) because ACs are potentially harmful to animals. Fish and marine mammals extensively metabolize most ACs in their livers and predominantly excrete them into bile. The pollution of water sources due to xenobites may play a major role in decline of aquatic animals. Increasing awareness of the adverse effects of

anthropogenic activities and pollution on aquatic environment has focused interest on health of fish populations and possibilities to utilize the health parameters for assessment of the quality of aquatic environment (Henry *et al.,* 2004). The African catfish of the genus *Clarias* is a highly esteemed group of fishes in tropical African and it commands high market value. Its hardy nature and possession of accessory air breathing organs enable it tolerate adverse aquatic conditions (Reed *et al.,* 1967). The high demand for the African catfish (*C. gariepinus*) due to its rich protein profile, fast growth, good size, taste and flavour informed the need to investigate the health parameters and survival of this choice fish species in polluted water.

Against this background, the tissue analysis of *C. gariepinus* adults treated with different concentrations of Bonny light crude oil was investigated. The aim was to inject graded concentrations of the oil pollutant into the fish and assess its impact on the tissue quality of the fish and on the fish survival. The criteria for assessment were: protein value, starch value, saponification and unsaponification values of tissues of *C. gariepinus* juveniles.

MATERIALS AND METHODS

Fish: One hundred and twenty (120) juveniles of C. *gariepinus* (90.60 ± 0.24) were purchased from a private fish farm (Aqua Fish Farm Limited) in Iporoto, Inyiamagu, Ikwo Local Government Area Ebonyi State, Nigeria. The fish were transported in five plastic containers (25 litres capacity) to the fisheries laboratory of Enugu State University of Science and Technology, Enugu. Water temperature during transportation was maintained at 28 ± 0.40°C by introduction of ice cubes preserved in a portable cooler.

The fish were acclimated for 14 days on a 38 % crude protein maintenance diet (Table 1) at 3% body weight daily ($bw.d^{-1}$). Subsequently, the fish were randomly stocked in 12 plastic bowls (25L) containing 24L of dechlorinated tap water at 10 fish per container. The fish were then starved for 24 hours prior to the commencement of the treatment.

BLCO Injection: Injection of fish with graded concentrations of BLCO was carried out via the dorsal anterior musculature of the fish. In all cases, injection was done intramuscularly with the aid of a 2.50 ml hypodermal needle just below the dorsal fin. The crude oil doses applied were: 25 μlg^{-1} (T_1), 50 μlg^{-1} (T_2) and 75 μlg^{-1} (T_3); while the control fish were left without crude oil injection 0.00 μlg^{-1} (T_4). The fish were then fed a 38 % crude protein maintenance diet (Table 1) at 3% bw.d"[1] for 96 hours (4 days) toxicity period and later at 5% $bw.d^{-1}$ for 42 days recovery period. Water temperature (27 ± 1.20°C) and pH (6.60 ± 0.20) were recorded with the aid of maximum - minimum thermometer and a pH meter (Model pH -1-2010L), respectively.

Table 1: Gross composition of the experimental diet fed to *Clarias gariepinus* juveniles stocked in crude oil polluted water

Feed ingredient	% Composition
Yellow maize	9.29
Soyabean meal	54.84
Fish meal	16.65
Blood meal	10.97
Palm oil	5.00
Salt	0.25
Vitamin premix[1]	0.60
Mineral premix[2]	2.40
Total	100.00
Nutrients	
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.80
Nitrogen-free extract	34.46
Total	100.00

(1) Vitamin premix provided the following constituents diluted in cellulose (mg/kg o diet): thiamine, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3,000; niacin, 150; vitamin B12 0.06; retinyl acetate (500,000 IU/g), 6; menadione-Na-bisulphate 80; inositol, 400; biotin, vitamin C, 200; alphatocopherol, 200; cholecalcipherol 1,000,000 IU/g. (2) Contained as g/kg of mineral premix: $FeSO_4.7H_2O$, 5; $MgSO_4.7H_2O$, 132; K_2SO_4, 329.90; KI, 0.15; NaCl, 45; Na_2SO_4, 88; $AlCl_3$, 0.15; $CoCl_2.6H_2O$, 0.50; $CuSO_4.5H_2O$, 0.50; $NaSeO_3$, 0.11; $MnSO_4.H_2O$, 0.70; and Cellulose, 380.97.

Tissue Analyses: The fish tissue analysis was obtained from each treated plastic container and control after 96 hours toxicity (4 days) and 42 days recovery periods. In all cases, fish samples from each plastic container (T_1, T_2, T_3 and T_4) were sacrificed decapitated and the visceral organs removed.

The remaining (fish tissue) was thoroughly washed and used for chemical analysis. Fish tissues were analyzed for their proximate composition using the method of Windham (1996). Tissue analysis of fish done at 96 hours (4 days) for the toxicity phase and 42 days recovery phase were: protein value (PV), starch value (SV), saponification value (SPV) and unsaponification value (USPV) (Windham, 1996).

Mortality: Daily records of the fish mortality and survival were taken during the 96 hours (4 days) toxicity and the 42 days recovery periods. The data were used in calculation of mortality and survival rates, respectively.

Statistical Analysis: All the data obtained were subjected to analysis of variance (ANOVA) to determine if statistical difference existed among treatment means at 5% level of significance.

RESULTS

The tissue analysis for the nutritional value of *C. gariepinus* injected with different concentrations (25.00 — 75 μlg^{-1}.) of BLCO and control indicated that the protein value increased with increasing concentration during the toxicity phase of this study and decreased at recovery period with increasing concentration (Table 2). The SPV, SV and USPV of the fish were not affected by crude oil concentrations and duration of the study. Both the toxicity and recovery phases of the study recorded significant differences in the PV and USPV ($P<0.05$) as well as in the SPV ($P<0.05$) of the fish tissue as the crude oil concentrations in the fish increased from 25.00 to 75.00 μlg^{-1}. The mortality rate (MR) of the fish during the 4 days toxicity and 42 days recovery periods increased as the concentration of injected BLCO increased from 50.00 to 75.00 μlg^{-1} (Figure 1). The MR of the fish under the control treatment had the same effect as that of fish injected with 25µl. g^{-1}. The fish subjected to 75.00μlg^{-1} BLCO injection generally had higher percentage mortality (33.00%) at the recovery period than those injected with 50μlg^{-1} BLCO.

The survival rates (SR) of the fish obtained decreased with increasing concentrations of BLCO. Data obtained from the toxicity and recovery periods showed that there were no significant differences ($P<0.05$) between the SR values of the fish injected with 25 μlg^{-1} BLCO and those under the control study. The fish survived better when exposed to 50 μlg^{-1} BLCO than when injected with 75 μlg^{-1} BLCO (Figure 1).

DISCUSSION

The PV of the fish in (50.00 and 75.00 μlg^{-1} BLCO) increased at toxicity period. This implied that the interactive effect of the crude oil and the tissue protein probably resulted in increased protein concentrations in the fish tissue. Secondly, the organ responsible for protein metabolism was better disposed to synthesize protein at toxicity period to boost the immune mechanism. The SV values of the fish under the control, for both the toxicity and recovery periods did differ significantly from those treated with the crude oil injections. These results also implied that the differences between the metabolism of starch in the injected fish and that in the control fish were significant and that the SV and SPV of the fish were affected by the presence of crude oil in the fish body. The saponification value of the fish tissue increased only in the control fish. The result from both the toxicity and the recovery periods indicated that the reactions of the BLCO concentrations in the fish reduced saponification value of the treated fish. At 75.00 μlg^{-1} BLCO concentrations, the fish were less resistant than those injected with 25.00 μlg^{-1} BLCO, and hence the highest mortality was recorded with fish injected with 75.00 μlg^{-1} BLCO. Lee (1975) reported that mortality increased with the increase in the crude oil concentration in the fish. The similar MR values recorded with 25.00 μlg^{-1} BLCO and

Table 2: Tissues protein, starch, saponification and unsaponification values of *Clarias gariepinus* injected with different concentrations of Bonny light crude oil

Duration	4 Days			42 Days			Control
Crude oil concentration (µlg^{-1})	75	50	25	75	50	25	0
Tissue Protein value (IU/l)	52.95	43.55	30.50	8.10	14.10	18.90	19.60
Tissue Starch Value (IU/l)	0.20	0.21	0.15	0.18	0.16	0.10	0.20
Saponification value (IU/l)	20.19	20.20	20.17	19.80	21.20	21.10	39.20
Unsaponification Value (IU/l)	0.40	0.35	0.30	0.45	0.40	0.35	0.18

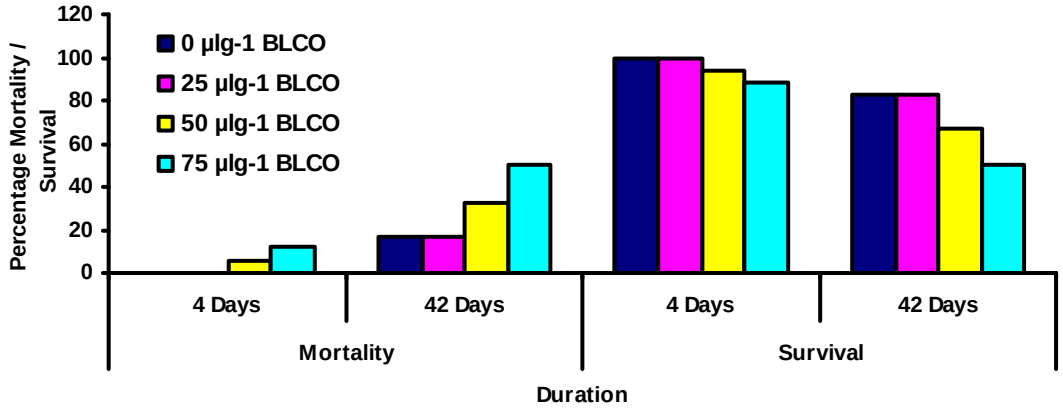

Figure 1: Percentage mortality and survival of *Clarias gariepinus* injected with different concentrations of Bonny light crude oil

the control signified that the 25.00 µlg^{-1} BLCO dosage was probably not enough to effect any change in the mortality rate of *C. gariepinus juveniles*. Mortality of *C. gariepinus juveniles* was directly proportional to BLCO concentrations. The *C. gariepinus* with 25.00 µlg^{-1} BLCO survived in the same manner as those under the control treatment. The least SR values recorded with the 75.00 µlg^{-1} BLCO injection at both periods of this study were consistent with the report of Bryan (1976). Survival of *C. gariepinus juveniles* was inversely proportional to BLCO concentrations.

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EFFECT OF ORAL ADMINISTRATION OF SUBLETHAL CONCENTRATION OF ATRAZINE ON THE HAEMATOLOGICAL PROFILE OF ALBINO RAT

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ABSTRACT

The study investigated the effects of 28 day exposure of albino rats to graded sublethal concentrations of atrazine on the haematological profile. Thirty six (36) male albino rats used for the study were divided into four groups of nine rats each. Group I served as the untreated control while groups II, III and IV were treated with atrazine at 150, 200 and 300 mg/kg body weight orally administered for 28 days. Packed cell volume (PCV), red blood cell (RBC) count, haemoglobin concentration (Hb), total white blood cell (WBC) count and differential WBC count were assessed on day 14, 21, and 28. The exposure to atrazine led to significant reductions in PCV, Hb and RBC of the treated groups especially on day 21 and 28. The treated groups also had significantly higher ($p<0.05$) total WBC counts all through the study, and group IV rats had significantly higher ($p<0.05$) lymphocytes and significantly lower ($p<0.05$) neutrophil on day 28 when compared with the control.

Keywords: Albino rat, *Rattus norvegicus,* Atrazine, Haematology

INTRODUCTION

The impact of man - made compounds on wild life has been critically assessed in recent times. The chemicals originated from a variety of compounds of anthropogenic origin such as pesticides, detergents and plasticizers. Atrazine (2-chloro-4 (ethylamino) 6-isopropylamino)-S-triazine) was a selective chloroatrazine herbicides used on agricultural crops. It was available as dry flowable liquid, water dispersible granular and wettable powder formulations. Atrazine and its primary metabolite, diethylatrazine, are the most commonly encountered groundwater contaminants, mixed use, undeveloped and urban - use areas, the primary justification for most immunotoxicological studies on environmentally relevant xenobiotics was to determine the potential risk of xenobiotic to the human beings (Kristinia *et al.*, 2012). Toxicological manifestations in the immune system following xenobiotic exposure in experimental animals may appear as: changes in lymphoid organ weights and/or histology; quantitative or qualitative changes in cellularity of lymphoid tissue, bone marrow or numbers of peripheral leukocytes; impairment of immune cell function; and increased susceptibility to infectious agents. Most laboratory animal - based immunotoxicology studies stimulate the animal's immune system so that the ability of its immune system to respond to an antigenic challenge can be measured. Mice and rats are the primary mammalian laboratory animals used in immunotoxicology studies because of the availability of a large number of antibodies for assessing the immune system. The existence of

inbred and knockout mouse strains provide additional resources not available in other animal models. The most common pathway for atrazine degradation involves the intermediate cyanuric acid, in which carbon was fully oxidized, thus, the ring was primarily a nitrogen source for aerobic micro - organisms. Atrazine was moderately to highly mobile in soils, especially where soils have low clay or organic content, because, it does not adhere strongly to soil particles and has a lengthy soil half-life (13 to 261 days) (Cai *et al.,* 2003). Atrazine was also subject to photodecomposition and volatilization, when high temperature and prolonged sunlight occur after precipitation, but these effects are not significant under formal field conditions. Atrazine can persist for longer than one year under conditions which are not conducive to chemical or biological activity, such as dry or cold climate. Low concentration of atrazine in water, food and air causes health problems that have endocrine disruptor, possible carcinogenic, immunotoxic effects and low sperm count in men (Birnbatum and Fenton, 2003). According to extension toxicology network, the oral median lethal dose or LD_{50} for atrazine was 3090 mg/kg in rats, 1750 mg/kg in mice, 750 mg/kg in rabbits and 1000 mg/kg in hamsters. The one hour inhalation LC_{50} was greater than 0.7 mg/l in rats (Ciba-Geigy, 1987). There was a growing awareness of the role, which changes in the blood indices of rat could play in the evaluation of the pollution status in the environment. Donna *et al.* (1989) noted that changes in the haematological parameters were useful tools in assessment of the physiological status of rat. Atrazine was absorbed from the gastrointestinal tract based on recovery of orally administered radio labeled material in rats (Bakke *et al.,* 1972). The measurement of haematological changes in blood and tissue of rat under exposure to toxicant may be used to predict the toxic effects of herbicides; detection of specific physiological abnormalities and provide an indication of any gross damage. Highly effective herbicides used on entering the environment may bring about multiple changes in the organism by altering the nutritional values, growth rate and behavioural patterns. Changes in haematological parameters (RBC count, Hb and haematocrit) were reported in Sprague-Dawley rats chemically exposed to 0, 10, 70, 500 or 1000 part per million (ppm) of atrazine for two years (Donna *et al.,* 1989). Haematological studies are essential because the blood has been regarded by man as the essence of life, the seat of the soul and progenitor of psychic and physical strength. The purpose of the study was to investigate the effects of atrazine administered orally on the haematological profile of albino rat (*Rattus norvegicus*) with particular reference to concentration and duration of exposure.

MATERIALS AND METHODS

The present study was done with 36 male *Rattus norvegicus* with mean body weight of 168.89 ± 22.61 purchased from Faculty of Veterinary Medicine, University of Nigeria, Nsukka. It was transported to Animal Genetics and Breeding Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were acclimatized for two weeks before the commencement of the study. During the acclimatization and experiment phases of the study, the rats were fed (Growers' Marsh, Grand Cereals Limited, Nigeria) and watered *ad libitum*. The rats were randomly divided into four groups of three rats each replicated thrice. The rats in group I were not treated with atrazine and served as the control. Group II, III and IV rats were treated with 150, 200 and 300 mg/kg of atrazine per body weight (bw), respectively for 28 days. Atrazine was administered based on the maximum tolerated dose of a pilot study conducted with various doses of atrazine (Narotsky *et al.,* 2001). The weekly weights of the rats were measured electronically. Blood samples were collected from the rats from each group on days 14, 21 and 28 post treatment and haematological parameters assayed.

Blood: Blood sample for haematological analysis were collected from the retro - bulbar plexus of the medial canthus of the eye of the rats. A microcapillary tube was carefully inserted into the medial canthus of the eye to puncture

the retro - bulbar plexus and thus enable outflow of blood into a labeled sample bottle containing ethylene diamine tetra - acetic acid (EDTA). The sample bottle was shaken gently to mix up the blood with EDTA and prevent blood clotting (Stone, 1954).

Haematology: Standard procedures were followed in all the haematological procedures. The haemoglobin concentration of the blood samples was determined by the cyano-menthaemoglobin method (Kachmer, 1970). The PCV was determined by the microhaematocrit method (Cole, 1986). A microcapillary tube nearly filled with the blood sample and sealed at one end with plasticine was centrifuged at 10,000 rpm for 5 minutes using a microhaematocrit centrifuge. After centrifugation, the PCV was read using a microhaematocrit reader.

Red blood cells were counted using an improved Neubaur haemocytometer chamber (Schalm *et al.,* 1975). Erythrocytes were counted in the loaded Neubaur chamber and total numbers were reported as 10^6 mm $^{-3}$ (Wintrobe, 1967). Total WBCs were counted using an improved Neubaur haemocytometer (Schalm *et al.,* 1975). The differential leucocyte count was done using the Leishman technique. The blood sample was shaken and a drop of blood was smeared on the slide using a cover slip to make a thin smear. The smear was air dried and thereafter stained using Leishman stain. The stained slides were examined with an immersion objective. 200 cells were counted by the longitudinal counting method and each cell type was identified and scored using the differential cell counter. The results for each type of WBC was expressed as a percentage of the total count and converted to the absolute value per microlitre of blood.

The data were analyzed using one way analysis of variance (ANOVA) and variant means were separated using least significant difference (LSD) post hoc test. Means were accepted as significant at $p < 0.05$.

RESULTS

After 14 days of treatment, the PCV of the control group (Group 1) was 39.00 ± 0.57% while that of the groups given atrazine (Group II to IV) were 37.66 ± 1.45, 36.33 ± 1.20, and 37.16 ± 0.60% for the groups II, III and IV, respectively (Figure 1). The PCV of the rat groups given atrazine were significantly lower ($p< 0.05$) than the control rats on day 14 post treatment. After 21 days of exposure to atrazine, the mean PCV of the groups (Groups 1, II, III and IV) were 41.00 ± 0.57; 38.66 ± 0.88, 37.66 ± 0.33 and 35.25 ± 0.73%, respectively. At 21 days of exposure, only the PCV of group IV rats was significantly lower ($P < 0.05$) than that of the control (Figure 1). The PCV results after 28 days of treatment for the groups were 44.66 ± 0.88, 36.66 ± 0.88; 35.66 ± 0.88, 35.33 ± 0.33% for group I, 11, III, and IV (Figure 1). The effects of the PCV were observed to be dose dependent and the PCV of all the rat groups exposed to atrazine was found to be significantly lower ($p<0.05$) than that of the control.

There were no significant variations ($p > 0.05$) in the haemoglobin concentration of all, the rat groups after 14 days of exposure of the treated groups to atrazine, but after 21 and 28 days of exposure the haemoglobin concentration of the groups exposed to atrazine was found to be significantly lower ($p<0.05$) than that of the control (Figure 2).

On all the days of assessment (day 14, 21 and 28), the total WBC count of the groups exposed to atrazine were significantly higher ($p < 0.05$) than that of the control group (Figure 3).

There were no significant variations ($p > 0.05$) in RBC counts between all the groups after 14 days of exposure of the treatment groups to atrazine (Figure 4). However, after 21 and 28 days of exposure to atrazine groups, their RBC counts of the treated groups were found to be significantly lower ($p < 0.05$) than that of the control (Figure 4).

Though the lymphocyte counts of the group IV rats was higher than that of other groups after 14 days of exposure to atrazine, there was however no significantly ($P > 0.05$)

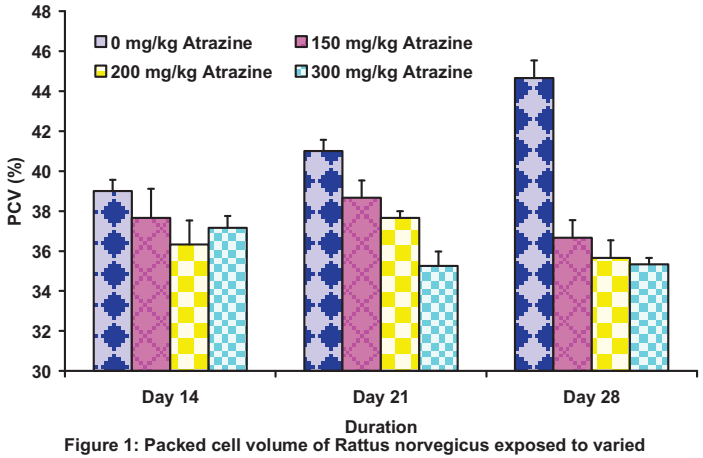

Figure 1: Packed cell volume of Rattus norvegicus exposed to varied concentrations of atrazine

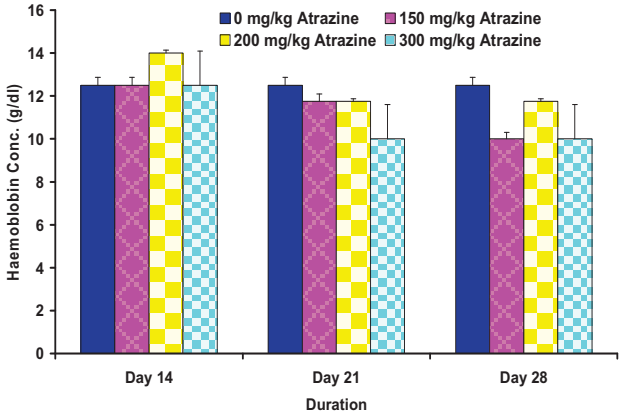

Figure 2; Haemoglobin concentration of *Rattus norvegicus* exposed to varied concentrations of atrazine

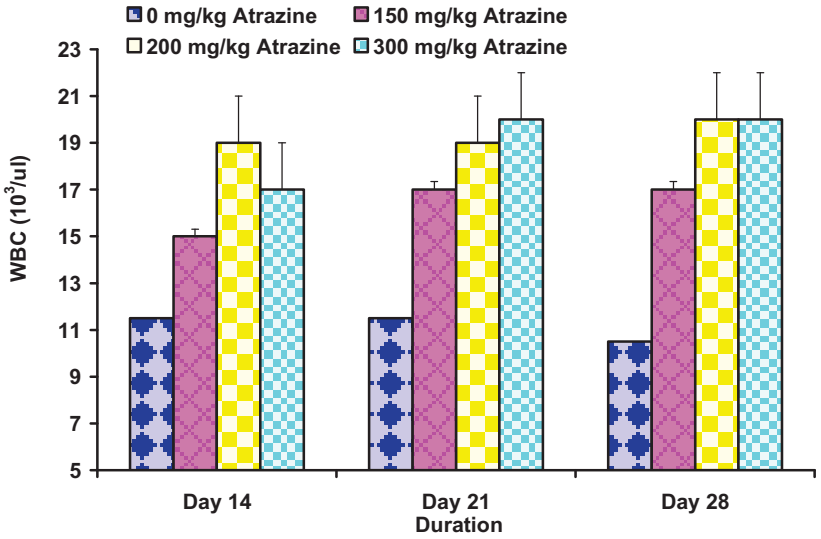

Figure 3: White blood cell counts of *Rattus norvegicus* exposed to varied concentrations of atrazine

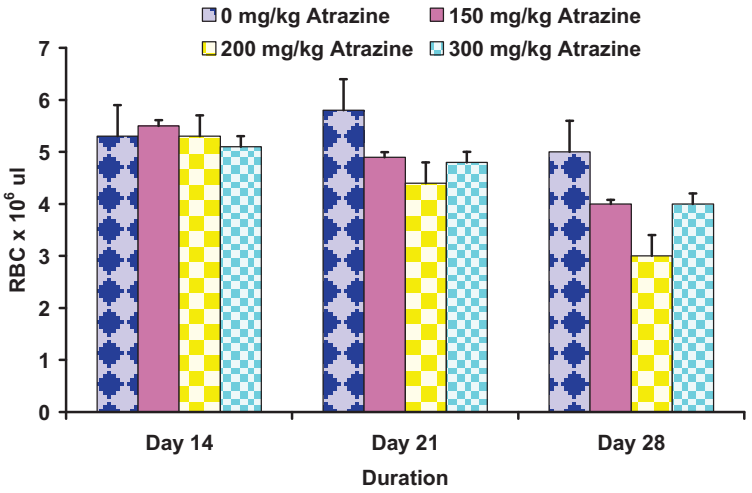

Figure 4: Red blood cell counts of *Rattus norvegicus* exposed to varied concentrations of atrazine

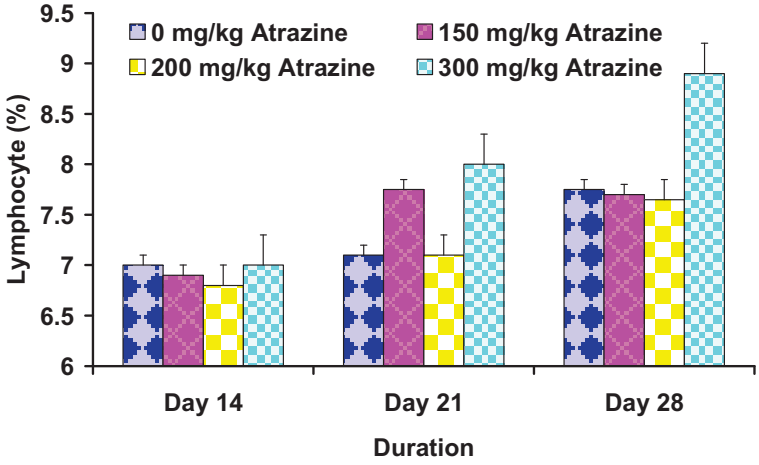

Figure 5: Lymphocyte cell counts of *Rattus norvegicus* exposed to varied concentrations of atrazine

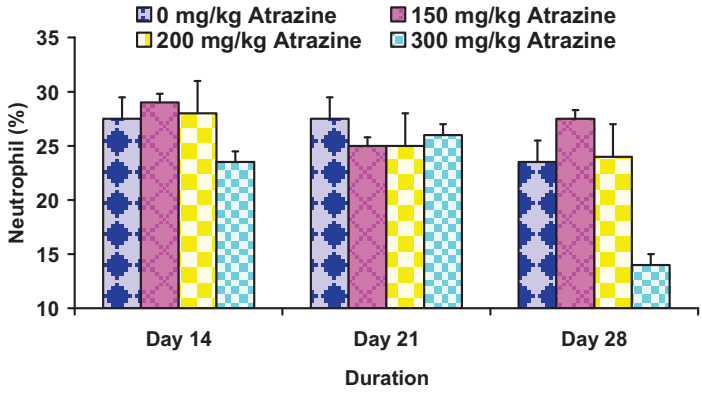

Figure 6: Neutrophil cell counts of *Rattus norvegicus* exposed to varied concentrations of atrazine

variation between all the groups (Figure 5).However the lymphocyte counts of group iv rats was significantly higher ($p < 0.05$) than that of all other rat groups on day 21 and 28 (Figure 5). After 14 days of exposure to atrazine, the neutrophil count of the group 4 rats was significantly lower ($p < 0.05$) than that of all other groups, but at day 21 of assessment, there were no significant variations ($p > 0.05$) between the groups (Figure 6). The day 28 assessment showed that the neutrophil count of the group IV was far lower and significantly lower ($p < 0.05$) than that of all the other groups, and that of group 2 was significantly higher ($p < 0.05$) than that of the control (Figure 6).

DISCUSSION

In recent years, haematological variables have been used more to determine the sublethal concentration of pollutants. In the present study, the results of the present investigation show that atrazine exposure as used in this study led to significant reductions in PCV, haemoglobin concentration and RBC counts. The recorded reduction in erythrocytic parameters in this study was in accordance with the findings of Ciba-Giegy (1987) in CF-1 mice and beagle dogs exposed to atrazine. This implied that exposure to atrazine at the level used in this study could lead to anaemia, probably due to decrease in rate of RBC production or outright impairment of erythropoiesis (Ihedioha and Chineme, 2004). Baroni and Sahai (1992) also reported that

haematological changes resulted in reduction of haemoglobin, RBC and WBC counts after administration of benzene hexachloride on *Rattus rattus.* The disturbances in the antioxidative capacity reported previously in rats' RBCs after sodium metavanadate (SMV; 0.125mgV/ml) intoxication (Ścibior *et al*., 2012) may suggest that oxidative stress could also be, in part, involved in the mechanism responsible for the development of anaemia.

In the present study there was increase in WBC counts in the atrazine exposed rats. This finding was in agreement with the reports of Alexander *et al.* (2008). WBCs play a major role in the defense mechanism of the body. The observed leukocytosis was an indication that the body defense mechanisms of the rats recognized and responded appropriately to atrazine as a foreign chemical agent. The observed leucocytosis in this study represented a physiological response and attempt of protection against damage by a chemical agent. Similar leucocytosis had been reported in fish exposed to pesticides (Allen, 1994). The recorded high lymphocyte counts of group IV rats implies that exposure of rats to atrazine of 300mg/kg bw may be immunogenic. The immunogenicity may be part of protective response or a stimulatory action (Ihedioha and Chineme, 2004). The finding of a significant reduction in neutrophil count of group IV rats was noteworthy. It suggests either a specific use - up of neutrophils or decreased production of these specific cells in rats given 300mg/kg atrazine. Based on the results of this study, it was concluded that exposure of rats to atrazine led to significant reduction in PCV, RBC counts and haemoglobin concentration of exposed rats, significant elevation of total WBC counts of exposed rats and significant increase in neutrophil counts of the rats exposed to 300 mg/kg bw. Some investigators are of the opinion that any immune alteration observed in rodents following xenobiotic exposure was of potential consequence for man (Bourdeau *et al.,* 1990; Kristinia *et al*., 2012). An alternative opinion was that only those immune alterations in rodents which are associated with hypersensitivity or altered host resistance to infectious agents or neoplastic cells are of major concern. The use of the immune system as a sensitive parameter for detecting sub clinical toxic injury was justified for several reasons: functionally immunocompetent cells are required for host resistance (Rooney *et al*., 2004).

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EFFECTS OF *Piliostigma thonningii* ETHYL ACETATE LEAF EXTRACT ON ALUMINIUM-CUM EXTRACT TREATED WISTAR RATS

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ABSTRACT

Recent research findings extol the medicinal significance of the different parts of Piliostigma thonningii. The present study investigated the hepatoprotective effect of its ethyl acetate leaf extract against $AlCl_3$-induced hepatocellular derangement in mature male rats. Thirty male Wistar rats (mean weight, 207 ± 11.01g) were randomly assigned to three groups: a control group treated with 0.5 ml of olive oil (vehicle for the extract) and 1 ml of saline (vehicle for the toxicant), a second group treated with 0.5 mg of $AlCl_3$ (toxicant) per kg of body weight (bwt) and a third group treated with 0.5 mg of $Alcl_3$ and 250 mg of P. thonningii extract per kg of body weight. Doses were administered for a period of 35 days at 24 h interval. Enzyme indices of liver functional and physiological integrity were analyzed using clinical test kits. The data obtained showed that rats treated with $AlCl_3$ expressed significant decrease in mean body weight gain ($p<0.05$) as well as increased serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) relative to the control group. $AlCl_3$ also caused a significant increase in the absolute weight of the liver of rats. The $AlCl_3$-induced derangements were almost completely reversed in rats co-treated with P. thonningii ethyl acetate leaf extract. Histopathological examination of thin sections of liver of rats in the different groups showed massive hepatocellular damage in rats exposed to $AlCl_3$ alone compared to rats co-treated with the extract. Conclusively, the current study indicates that P. thonningii ethyl acetate leaf extract at the dose administered (250 mg/kg bwt po) protected rats against $AlCl_3$-induced liver damage.

Keywords: *Piliostigma thonningii*, Ethyl acetate, Aluminium chloride, Liver damage, Histopathology

INTRODUCTION

Liver diseases are arguably the most sever ailments and major cause of death globally. Hepatotoxic agents range from chemicals (carbon tetrachloride, excess consumption of alcohol), chemotherapeutic agents (high doses of paracetamol), microbes (virus) to peroxidised oils (Maheswari *et al.,* 2008). Aluminium in its different salt forms has been reported to be hepatotoxic (Hassoun and Stohs, 1995; Chinoy and Memon, 2001; El-Demerdash, 2004). Most of these studies reported intraperitoneal administration of this compound, which does not represent the main route of human exposure. Pulmonary and oral are the major routes through which aluminum enters the body (Testolin *et al*., 1996). Although only a small portion of aluminum is absorbed through the gastro intestinal tract (GIT), oral intake is associated with the greatest toxicological implications (Testolin *et al*., 1996). Nevertheless, in this study, $AlCl_3$ was administered to the experimental animals

through oral route. More than ever before, humans are continually exposed to aluminium through cooking utensils (Sharma and Mishra, 2006), food products and drinking water (Yokel and McNamara, 2001), food addictives and toothpaste (Abbasali *et al*., 2005) and through medicines such as antacids, phosphate binders, buffered aspirin, vaccines and injectable allergens (Lione, 1985; Kowalczyk *et al.,* 2004). Environmental pollution from a variety of aluminium-containing waste, especially from industrial waste water, increases human exposure beyond normal levels (Kloppel, 1997). Orthodox anti-hepatotoxic or hepatoprotective agents are expensive and unaffordable by a large size of the populace in developing countries. Although orthodox drugs are generally preferred, alternative medicine is very much relied on all over the world (O'Brien, 2004; Leckridge, 2004). According to the World Health Organization estimates, almost 80% of the people in developing countries rely entirely on traditional medicine for their primary health care and 85% of such traditional medicine involves the use of plant extract (Farnsworth, 1988).

Piliostigma thonningii (Figure 1) is reported to be rich in different antioxidant molecules (Aderogba *et al.,* 2004; Akindahunsi *et al.,* 2005; Ighodaro *et al*., 2012). Many plants known to possess antioxidant properties have been proposed in the treatment and prevention of different pathologies induced by oxidative stress (Seigler, 1998). *P. thonningii* is a leguminous plant which belongs to the family, Leguminosae-Caesalpiniodae that comprises trees, shrubs or very rarely scramblers. The tree is perennial in nature and its petals are whitish to pinkish in color, produced between November and April (Jimoh and Oladeji, 2005). Its various organs: root, bark, seed, fruit, leaves, have been used for various medicinal purposes. Preliminary phytochemical studies on *P. thonningii* reveals high levels of flavonoids, tannins and alkaloids as well as nutritionally important vitamins (such as C, E and beta-carotene) all of which contributes to its strong antioxidant properties (Aderogba *et al.,* 2004; Akindahunsi *et al.,* 2005; Ighodaro, *et al*., 2012). In many African countries *P. thonningii* is used to treat wound, ulcers, gastric/heart pain, gingivitis and as an antipyretic. In Tanzania and Zimbabwe, a cough remedy is prepared from root bark; this fraction exhibits significant anti-inflammatory/analgesic activity in some situations (Silva, 1997). It has also been reported that certain compounds isolated from its leaves have anti-inflammatory (Silva, 1997) and antibacterial activities (Akinpelu *et al.,* 2000). The present study is designed to evaluate the possible hepatoprotective effect of *P. thonningii* ethyl acetate leaf extract on aluminium-induced hepatotoxicity in Wister rats.

MATERIALS AND METHODS

Extract: *Piliostigma thonningii* leaves were collected from the Botanical Garden, University of Agriculture, Abeokuta, southwest of Nigeria. The harvested leaves were freed of extraneous materials; air dried at room temperature and was milled into a powdery form. Five hundred grams of the powdery sample was dissolved in 2.5 litres of distilled water. The mixture was allowed to stand for 48h and stirred intermittently to facilitate extraction. The mixture was sieved using a muslin cloth. The resulting volume on sieving was reduced with a rotary evaporator at 60 ± 1 ^{0}C. Final solvent elimination and drying was done using a water bath at 40 ^{0}C. A solution of the extract was prepared and appropriate aliquots were taken when required.

Animal: Thirty six male Wistar albino rats (mean weight, 207 ± 11.01g) used for the study were purchased from a local breeder at Oje, Ibadan, southwest of Nigeria. The animals were handled humanely, kept in metallic suspended cages in a well ventilated and hygienic rat house under standard conditions of temperature and humidity. They were maintained on normal laboratory chow (Ladokun feeds) with water *ad libitum,* and subjected to natural photoperiod of 12 hours light/12 hours dark cycle. The rats were randomly assigned to three treatment groups replicated thrice (4 rats per replicate). All animals were weighed before and at the end of the experiment.

Experiment: Group I (control) rats were given 0.5 ml of olive oil (vehicle for the extract) and 1ml of saline (vehicle for the extract). Group II rats were treated orally with 0.5 mg of $AlCl_3$ (toxicant) per kg bwt. Group III rats were co-treated orally with 0.5 mg of $AlCl_3$ and 250 mg/kg of *P. thonningii* extract per kg bwt. The administrations of the toxicant and the plant extract were done for a period of 35 days at 24 h interval. Animals in all the groups were given equal access to laboratory chow and water *ad libitum.*

Biochemical Analysis: After the last treatment, the animals were fasted overnight and weighed. Blood was collected from the retro orbital sinus of the eye by ocular puncture into non-heparinised tubes, allowed to clot at room temperature for 30 minutes and the serum was separated by centrifugation at 3000x g for 10 minutes dispensed in aliquots for biochemical analyses. The activity of alanine amino transferase (ALT: EC. 2.61.2.1) and aspertate amino transferase (AST: EC. 2.6 1.1) in blood samples were estimated by the use of end point colorimetric diagnostic kit (Randox Laboratories Limited, England) and standardized using (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) activity was determined by the use of sigma diagnostic kits (Sigma Diagnostic, USA) and standardized using (Englehardt *et al.*, 1970).

Histopathology: The rats were then sacrificed by cervical dislocation and the target organ, liver was quickly excised from each rat and separately washed in ice-cold 1.15% KCl solution, blotted and weighed. Parts of the liver was fixed in 10% formalin and processed for paraffin embedding using the standard micro technique. Thin sections (3µm) of the liver from rats in different groups were histologically processed, stained with Haematoxyln and Eosin and observed with light microscope for histopathological changes.

RESULTS AND DISCUSSION

Body and Organ weights: There was a slight increase in the body weight of rats in all the groups and the gain was lowest in rats treated with $AlCl_3$ alone. There was however no significant differences in mean weight gain among the groups except for rats treated with $AlCl_3$ (Table 1). $AlCl_3$ treatment in the current study significantly lowered the mean body weight gain of rats (by 53.7 %) when compared to the control group (Table 1). $AlCl_3$ is a heavy metal and interferes with the absorption and utilization of essential nutrients in body cells. Particularly, it competes with Mg, Zn and Ca for absorption, and as a trivalent cation (Al^{3+}), it binds readily to negatively charged groups such as phosphate groups, nucleic acids and phosphorylated proteins. Deficiency in calcium and phosphate in the body cells affects bone formation (Szilagyi *et al.,* 1994) and reduces bone density which is an integral part of body mass. Moreover, binding of aluminium to phosphate groups severely reduces DNA and RNA synthesis (Nicholls *et al.*, 1995; Yumoto *et al.,* 2001) and inhibits protein synthesis and cell proliferation. This probably explained the significant decrease in mean body weight gain ($p < 0.05$) associated with rats treated with $AlCl_3$ alone. This observation corroborated with reports from earlier studies (Yousef *et al.*, 2005; Guo *et al.*, 2005) that aluminium intake results in remarkable decrease in body weights gain in rats. Kowalczyk *et al.* (2004) particularly noted significant reduction in water and food intake and transient diarrhoea, which resulted in lowering of final body mass of male rats treated with aluminium chloride for three months relative to the control group. Rats which were co-treated with *P. thonningii* extract and $AlCl_3$ were apparently protected against nutritional deficiency induced by $AlCl_3$ intake, as evident in the similar mean body weight gain of the rats (29.41g) and that of the control rats (32.25g) (Table1). This is probably due to the ability of the extract to curtail aluminium toxicity or contribute to the nutrition of the animals. $AlCl_3$ ingestion caused a marked increase in absolute weight of the liver of rats, and this change was almost completely reversed in rats co-treated with the extract (Table1). The liver is the central metabolic site of the body. Virtually all toxicants, drugs or xenobiotics are metabolized in it; this makes the organ highly susceptible to harmful

Figure 1: *Piliostigma thonningii* leaves

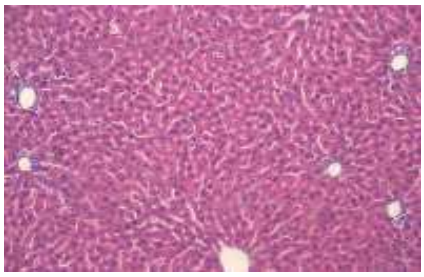

Figure 2: Tin section of liver of control rat treated olive oil and saline (vehicles for extract and toxicant showing normal histology

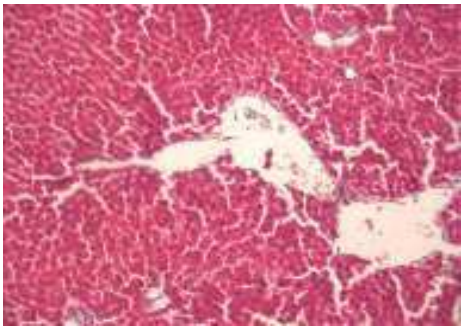

Figure 3: Tin section of liver of rats treated $AlCl_3$ showing signs of fatty degeneration, congested sinusoids and lymphocyte infiltration

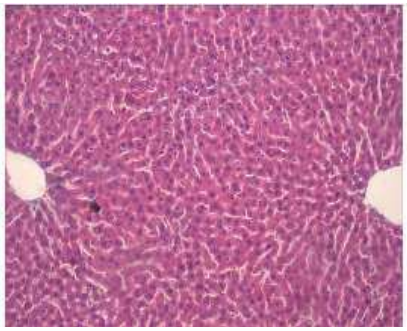

Figure 4: Tin section of liver of rats co-treated with $AlCl_3$ appearing almost as normal as that of the control rat

Table 1: Effects of $AlCl_3$ and *P. thonningii* ethyl acetate leaf extract on the body weight gain and weights of selected organs of rats

Organ	Control	$AlCl_3$ alone	EA. Extract + $AlCl_3$
Body weight gain (g)	32.25 ± 5.30	14.92 ± 4.91*	29.41 ± 3.31**
Liver (g)	6.03 ± 1.08	6.49 ± 1.32*	5.84 ± 0.89**
Liver (g/100g bwt)	3.20 ± 1.10	3.31 ± 1.02	3.0 ± 0.56

*Values are expressed as Mean ± SD (n=12). *P < 0.05 against control, ** P < 0.005 against group treated with $AlCl_3$ alone. n= number of rats per group,* EA=Ethyl acetate *SD= Standard deviation*

Table 2: Effects of $AlCl_3$ and *P. thonningii* ethyl acetate leaf extract on liver enzymes activity in serum of rats

Enzyme	Control	$AlCl_3$ alone	EA. Extract + $AlCl_3$
ALT (U/L)	10.62 ± 2.31	18.25 ± 3.10*	12.12 ± 2.60**
AST (U/L)	13.19 ± 1.63	29.12 ± 1.90*	16.30 ± 3.42**
ALP (U/L)	119.62 ± 5.31	228.01 ± 3.52*	213.56 ± 5.81

*Values are expressed as Mean ± SD (n = 12). *P < 0.05 against control, **P < 0.05 against group treated with $AlCl_3$ alone. n = number of rats per group.*

metabolites generated in the process of metabolism. The increase in the weight of the liver of rats following oral ingestion of $AlCl_3$ is likely as a result of inflammation of the hepatocytes which may be associated with $AlCl_3$ metabolism or direct toxicity.

Liver Enzymes and Histopathology: Compared to the control rats, $AlCl_3$ ingestion significantly ($P<0.05$) raised the activities of liver enzymes, ALT, AST and ALP in rats (Table 2). Elevated serum level of these enzymes is indicative of liver injury, as they are often present in large amounts in the liver and increase in the serum following hepatocellular

damage or injury. In this study, Exposure of rats to $AlCl_3$ caused significant ($P<0.05$) increase in ALT, AST and ALP activities by 71.8%, 120.0% and 90.6% respectively in the serum of rats treated with $AlCl_3$ as compared to the corresponding group of control rats. This is substantiated by the photomicrographs of the liver of rats in the different groups which showed high degree of fatty degeneration and lymphocyte infiltration in the liver of rats treated with $AlCl_3$ alone as compared to other groups (Figures 2, 3 and 4). Upsurge in ALT, AST and ALP activities was prevented on co-administration of *P. thonningii* extract by 33.6%, 44.02% and 6.34% respectively (Table 2). These reductions except in ALP are significant and suggest that the extract protected the rats against $AlCl_3$ mediated oxidative stress. The relatively lower decrease in serum ALP by the extract is unclear. The protective effects of *P. thonningii* could be attributed to its antioxidant properties or chelating effect on aluminium. *Piliostigma thonningii* is rich in flavonoids, tannins and alkaloids as well as nutritionally important vitamins such as C, E and beta-carotene and minerals such as calcium, magnesium, zinc and potassium, all of which contributes to its strong antioxidant properties (Aderogba *et al.,* 2004; Akinpelu, 2005; Ighodaro *et al.,* 2012).

Conclusion: The current study indicates that $AlCl_3$ ingestion at a dose of (0.5 mg/kg bwt po) caused decrease in body weight gain, increase in absolute weight of the liver and in hepatocellular damage in rats. Treatment with *P. thonningii* ethyl acetate leaf extract at a dose of 250 mg/kg bwt po) showed significant hepatoprotective potential against liver toxicity. This pharmacological property may be adduced to antioxidant molecules (polyphenol, vitamins and minerals) present in *P. thonningii* ethyl acetate leaf extract. We thus recommend that the required dietary allowance of aluminium in foods, water and medical drugs should be critically reviewed.

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COMPARATIVE ANALYSIS OF ACON-*Plasmodium falciparum* RAPID MALARIA DIAGNOSTIC TEST WITH ROUTINE MICROSCOPY AMONG SCHOOL CHILDREN AND PREGNANT WOMEN IN A RURAL COMMUNITY IN ENUGU STATE, NIGERIA

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ABSTRACT

There are currently two methods for the direct diagnosis of malaria: the thin blood smear and the thick smear. A third method called the Acon-Plasmodium falciparum (Acon-Pf) (a new cheap malaria rapid diagnostic test) has been developed. This was the first study comparing the three methods in rapid malaria diagnosis among school children and pregnant women in Enugu State, Nigeria, using qualitative and quantitative questionnaires that address their knowledge, attitude, behaviour and practice. In this study, 352 whole blood samples of individuals with suspected falciparum malaria were investigated among pregnant women attending antenatal clinic and school children in rural communities in Enugu State. The following parameters were determined: intrinsic validity, predictive values, species diagnostic power and logistic factors. Acon-Pf had the following characteristics: 91.5 % sensitivity, 87.2 % specificity, 64.3 % positive predictive values, 97.6 % negative predictive values, high malaria infection rate determination of 58.7%, reduced mean packed cell volume (0.25 mmol/L) in malaria patients, correct species diagnosis including both the trophozoite and gametocyte stages of Plasmodium falciparum. The Acon-Pf positive test result ranged from light to thick coloured bands and the time required for the test was 1.3 – 15 ± 5 minutes. The cost per Acon-Pf test cassette at bulk purchase was ₦112.50 (US$0.90) without the service charge as compared with thick smear (₦ 300.00, US$ 2.50) and ₦ 350.00 (US$ 2.90) for thin smear. The study found that Acon-Pf is suitable along side microscopy in the accurate diagnosis of malaria in Enugu State. The use of Acon- Pf and thick smear tests in parallel, first collecting the Acon-Pf results, as it contributes in reading the thin smear result for confirmation of species, diagnosis and assessment of parasitaemia. Thus, Acon-Pf test device is a viable cost effective adjunct to routine microscopy and a reliable option for malaria diagnosis in remote and emergency situations.

Keywords: Acon-Pf, Routine microscopy sensitivity, Specificity, Intrinsic validity, Predictive values, Parasitaemia, Trophozoite, Gametocyte, Diagnostic power, Epidemiology, Logistic factors

INTRODUCTION

Malaria remains a major public health problem in Nigeria, despite decades of a sustained national control programme. One of the major setbacks of this control programme is the lack of a rapid, accurate, affordable and simple means for malaria diagnosis. The two diagnostic approaches currently used i.e. clinical and microscopic diagnosis, however, do not allow for a satisfactory diagnosis of malaria. Clinical diagnosis is the most widely used; however, the symptoms of malaria are very nonspecific and overlap with those of other febrile illnesses (WHO, 1999). A diagnosis of malaria based on clinical grounds alone is therefore unreliable and, when possible, should be confirmed by laboratory tests. Microscopic examination of thick blood film is currently the standard method for malaria diagnosis. This method is relatively simple and has low direct costs, but its reliability is questionable, particularly at low levels of parasitaemia and in the interpretation of mixed infections (Molyneux and Fox, 1993; WHO, 1996). Recently, rapid antigen detection methods have been developed for situations in which reliable microscopy may not be available. These tests are based on the detection of antigen(s) released from parasitized red blood cells (Moody, 2002). In the case of *Plasmodium falciparum*, these new methods are based on detection of *P. falciparum* histidine-rich protein 2. Diagnoses in malaria endemic rural areas are based on clinical symptoms which are non-specific and often difficult to interpret (WHO, 1990; Gilles and Warrell, 1993). This inevitably leads to considerable misdiagnosis and cause less than half of the people with malaria not to receive adequate and effective antimalarial treatment (WHO, 1984; 1990; 2000a).

Research showed that malaria kills as many as 3 million people a year and weaken nearly 300 – 500 million more, mostly in Africa. Children under five years and pregnant women are the most vulnerable to this menace (WHO, 2000b). The burden of malaria in Africa is particularly dangerous, causing 900,000 deaths, and every 30 seconds an African child dies (WHO, 2000b). Everyday, at least 1000 Africans dies of malaria and at least 20 % of all are children under five. The morbidity is high with about 500,000 African children suffering from cerebral malaria and high incidence of mortality during pregnancy and neonatal deaths (within the first 28 days of birth), about 8-14% low birth weight and 3 – 8% of infant deaths in endemic areas of Africa (ASPAD, 2004). Malaria rapid diagnostic tests have the potential to significantly improve the diagnosis of malaria in developing countries (Center for Human Services, 2000). Malaria can be suspected based on a patient's symptoms and physical findings at examination. However, for a definitive diagnosis to be made, laboratory tests must reveal the malaria parasites or their components (WHO, 2005).

Although the peripheral blood smear examination that provides the most comprehensive information on a single test format has been the "gold standard" for the diagnosis of malaria, the immuno-chromatographic tests for the detection of malaria antigens, developed in the past decade, have opened a new and exciting avenue in malaria diagnosis (Chiktara and Ahmed, 2004). However, their role in the management and control of malaria appears to be limited at present.

For monitoring the effectiveness of treatment, the malaria rapid diagnostic tests could therefore be a useful alternative to microscopy, particularly (i) in places where the facilities for microscopy are poor or non-existent and (ii) among hospitalized patients with severe, complicated malaria (in whom parasitaemia and drug response need to be followed very carefully) (WHO, 2000b; Singh *et al.,* 2003). Malaria rapid diagnostic tests can be used by relatively inexperienced persons to diagnose malaria infection in rural areas where facilities for microscopy are not available. In the case of pregnancy, malaria parasites that are sequestered in the placenta may not be detected by smears prepared using peripheral blood samples (Duffy and Fried, 2006). This study investigated evaluated the effectiveness of Acon-Pf rapid malaria diagnostic test and routine microscopy among school children and pregnant women in Enugu State, Nigeria in malaria diagnosis.

MATERIALS AND METHODS

Study Area: Affa is a rural community in Udi Local Government Area of Enugu State, Nigeria. Affa has one of the largest land mass and population of 58,000 people living in scattered farm communities and villages. Semi-subsistence agriculture is the economic mainstay of the Affa people. The inhabitants of Affa lack access to both microscopic diagnosis and malaria rapid diagnostic tests of malaria in the rural health centres. The community is about 70 kilometers away from the district and specialist hospitals located in Udi Local Government Area and Enugu metropolis, respectively. Traveling to these health facilities is difficult and expensive for the poor farmers. The study sites were mapped using the Ach-GIS Global Positioning System (GPS) instrument as follows: Amofia-Agu Dispensary, Affa (Long. 7.25117 E and Lat. 6.56483 N), Affa Health Centre, Affa (Long. 7.32474 E and Lat. 6.60107 N), Cottage Hospital, Affa (Long. 7.32509 E and Lat. 6.60112 N) and Enugu State University of Science and Technology (ESUT) Teaching Hospital (Long. 7.49450 E and Lat. 6.46047 N).

Study Population: A total of 352 subjects were sampled comprising of primary school children aged between (6 and 17) years and pregnant women attending ante-natal clinic. The study was conducted between August and December, 2007.

Ethical Approval: Ethical approval for the study was obtained from the Ministry of Health, Enugu State. At each study site, participation was voluntary and verbal informed consent sought from the subjects and from the parent/guardians of the children to be investigated, before an individual was recruited into the study. The research team consisted of a Medical Practitioner to facilitate the administration of drugs and treatment of infected population. Coartem was administered free of charge, to symptomatic patients who tests positive to the Acon-Pf in the field and fit a standardized diagnostic algorithm that includes fever and headache and/or chills or rigors occurring within the preceding three days, according to World Health Organization standards. Children who were recruited for this study were generally dewormed after being tested, irrespective of the status of the malaria test result with albendazole regimens received from the United Children Education Fund (UNICEF) as a benefit for volunteering towards the survey. There was a strict adherence to all universal precautions and provision of Post Exposure Prophylaxis accordingly as the need arises. Bio-safety issues on handling of needles, disinfections, control of haemorrhages, containment of complications and disposal of equipment and leftovers was strictly adhered to as directed. All sampled data and the research documents were guided with utmost confidentiality.

Study Design: This is an evaluation study in which the results from *falciparum* malaria diagnosis by the (Acon-Pf test cassette) were compared against blood film microscopy and the practicability of the new test in rural and malaria endemic areas assessed. The symptomatic diagnosis of malaria was based on the presence of fever (axillary temperature > 37.5°C) at the time of presentation to the health facilities or within the previous 48 hours, coupled with a history of recent bout of mosquitoes at home and farmlands.

Patients were questioned regarding the nature and duration of recent symptoms. Malaria rapid diagnostic tests and slides were prepared and the malaria rapid diagnostic tests were read. Acon-Pf test cassette, a rapid diagnostic test device manufactured by ACON Laboratories, Incorporated, USA, was used for immunochromatography test. The immuno-chromatographic test (ICT) is based on the detection of histidine rich protein-2 (HRP-2) in the blood. The intensity of malaria rapid diagnostic tests antigen bands were graded 1 (faint) to 3 (equal to or darker than the control band). The slides were re-read by an experienced microscopist, who was not aware of the results of the malaria rapid diagnostic tests, in accordance with WHO criteria (100 thick film fields before negativity declared (WHO, 2001). This was done after storage and transportation

for between two and four weeks at ambient temperature and humidity, as was usual in the area. The ambient temperature of the locality was within the range of 27°C to 29.5°C during the period of the study.

The packed cell volume (PCV) of the pregnant women was determined using Micro-Haematocrit Centrifuge (LAB–TECH, India) at 1000 rpm for 5 minutes to ascertain the effect of the *P. falciparum* malaria on the haemoglobin level of the patients.

The logistic factors that affect the Acon-Pf test cassette which include ambient temperature and humidity, and the effects on transportation and storage on the test cassette. The average times taken for the detection of parasite antigen by the Acon-Pf test cassette, and the type, stage and morphology by the routine microscopy were recorded, respectively.

The comparative cost benefit of using microscopy or Acon-Pf test cassette in diagnosis of malaria was estimated in Naira.

Questionnaires: Questionnaires were administered among the population samples comprising school children and pregnant women to determine their knowledge, attitude, belief and practice (KABP) on malaria prevention and intervention strategies. In an in-depth interview, each schoolchild was asked if he or she had a recent history of fever and, if so, how was the fever treated.

Blood Samples: The venous blood samples were collected from asymptomatic school children and pregnant women to prepare thin and thick smears and diagnose for malaria parasites (WHO, 1991). A tunicate was tied at the upper cubic-forsa of the left arm and using a 5-ml syringe; 2 ml of venous blood was draw and placed in an EDTA bottle. The samples were well packed in the rack and transported in compliance with all ethical regulations. The routine examination included the packed cell volume (PCV) to determine the effect of malaria parasitaemia on the Haemoglobin (Hb) level of patients. Epidemiological and variation rate of parasitaemia was also determined. Careful attention to technique was necessary in the collection of blood and the preparation of blood films. During the study, caution was placed on collection and handling of samples due to a number of viral, bacterial, and parasitological diseases that may be transmitted through the blood (WHO, 1991).

Microscopic Diagnosis: Conventional light microscopy is the established method for laboratory confirmation of malaria (WHO, 1991). The careful examination by an expert microscopist of a well prepared and well stained blood film remains currently the practices for detecting and identifying malaria parasites. The procedure used consists of collecting a finger-prick blood sample, preparing a thick and a thin blood smear, staining of the smear and microscopic examination (preferably with a 100X oil-immersion objective) of the blood smear for the presence of malaria parasites.

The cells in the thin blood smear were chemically fixed to the slide, and the slide is stained with alpha-phenolphthalein stain which better preserves parasite morphology and facilitates detection of parasites in the blood film. The thick blood film increases the sensitivity of the test. The thin blood film was used to quantify and identify parasites to the species level. A high-power microscope (400 times to 1,000 times magnification, with an oil immersion objective) was used to read thick and thin blood films. Between 100 and 200 microscope fields was examined to rule out the presence of parasites in a thick blood film (Cheesbrough and Precott, 1987; Payne, 1988).

Blood film: For routine malaria microscopy, a thin and thick blood smears were made on the same slide after patient information has been recorded in the appropriate register as follows: With the patient's left hand, palm upwards, the third finger was selected. A cotton wool lightly soaked in alcohol was used to clean the finger using firm strokes to remove dirt and grease from the ball of the finger. A clean cotton towel was used to dry the finger, by applying firm strokes to stimulate blood circulation on the finger (WHO, 1991).

With a sterile lancet the ball of the finger was punctured, using a quick rolling action. By applying gentle pressure to the

finger, the first drop of blood expressed was wiped away with dry cotton wool making sure no strands of cotton remain on the finger.

Quickly and by handling clean slides only by the edges, a single and small drop of blood, about this size o was collected by applying gently pressure to the finger, and dropped on to the middle of the slide for the thin film. A thick film was gotten by applying further pressure to express more blood to collect two or three larger drops, about this size o, on to the slide about 1 cm from the initial drop intended for the thin film. The remaining blood was wiped away from the finger with cotton wool (WHO, 1991).

Thick film: Using the corner of the spreader, the two or three more drops of blood were quickly joined and spread to make an even thick film. The blood was not excessively stirred, rather was spread in a circular or rectangular form with 3 – 6 movements.

The smear was allowed to dry in a flat, level position protected from flies, dust, and extreme heat. The dry film was labeled with a pen or marker pencil and not ball point pen was used for writing across the thicker potion of the film, the patients' name or number and date. The dry slides were wrapped in clean dry paper, and dispatch to the laboratory as soon as possible for diagnosis. The slide used for spreading the blood films was routinely disinfected with a spirit-moisted cotton wool to be used on the next patient, another clean slide from the pack being used as a spreader (WHO, 1991).

Thin film: Using another clean slide as a "spreader", and with the slide the blood drops resting on a flat, firm surface, touch the small drop with the spreader and allow the blood to run along its edge. Haven maintained an even contact of blood sample, the spreader is firmly pushed along the slide, away from the largest drops, keeping the spreader at an angle of 45° and not extending to the edges of the slide in order to prevent autoinfection (WHO, 1991).

Staining Blood Films: The blood films have dried overnight and fixed by dipping it in a container of methanol for a five seconds (WHO, 1991). The thin film with prolonged fixation, it may be difficult to demonstrate Schuffner's dots and Maurer's dots. To permit dehaemoglobinization, the thick film was not fixed; exposure of the film to methanol or methanol vapour was greatly reduced. The slides were placed back to back across an iron bar placed over a staining dish. 3% Giemsa solution was prepared in pH 7.2 buffered distilled. Using a dropper, the stain was gently dropped on the films, until the slides are totally covered. Each stained slide was allowed to stain for 30 minutes. The slides were gently immersed into clean water to float off the iridescent scum on the surface of the stain. The remaining stain was gently poured off, and rinse again in clean water for a few seconds and poured off the water. The slides were removed one by one and placed in a slide rack to drain and dry. The film side was downward, ensuring that film does not touch the slide rack (WHO, 1991).

Rapid Diagnostic Test Procedure (Acon-Pf): The kits were stored at room temperature (27 – 30°C). The test device remained in the sealed pouch until use and was not used beyond the expiry date. The device was not freezed, though could be refrigerated at 2°C as directed by the manufacturer. Schematic representation of the direction for use of the Acon-Pf cassette is shown in Fig. 1. The test procedure recommended by the manufacturers for Acon-Pf test cassettes was followed. The test device, specimen, buffer, and/or controls were allowed to equilibrate to room temperature (15 – 30°C) prior to testing. The test device was removed from the foil pouch and was used as soon as possible to perform the assay within one hour to obtain the best result. The test device was placed on a clean and level surface.

Both finger stick whole blood and venipuncture whole blood were used depending on the situation. The finger stick was mainly used during emergency cases and for children, while the venipuncture was preferable for normal diagnosis and epidemiological purposes (WHO, 2000). Each patient's hand was cleaned with an alcohol swab and allowed to dry. The

hand was massaged without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger. A puncture of the skin was made with a sterile lancet. The first sign of blood was wiped away. Gently the hand was rubbed from wrist to palm to finger to form a rounded drop of blood over the puncture site. An additional 2 to 3 drops of blood were collected by rubbing the finger further over the puncture site. Testing was performed immediately after specimen collection, without allowing the specimen at room temperature for prolonged periods. The whole blood collected by venipunture was stored at (2 - 8°C) in an EDTA bottle if the tests were to be run within 2 days of collection. Such specimens mentioned above, were brought to room temperature prior to testing. The specimens that got frozen were completely thawed repeatedly for more than three times before use. The specimens that were meant to be transported from the remote areas to the diagnostic laboratory were packed in compliance with ethical regulations covering the transportation of aetiological agents. Using a disposable specimen dropper was held vertically; the blood sample was drawn up to the fill line (approximately 10 µL). The blood sample was transferred to the specimen well (S) of the test device and 3 drops of the buffer (approximately 20 µL) was added. The stopwatch timer was then started. A waiting period of 15 minutes was allowed for the coloured line(s) or band(s) of the Acon-Pf test to appear and the result read. The coloured lines appeared with varied intensities and test antigen bands were graded 1 (faint) to 3 (equal to or darker than the control band). No results were interpreted after 20 minutes.

Interpretation of results

Positive: Two distinct coloured lines appear to confirm a positive result. One line was in the control region (C) and another line was in the test region (T). Note that the intensity of the colour in the test line region varied depending on the concentration of the *P. falciparum* present in the specimen. Therefore, any shade of colour in the test region (T) was considered as a positive test result.

Negative: One coloured line appeared in the control region (C). No apparent coloured line appeared in the test region (T).

Invalid: Control line failed to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. In such instance, the procedure was reviewed and the test was repeated with a new test device to rule out all doubts, and otherwise the test would have been discontinued.

Data Analysis

All data were analyzed by Statistical Package for Social Sciences (SPSS). In order to ascertain whether there is any significance differences in the study, data was analyzed using one-way analysis of variance (ANOVA) with a Scheffe and Duncan's New Multiple Range Test post hoc multiple comparison to separate the means that were statistically different. The following key variables were measured:

Disease prevalence: The total number of the individuals infected at the particular period of the study = Number infected / a + b = Total Number examined (n) / N (Okafor, 2005).

Sensitivity: The ability of the test to detect infected individuals as positive, calculated as TP/(TP+FN) (Mharakurwa *et al.,* 1997).

Specificity: The ability of the test to detect individuals without infection as negative, calculated as TN/(TN+FP) (Mharakurwa *et al.,* 1997).

Positive predictive value: The proportion of the test's positive readings which are truly negative, calculated as TP/(TP+FP) (Mharakurwa *et al.,* 1997).

Negative predictive value: The proportion of the test's negative readings which are truly

negative, calculated as TN/(TN+FN) (Mharakurwa *et al.,* 1997).

False positive rate: The proportion of individuals without infection being missed by the test and falsely ascribed a positive status, calculated as FP/(FP+TN) (Mharakurwa *et al.,* 1997).

False negative rate: The proportion of infected individuals being missed by the test and falsely ascribed a negative status, calculated as FN/(FN+TP(Mharakurwa *et al.,* 1997).

The J-index or overall measure of reliability of a diagnostic test which summarizes both sensitivity and specificity, calculated as (TP×TN-FP×FN)/(TP+FN)(TN+FP). The J-index values lie between 0 and 1, and as the value approaches the ideal; diagnostic ability is poorer the more closely the J-index values approaches 0 (Mharakurwa *et al.,* 1997), where TP = true positives, TN = true negative, FP = false positives and FN = false negatives.

RESULTS

Prevalence: The prevalence data of positive test results of the Acon-Pf and slide microscopy carried out on the same blood sample of children and adult population showed that the prevalence of malaria among the children population below the age of 5 years was highest with parasitaemia levels of 33.8% for Acon-Pf and 28.7% for microscopy. The prevalence rate of the adult population was lower with parasitaemia levels of 21.1% for Acon-Pf and 26.7% for microscopy. A total of 71 and 101 patients tested positive to the Acon-Pf and microscopy, respectively out of the 352 sample size. It also shows that more than half of the sample population of 180 patients tested negative for both the Acon-Pf and routine microscopy (Table 1).

The schematic representation of an Acon-Pf test cassette results conducted showed that the negative test result of patient had only one coloured line which appeared in the control region (C) and there was no apparent coloured line in the test region (T), indicating an actual negative test result. All the positive test results had two distinct coloured lines that appeared in the control region (C), and the other coloured line in the test region (T), indicating an actual positive test result. There was no invalid Acon-Pf test cassette result observed in the study, showing that one coloured line of all the cassettes used appeared in the control region (C) (Figure 1).

***P. falciparum* Stages:** The routine microscopy of thin blood film revealed an early stage a *P. falciparum* trophozoite. It is a ring stage with fine chromatin dots and frequently two, but some have one dot. The developing stages can appear two in a red blood cell and in a small, compact and coarse pigmentation present (Figures 2 and 3).

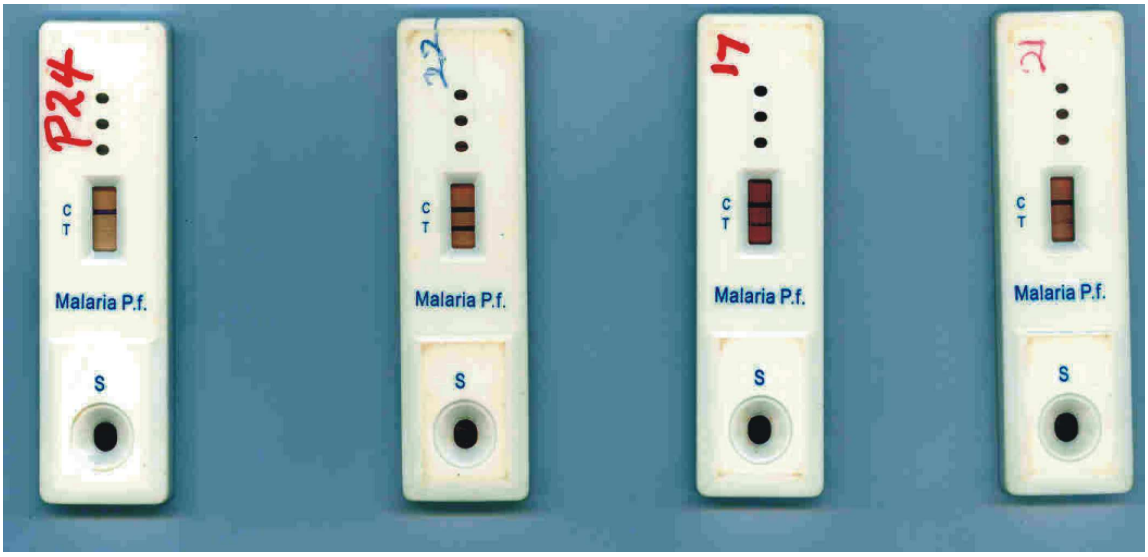
Sensitivity and Specificity of Diagnostic Test Devices: The validity of the both Acon-Pf test cassette and routine microscopic test were considered for same sample size of 352 patients using sensitivity and specificity. The standard deviation validity test was significantly higher in the routine microscopy than the Acon-Pf test cassette (0.71135 versus 0.40184; $p < 0.05$) at (0.1596 – 0.2438) confidence interval, which means Acon-Pf test was more reliable due to minimum variance.

Out of the 352 samples studied, 281(79.8%) tested negative for Acon-Pf test cassettes mean value and standard deviation 0.217 ± 0.591, respectively and 251(71.3%) for routine microscopy with mean value and standard deviation 0.024 ± 0.153, respectively, while 71(20.2%) tested positive for Acon-Pf test cassettes and 55(15.3%) for microscopic test and Acon-Pf test cassettes showed no measurable scanty result, but routine microscopy showed 46(13.1%) scanty results. The mean sensitivity rate was significantly higher in Acon-Pf test cassettes than in microscopic tests (1.21 ± 0.583 versus 0.800 ± 0.403; $P<0.05$) (Table 1).

Out of the 352 sample studied, the sensitivity rate of the Acon-Pf test cassette was 91.5%, the specificity rate was 87.2%, with positive and negative predictive values at 64.3%

Table 1: The prevalence data of positive test results of the Acon-Pf and slide microscopy

Subjects	Positive		Negative	
	Acon-Pf	Microscopy	Acon-Pf	Microscopy
352	71(20.2%)	101(28.7%)	281(79.8%)	251(71.3%)



Negative Test Result Thickly Positive Test Result Light Positive Test Result Lighter Positive Test Result

Figure 1: Schematic representation of the direction for use of the Acon-Pf cassette (C = control, T = positive test result and S = well).

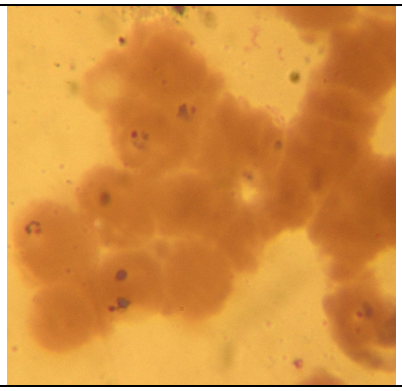


Figure 2: Appearance of *Plasmodium falciparum* Trophozoite stage and species identification in Giemsa's stained thin blood films X1000. (Many parasites are present, mainly small and delicate rings, few rings are with double chromatin dots, the chromatin dot is stained in red colouration, the ring is stained in purple colouration, many rings signifying heavy infection, the size of each trophozoite ring cover about 1/5 of one red blood cell and red blood cell not enlarged. Host red cell are not enlarged, no Schuffner's dots, but cells containing late stage trophozoites show irregular red-mauve staining Maurer's dots (clefts) and more than one parasite in a cell).

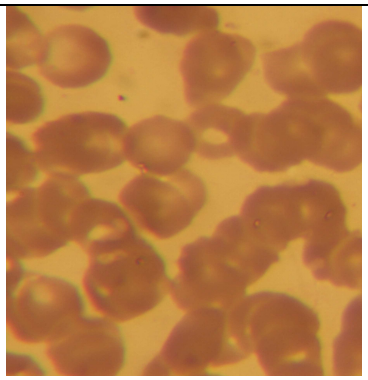


Figure 2: Appearance of *Plasmodium falciparum* gametocyte stage and species identification in Giemsa's stained thin blood films X1000. (Crescent (banana) shaped with rounded or pointed ends, but few are oval forms. The chromatin dot is stained in red colouration, chromatin appear as a compact mass near the centre for the female gametocyte (macrogametocyte), chromatin where present, are fine granules scattered throughout the male gametocyte and red blood cell not enlarged.

Table 2: Sensitivity, specificities and positive and negative predictive values recorded for Acon-Pf malaria diagnostic test in the health facilities studied

Type	Subject No	Sensitivity (%)	Specificity (%)	Predictive Value (%) Positive	Predictive Value (%) Negative	FPR (%)	FNR (%)	J-index (%)
Acon-Pf	352	91.5	87.2	64.3	97.6	12.8	8.0	78.7

and 97.6% respectively. The Acon-Pf test cassette used was highly reliable at 78.7% of the sensitivity and specificity tests as indicated through the J-index (Table 2).

Symptomatic diagnosis of the 352 pregnant women and children, showed that 260(79%) did not manifest any of the above symptoms within the past three months as at the time of the study, 20 patients had recent fever alone highest at (5.7%), chill and blisters 0.9% each, headache, dairrheoa and joint pain 0.3% each, weakness and anorexia 0.6% each. Recent fever combined with chill was highest 2.8%, followed by anorexia and weakness 2.0%, fever with headache, and weakness 1.7%, fever with anorexia and/ or vomiting 1.4%, fever with chill and headache and/ or amber urine or joint pain or anorexia or frequent sleeping 1.1%, headache with weakness 1.1%.

Forty six (46) antenatal patients out of the 352 population sampled for symptomatic diagnosis to predict malaria infection in symptomatic patients with recent history of treatment showed low sensitivity (41.6%), but highly specific (80%), low positive value (45.7%), high negative predictive value (77.3%), low false positive rate (20%), moderately high false negative rate (58.4%) and a high J-index (78.7%). The axillary temperature was poorly predictive for negative samples with normal axillary temperatures. The mean axillary temperature among children with Acon-Pf positive at (37.0°C) and Acon-Pf negative at (36.4°C), antenatal patients with Acon-Pf positive at (36.5°C) and antenatal patients with Acon-Pf negative at (35.1°C).

Infection Rate: The analysis of the KABP showed that out of the 46 respondents, 27 antenatal care patients showed the highest frequency of malaria prevalence at an above average percentage of 58.7%, followed by a combination of malaria and typhoid diseases at 13.0%, other infections have less rate of prevalence compared to malaria and typhoid which are two common and serious tropical diseases (Table 3).

Interviews Results: Eighteen laboratory scientists from the health facilities gave responses to the two alternative diagnostic techniques, knowledge of the Acon-Pf test device and trained on the use of RDTs. All the respondents would use routine microscopy, 88.9% prefer Acon-Pf test device, 38.9% would combine both techniques, 88.9% had knowledge about the rapid malaria diagnostic tests devices and 11.1% have had training on the use of the malaria diagnostic tests devices (Table 4).

Table 3: Diseases associated in the diagnosis and management of malaria among antenatal patients in a semi urban tropical community in Enugu State, Nigeria

Associated Diseases	Frequency	Percentage (%)
Vomiting	2	4.3
Malaria	27	58.7
Diarrhea	2	4.3
Pinworm	2	4.3
Malaria + typhoid	6	13.0
Malaria + Diarrhea	4	8.7
Malaria + Headache	3	6.5
Total	46	100.0

Table 4: Responds on the knowledge of the Acon-Pf test device and training on the use of RDTs

	Frequency	Percentage (%)
First choice Slide Microscopy	18	100
Second choice Acon-Pf	16	88.9
Combined choice	7	38.9
Knowledge of RDTs	16	88.9
Trained on RDT use	2	11.1

The performance of the Acon-Pf test and the test procedures were easy to master and use, with minimum time required. The mean time required for the collection of venous blood samples by a trained phlebotomist were 1.16 minutes and 1.38 minutes from women attending antenatal care and their children, respectively. However, the actual range of time required for a positive test was 0.30 seconds to 15 minutes, and the control band showed at average time of 1.26 minutes depending on the level of parasitaemia and experience of the user, as shown by the intensity of the developing colour.

Logistic Factors: The Acon-Pf test cassettes could detect antigens from the oxalated blood stored for over three days at 30ºC, but exposure to direct sunlight was avoided. Acon-Pf test cassettes were stored in two different environments up to 8 months at a mean ambient temperature of (29.0 ± 3.5 °C) which did not show any adverse effect, thus the procurement, transportation during field work and storage of the Acon-Pf test cassettes did not pose any problem to the quality of the test, and hence does not need a cold chain.

Cost Benefit: The cost per Acon-Pf test cassette at bulk purchase = ₦112.50 ≡ US$0.90, without the service charge (as compared with thick smear = ₦ 300.00 ≡ US$ 2.50; and ₦ 350.00 ≡ US$ 2.90 for thin smear). The cost of a packet of Acon-Pf test device which contains 40 cassettes was ₦ 4, 500.00 ≡ US$ 36.00 as at the time of the study.

DISCUSSION

Despite several *P. falciparum* malaria diagnostic techniques which have been developed currently for the prevention of malaria morbidity and mortality, the incidences of malaria are devastating and decimating the global population, especially among the poor people and the developing countries of the tropical regions. Thus, the rationale for the investigation of the reliability of the Acon-Pf test cassette as an adjunct to the microscopic devices as a simple, inexpensive and sensitive malaria rapid diagnostic test for field and remote areas.

The prevalence of malaria parasitaemia among the sample populations was higher among children less than 5 years, than what was found among the adult population. This may be contributed to low level of malaria induced immunity, late and poor clinical diagnosis especially among those residing in remote and rural communities of study area. The prevalence rates in children were as high as for Acon-Pf (33.8%), slide microscopy (28.7%) and sharply followed by those of the adult population, Acon-Pf (21.1%) and slide microscopy (26.7%), respectively. A mean age of 15.78 years constituted the study population. The malaria parasite species found was *P. falciparum* and two stages were identified as trophozoites and gametocytes. The red blood cells were not enlarged even as the parasites are found in the infected red blood cells. One or more parasites were found in parasitized the red blood cells in slide thin films. The trophozoites showed various shapes which include the ring and comma; while some are rounded. The gametocytes identified showed crescent (banana) shape with the presence of a chromatin on one of the red blood cells.

The Acon-Pf test results showed some negative and positive test results and no invalid test resulted was recorded, which confirm the reliability of the new rapid diagnostic test cassette for prompt malaria diagnosis. This work did not study the quantitative analysis of parasites, however, some of the Acon-Pf

positive test results showed bands which were light to thickly coloured. The intensity of the colour in the test line region (T) may vary depending on the level of the parasitaemia of the *P. falciparum* present in the specimen. More so, in slide microscopy, there were cases with high parasitaemia with asexual stages which turned out to be negative by Acon-Pf test results. The failure of a *P. falciparum* histidine-rich protein-2 that is produced by the asexual stages and gametocytes of *P. falciparum*, which is expressed on the red cell membrane surface, to detect any positive result, may be attributed to low level of parasitaemia and/or error in the interpretation of the colour changes of the rapid diagnostic test result. According to Kakkilaya (2003), other factors that can limit that sensitivity of a rapid diagnostic test result include genetic heterogeneity of *P. falciparum* histidine rich protein-2 expression, deletion of histidine rich protein-2 gene, presence of blocking antibodies for *P. falciparum* histidine rich protein-2 antigen or immune-complex formation, prozone phenomenon at high antigenemia or to unknown causes. In addition, that false negative tests have been observed even in severe malaria with parasitaemia >40000 parasites/µl. Therefore, in cases of suspected severe malaria or complex health emergencies, a positive result may be confirmatory but a negative result may not rule out malaria, especially in cases of asymptomatic malaria. It should be emphasized that *P. falciparum* malaria, a resistant tropical disease, must not be missed because of a false-negative rapid diagnostic test. It has been suggested that in such cases, 1 in 10 dilution of a negative sample with 0.9% sodium chloride solution may help to exclude the prozone phenomenon. Furthermore, any doubtful negative rapid diagnostic test result should always be confirmed by microscopy.

The Acon-Pf was strongly positive and correlated well with the trophozoites stages and degree of parasitaemia. In the blood specimen from patients showing only gametocytes in smear examination, the test was either weakly positive or negative. The weak positivity perhaps was due to persistent low antigenemia. Whether Acon-Pf can detect the early gametocytic stage of parasitaemia is still unclear. This limitation of the test must be appreciated as the patients are in infective stage. The detection of gametocytes by microscopy is relatively easy due to their large size and peculiar morphology. The test could not detect antigen properly from haemolyzed blood or from serum samples.

Rapid diagnostic tests achieved high sensitivity (91.5%), but few cases indicated as positive by rapid diagnostic tests were negative by microscopy. Results of above mentioned diagnostic tests are similar to earlier workers findings of Quitana *et al.* (1998) and Iqbal *et al.* (2001). Sensitivity of a diagnostic test indicates its ability to detect all those infected. The more false negative the test result, the lower the sensitivity of the test. The specificity of a diagnostic test indicates its ability to distinguish one particular infection from the other infections or conditions. The more false positive test result, the lower the specificity of the test. Further analysis of these cases indicated that Rapid Diagnostic Tests were detecting low-level parasitaemia missed by microscopy, and that local slide microscopy in poor health facility without a trained and experienced microscopist, had poor accuracy.

The rapid malaria diagnostic test conducted by experienced personnel demonstrated good sensitivity and negative predictive values in comparison with locally read slides and read in a controlled environment. Specificity and positive predictive values for rapid diagnostic tests are difficult to assess when comparison is made with microscopy as a confirmatory test, which itself has poor sensitivity at low parasite densities. They were probably much higher than this study indicated, for the following reasons. The persistent of the gametocytes of *P. falciparum* following chemotherapy without implying drug resistance and some versions of the test kits targeting Histidine Rich Protein-2 of *P. falciparum* have given false positive results in patients with rheumatoid factor as reported in the work of Whitty *et al*. (2000). Available researches show that Histidine Rich Protein-2 of *P. falciparum* antigen, which is detected by the ICT Malaria Pf/Pv test, may persist for up to 7 to 10 days

after asexual parasite clearance (Shiff *et al.,* 1993; Igbal *et al.,* 2001).

False negative Acon-Pf test may have resulted at parasitaemia < 500/ µl. This can potentially be dangerous, as to miss the diagnosis of malaria in an ambulant, febrile patient may mean that complications develop because appropriate treatment was not instituted in time. False negative rapid malaria diagnostic test results in samples with higher parasitemia have been observed in earlier studies, but the underlying reason was not stated (Anon, 1996; Karbwang *et al.,* 1996; Humar *et al.,* 1997; Palmer *et al.,* 1998; Van den *et al.,* 1998; Hunt-Cooke *et al.,* 1999; Igbal *et al.,* 2002).

In addition, false-negative results by microscopy can occur if patients have undertaken self-medication prior to presentation. It is likely that some of our patients with false-positive results may have performed self-medication with antimalarial drugs during an attack of fever. However, it is unlikely that these factors account for the entire set of false-positive cases. It is more probable that most of the false-positive cases were true positives which were not detected by microscopy, due to sequestration limiting the number of circulating parasites at the time of blood collection or due to the parasitaemia being below the detection limit of approximately 50/µl by microscopy.

The disease infection rate among 46 antenatal patients showed that malaria infection has the highest prevalence rate at an above average percentage of 58.7%, followed by a combination of malaria and typhoid diseases at 13.0%. The public health importance of this high infection rate may increase the level of anaemia, mortality and morbidity rate among children under the age five and pregnant women. This high prevalence rate of malaria demonstrated among the rural subsistence farmers in Enugu State may have exposed a large population of Nigerians to the risk of malaria infection. Therefore, there should be prompt and grass-root intervention programmes to reduce the socio-economic effects on the people through massive education and universal access to malaria control facilities.

The general sensitivity of the Acon-Pf test was found to be high in the sampled sites and communities. The desire for an easy, rapid, cost effective and accurate tests for the detection of resistant malaria *falciparum* infections would never be over emphasized, as Malaria Rapid Diagnostic Test was highly valuable in the communities and among the Health workers and may likely increase the compliance and treatment-seeking behaviour of patients. Rapid diagnostic tests were well accepted and accurately performed by the health workers in the facilities. The high rate of a febrile parasitaemia in this study indicated that large reservoir of barely symptomatic cases existed in the communities.

The symptomatic malaria diagnostic method is not reliable at 21.6% for the sensitivity and specificity test as indicated through the J-index. This is because there could be other infections other than malaria that have similar symptoms. The test will help in early diagnosis of *P. falciparum* malaria, thus enabling the physicians to institute specific treatment of those suffering from *P. falciparum* malaria, and reduce the case fatality rate. Interventions were carried out in the field, thus a presumptive treatment was given to all fever cases and treatment for Pf malaria to those testing positive for *P. falciparum* malaria.

The asymptomatic stage does not automatically indicate that one has no malaria infection. In this same vein, blisters on the lips of the mouth alone can not indicate malaria, all the major symptoms – fever, chill, headache, anorexia and joint pain are indicators of malaria infection. However, blood test using rapid malaria diagnostic test kits and routine microscopy can only indicate infection proper. This is because other infections present similar symptoms like malaria infection.

In the remote and endemic areas of the study, the accuracy of symptom-based diagnosis was poor, as it has been reported by other researchers although specificity in this self-referring sick population should be higher than in the communities as a whole. Local transmission rates and therefore immunity, were low, and this should have increased the symptomatic malaria sensitivity. The

observation of fever alone, and/or fever in combination with chills and/or headache, achieved quite high sensitivities, but both criteria resulted in high rate of over-treatment in symptomatic patients. Any narrower combination of symptoms resulted in sensitivities unacceptable in relation to the detection of a life-threatening illness. The measurement of axillary temperature failed to achieve sufficient sensitivity or specificity to be useful. The data on symptomatic malaria diagnosis shows that children may have higher axillary temperature than adult antenatal patients, reason may be because adults have developed asymptomatic immunity to malaria due to repeated exposure to malaria infection. This study suggested that the symptoms identified can be used as an algorithm for the future identification of symptomatic malaria diagnosis for presumptive treatment in remote and malaria endemic regions.

The attitude of the antenatal patients to diagnosis during the study demonstrated the importance of providing patients with a reliable explanation for their illness. The responses of the rural community members and antenatal women suggested that there is an improved treatment-seeking behaviour and drug compliance. The KABP analysis also showed that there is poor knowledge of the, causes, prevention and control strategies of malaria, such as the use of Long Lasting Insecticide treated bed nets (LLIN). Further findings showed that there was poor attitude to the use of the Long Lasting Insecticide treated bed nets provided to the antenatal patients in the health facilities in the rural community. A rapid blood-based diagnosis at some cost was preferred by rural community members and antenatal patients to both the delayed free slide diagnosis and symptom-based diagnosis, despite the cost to the patient. This cost, approximately the difference between the estimated cost of microscopy to the health service and the bulk wholesale cost of the rapid diagnostic tests, was a substantial sum to families engaged in semi-subsistence agriculture.

The cost-effectiveness of symptom-based diagnosis, rapid diagnostic tests and microscopy, and the proportion of the costs at which the health workers and community members may wish to be buying the Acon-Pf were not investigated, that could be suggested for research.

They can be expected to vary with transmission rates and health service access. In the long term, improved compliance and treatment-seeking behaviour may bring additional economic benefits from rapid diagnostic tests through a reduced burden of illness. The detection of persistent antigen in asymptomatic infection, when fluctuating parasitaemia reduces the sensitivity of microscopy, also offers new possibilities for rapid screening of communities at risk.

In contrast to slide smear that takes up to 60 minutes (30 minutes for filming and staining, and 15 – 30 minutes for microscopy). Microscopy is to great extent subjective and sometimes can lead to misdiagnosis. Investigations on the performance rate of Acon-Pf to microscopy showed high level of awareness of rapid diagnostic test kit to the level of 77.8%, while ignorance to the use of the new rapid diagnostic test kit as much as 77.8%. Those advocating for the new Rapid Diagnostic Test Kit to be made accessible and affordable to Health workers are about 88.9%. The new technique was ethically and culturally acceptable to the volunteers, and may have overwhelming demand at the remote and rural communities in the tropical regions.

This study clearly shows that malaria parasitaemia cannot be easily identified by symptoms alone and that microscopy is a challenge and unreliable in remote areas. The rapid diagnostic test was well accepted by Health workers and community members and was performed accurately by the former and fairly by the latter after little training. It markedly improved diagnostic accuracy and met a desire in the communities for rapid blood-based diagnosis.

The complementary use of immunochromatographic rapid diagnostic tests may be useful, especially during emergency blood transfusion and on-call hours when expert microscopists may not be readily available. The malaria rapid diagnostic tests would reduce the risk of malaria transmission among

asymptomatic and unscreened blood donors, non-immune travelers and in the case of emergency blood transfusion. However, in order to ensure correct interpretation of rapid diagnostic test devices, their inherent limitations have to be well known.

The Acon-Pf could detect antigens from the oxalated blood stored for over 72 hours at 30°C ambient temperature. There was no significant difference in the Acon-Pf test cassette stored in two different environments at relative humidity and temperature as specified in the direction for use by the manufacturer. The Acon-Pf did not demonstrate any adverse effect on the procurement, handling, transportation during field work and storage of the Acon-Pf test cassettes retained the quality of the test, and hence does not need a cold chain. The test result colour lines (T) can remain as long as the control line (C), without fading away.

Depending on the local number of malaria cases seen, laboratory staff should have a low threshold for the decision to perform unsolicited malaria diagnostic tests on suspicious samples, especially if other laboratory tests are abnormal (e.g. thrombocytopenia, presence of atypical lymphocytes, or raised lactate dehydrogenase). The detection of intraleukocytic haemozoin during automated full blood counts is a promising new way to avoid misdiagnosis of clinically unsuspected malaria.

Irrespective of all its advantages, immunochromatographic test device will not be able to replace microscopy. Microscopy is still more flexible and offers the immense advantage of providing species diagnosis and exact parasite densities. The sensitivity of microscopy generally varies with the skill and experience of the slide reader, quality of smear preparation and staining, microscope quality, magnitude of parasitemia, and number of fields read. The potential role of a National Agency for Food and Drug Administration and Control (NAFDAC) – to control and facilitate access to approved rapid diagnostic tests particularly in settings where malaria microscopy is either not available or of poor quality should therefore not be underestimated.

Conclusion: The study shows that Acon-Pf is a reliable test kit for malaria diagnosis in remote areas and for epidemiological studies. The rapid diagnostic test was highly valued in the community and among the Health workers and may increase the compliance and treatment-seeking behaviour of patients. We recommend. A post-donor screening for malaria parasites using the Acon–Pf device is recommended to reduce malaria transmission by transfusion and among travelers. The study advocates for governmental support to researchers for the cultivation of in-vitro rapid diagnostic tests strips here in Nigeria, which may be more sensitive, increase access and may be more tolerant to the prevailing temperature and humidity in the tropical regions. Routine microscopy of thin blood film is still the first choice method for diagnosing malaria because it is specific to all *Plasmodium* species and offers differentiation between parasite growth stages, which are essential for prevention and intervention strategies. If a rapid diagnostic test result is negative and clinical symptoms persists, additional investigation using other clinical methods are recommended. A negative result does not at any time preclude the possibility of malaria infection. Hence, it is recommended that Federal, State, Local authorities and Developmental Partners should assist in promoting and increasing health facility and community access to the current, highly sensitive and specific rapid diagnostic test devices for malaria.

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REFERENCE VALUES FOR THE HAEMATOLOGY PROFILE OF CONVENTIONAL GRADE OUTBRED ALBINO MICE (*Mus musculus*) IN NSUKKA, EASTERN NIGERIA

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ABSTRACT

This study established reference values for the haematology profile of the conventional grade out-bred albino mice (Mus musculus) in Nsukka, Eastern Nigeria. A total of 336 apparently healthy mice, made up of 168 males and 168 females, were used for the study. Mice of 4, 8, 12, 16, 20, 24, 30 and 40 weeks of age (eight age sets) were studied, and for each age set the haematology profile of 21 males and 21 females were assessed following standard manual procedures immediately upon blood sample collection from the orbital sinus. Results showed that there were significant ($p < 0.05$) age related variations in the erythrocyte counts (EC), haemoglobin concentrations (HbC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leukocyte counts (TLC) and absolute lymphocyte counts (ALC) of males and females and mean corpuscular haemoglobin of males only. There were significant differences ($p < 0.05$) between the males and females in their TLC at weeks 4 and 40 of age, ALC at week 24 of age and absolute neutrophil counts (ANC) at weeks 4, 20 and 24 of age. The absolute values obtained in this present study were for some parameters different from the reference values documented for mice in the temperate countries, but the trend of age related variations and differences between the sexes were nearly the same except for the ANC.

Keywords: Haematology, Reference values, Mice, *Mus musculus*, Nsukka, Nigeria

INTRODUCTION

Population reference values, formerly known as normal values, is defined as a set of values of a certain type of quantity obtainable from a group of apparently healthy individuals (reference population) corresponding to a spelt-out stated description with a specified criteria for inclusion and exclusion of individuals measured (Elvebach, 1973; Dybkaer and Grasbeck, 1973; Sunderland, 1975; Stockham and Scott, 2008). Population based reference values thus describes the variation of values that can be expected in healthy individuals or animals of a population from which samples are submitted to the laboratory, such that measured values outside the reference intervals (that serve as guideline) are unlikely to originate from a

healthy individual/animal (Solberg and Grasbeck, 1989; Solberg, 2008; Kjelgaard-Hansen and Jensen, 2010). The term "reference values" was chosen to replace the older term "normal values" on semantic and scientific grounds in order to overcome the conceptual problems and syleptic ambiguities that arose as a result of the use of the term "normal", taking into consideration the fact that absolute health does not exist (Amador, 1975; Sunderland, 1975; Lumsden, 2000).

The laboratory mouse (*Mus musculus*), family *Muridae*, is a common experimental animal in the biomedical sciences and psychology, and it is the most commonly used mammalian model organism (Austin *et al*, 2004; Foster *et al*., 2006; NJABR, 2011; Sellers and Ward, 2012). Animal models are necessary for biomedical research because it is impractical and unethical to use humans in most aspects of research on diseases. It is generally accepted that it would be wrong to deliberately expose humans to health risks in order to observe the course of disease processes (Gallagher, 2003; NJABR, 2011, Prieto *et al*., 2011). Among all model organisms, the mouse offers particular advantages for the study of human biology and diseases because the mouse is a mammal, and its development, body plan, physiology, behavior and diseases have much in common with those of humans; almost all mouse genes (99%) have homologs in humans (Austin *et al*., 2004; Foster *et al*., 2006; NJABR, 2011; Sellers and Ward, 2012). Further, mice are small, inexpensive, easily maintained, generally very docile if raised from birth with sufficient human contact and can reproduce quickly making it possible to observe several generations within a relatively short period of time (Foster *et al*., 2006). The mouse had played a prominent role as a model organism for the study of human diseases for more than a hundred years, and still serves as an important animal model for preclinical, pharmacological and toxicological evaluation of drugs and the investigation of various diseases and disease mechanisms. It had been shown that studies on mice provide clinical toxicity predictions that in many respects may be comparable or perhaps superior to predictions from dog or monkey studies (Goldsmith *et al*., 1975; Austin *et al*., 2004; Foster *et al*., 2006; Sellers and Ward 2012).

Blood is the major transport system of the body, and both input and output substances of almost all the body's metabolic processes and deviations from normal caused by disease pathogens, injuries, harmful substances, deprivation and stress are commonly reflected by changes in the blood picture (Ihedioha *et al*., 2004; Poiout-Belissent and McCartney, 2010). A comprehensive haematology, also known as completed blood count, is the foundation of the evaluation of the haematopoietic system response in preclinical and clinical trials, and is a basic requirement for the preclinical assessment of drugs and drug candidates for toxicity (Harrison *et al*., 1978; Reagan *et al*., 2010). The assessment of haematological parameters plays a critical role in diagnosis, prognosis and characterization of diseases and phenotypes in clinical and research situations (Everds, 2006; Forbes *et al*., 2009).

Amongst all other factors that affect the haematology profile, variations in the climatic and geographical location factors such as temperature, humidity, altitude and day length make it imperative that reference haematology values should be established for specific geographical locations (Coles, 1986; CLSI, 2008; Stockham and Scott, 2008; Kjelgaard-Hansen and Jensen, 2010). To date, the reference haematology values established for albino mice bred and raised in the temperate developed countries of Europe and America are being used by researchers in Nigeria. There are no reference haematology values for albino mice bred and raised in Nigeria in available literature. Yet massive amounts of preclinical pharmacology, toxicology and pathology studies utilizing albino mice bred and raised in Nigeria had been going on as evidenced by the large number of publications of such studies in numerous local and international journals in which reference values generated from temperate countries are referred to. The objective of this study was to establish reference values for the haematology profile of the conventional grade out-bred albino mice in Nsukka, Eastern Nigeria.

MATERIALS AND METHODS

The mice used for the study were the conventional grade UN-FERH:NS outbred strain of albino mice (*Mus musculus*) bred and raised at the Laboratory Animal Facility of the Foundation for Education and Research on Health (FERH), Nsukka Nigeria. This strain was adapted from the mouse colony bred and maintained at the Faculty of Veterinary Medicine Laboratory Animal House, University of Nigeria, Nsukka.

The study location (Nsukka) is situated within the derived savannah belt of Eastern Nigeria, with coordinates 6º 51′ 24″ north and 7º 23′ 45″ east and an average elevation of approximately 550 m (1,810 ft) above sea level with an ISO 3166 code of NG.EN.NS. Nsukka is an area of fairly high temperature with a yearly minimum and maximum of 23.6º C and 34.2º C, with a mean of 27.8º C (FMANR, 2011). The angle of the sun's ray over Nsukka is near vertical, and the difference between the longest and shortest days in the year is only 48 minutes (FMANR, 2011). Nsukka experiences two seasons – a rainy season from March to October and dry season from November to February, with a yearly average rainfall of 120.5 mm. The relative humidity in Nsukka is about 70% during the rainy season and about 20% during the dry season (FMANR, 2011).

A total of 336 apparently healthy albino mice were used for the study, made up of 168 males and 168 females. Eight age sets (mice of 4, 8, 12, 16, 20, 24, 30 and 40 weeks of age) were studied. For each age set, 21 males and 21 females were used for the study. Mice showing any signs of abnormality were excluded from the study. The mice included in the study group were kept in clean cages in a fly-proof animal house and were fed with mice chow (Grand Cereals Nigeria Limited, Jos, Nigeria) formulated to meet the nutritional requirements of mice (NRC, 1995) and provided with clean drinking water *ad libitum*. Guidelines for the humane use and handling of laboratory animals for research (NAS, 2011) were followed all through the study. Blood samples for haematology were obtained from the orbital sinus using the orbital bleeding technique (Bolliger and Everds, 2010). The blood samples were collected in the morning periods between 7.30 am and 9.30 am on each day of study. The study period was March to September, 2011. About 1 ml of blood was collected from each mouse, and the anticoagulant used was sodium ethylene diamine tetra acetic acid (EDTA). Each mouse was bled only once. All the haematological determinations were conducted immediately after blood sample collection, following standard procedures.

The packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002), while the haemoglobin concentration was determined following the cyanomethaemoglobin method (Higgins *et al.*, 2008). The erythrocyte and total leukocyte counts were conducted following the haemocytometer method, while the differential leukocyte counts were done on air-dried thin blood smears stained by the Leishman technique and enumerated by the meander method (Thrall and Weiser, 2002). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of the erythrocytes was calculated using the standard formulae (Thrall and Weiser, 2002).

Data generated from the study were subjected to appropriate statistics using the statistical package for social sciences (SPSS) version 16 software package. Data on the sexes (males and females) were compared using student's t – test, while age related variations were subjected to one way analysis of variance (ANOVA). Variant means were further separated using the least significant difference (LSD). Significance was accepted at $p < 0.05$, and the results were presented as means ± standard deviation, along with the minimum and maximum values obtained.

RESULTS

The mean PCV of all the mice (both males and females) ranged from 41.07 ± 1.57 % recorded for the 4-week old females to a maximum of 46.29 ± 2.00 % recorded for the 24-week old females, and there were no significant ($p > 0.05$) variations in PCV across the age sets

(Table 1). There was no significant difference ($p > 0.05$) between the PCV of males and females for all the age sets studied (Table 1). The erythrocyte count and haemoglobin concentration of both the male and female mice significantly ($p < 0.05$) increased from their lowest values obtained for the 4-week old mice to a relatively stable value recorded for the 8-week old mice, and did not further vary significantly ($p > 0.05$) across the age sets up to 40 weeks of age (Table 1). There were no significant differences ($p > 0.05$) between the sexes (males and females) in their erythrocyte counts and haemoglobin concentration all through the age sets studied (Table 1).

The erythrocyte MCV of the male and female mice were highest in the 4-week old mice, and values significantly ($p < 0.05$) lower than that obtained for the 4-week old mice were recorded for male mice of 8, 12 and 40 weeks of age and female mice of 8 and 40 weeks of age (Table 2). There were no significant differences ($p > 0.05$) between the MCV of the males and females across the age sets studied (Table 2). The erythrocyte MCH of both the male and female mice was highest in the 4-week male and female mice (Table 2). The MCH of the 8, 12 and 40-week old male mice were significantly ($p < 0.05$) lower than that recorded for the 4-week olds, but that of male mice aged 16, 20 24 and 30 were not significantly ($p > 0.05$) different from that of other age sets (Table 2). There were no significant ($p > 0.05$) age-related variations in the MCH of the females all through the age sets studied (Table 2). The erythrocyte MCHC rose from its lowest value recorded for the 4-week old male and female mice up to the highest values obtained for the 24-week old male and female mice, which was significantly ($p < 0.05$) higher than the values recorded for the 4-week olds (Table 2). There were no significant ($p > 0.05$) differences between the MCHC of males and females all through the age sets studied (Table 2). The total leukocyte counts (TLC) of the male mice increased from the value obtained for the 4-week old mice up to a significantly ($p < 0.05$) higher value recorded for the 16, 20 and 24 old males, and later significantly ($p < 0.05$) decreased in the 40-week old males (Table 3). The TLC of the females rose from its lowest value obtained for the 4-week olds up to its highest value recorded for the 16-week old females, which was significantly ($p < 0.05$) higher than the values obtained for the younger age sets (Table 3). The TLC of female mice of 20, 24 and 30 weeks of age were not significantly ($p > 0.05$) different from that of other age sets, but that of the 40-week olds was significantly ($p < 0.05$) higher than that recorded for the 4, 8 and 12 week old females (Table 3). The TLC of the 4-week old males was significantly ($p < 0.05$) higher than that of their female age mates, but that of the 40-week old females was significantly ($p < 0.05$) higher than that of their male age mates (Table 3). There were no significant ($p > 0.05$) differences between the TLC of males and females for age sets between 8 and 30 weeks of age (Table 3).

There were no significant variations ($p > 0.05$) in the absolute lymphocyte counts (ALC) of 4, 8, 12 and 30 week old male mice, but that of the 16 and 24 week old male mice were significantly ($p < 0.05$) higher while that of the 40-week old males were significantly ($p < 0.05$) lower (Table 3). The ALC of the female mice rose from its lowest value recorded for the 4-week old females to a significantly ($p < 0.05$) higher value obtained for the 12, 16, 20, 24 and 30 week old females (Table 3). There were no significant ($p < 0.05$) differences between the ALC of the males and females all through the age sets studied, except for the 24-week old age set in which the ALC of the males was significantly ($p < 0.05$) higher than that of the females (Table 3). There were no significant variations ($p > 0.05$) in the absolute neutrophil counts (ANC) of all the age sets studied (both males and females), but the ANC of the 4, 20 and 24-week old male mice were significantly ($p < 0.05$) higher than those of their female age mates (Table 3). The absolute monocytes, eosinophil and basophil counts obtained for all the age sets of mice studied were relatively very low when compared to the absolute numbers of lymphocytes and neutrophils (Table 4).

Table 1: The packed cell volume, erythrocyte counts and haemoglobin concentration of albino mice of varied ages and sexes

Age (weeks)	Packed cell volume (%)		Erythrocyte counts (10^6/µl)		Haemoglobin concentration (g/dl)	
	Males	Females	Males	Females	Males	Females
4	41.29±1.70 [38.00–43.00]	41.07±1.57 [39.50–43.50]	5.49±0.91 [a] [4.25–5.75]	6.63±1.56 [a] [4.30–7.80]	13.02±1.52 [a] [10.42–15.26]	12.94±0.63 [a] [12.28–13.77]
8	41.43±1.79 [39.00–44.00]	42.57±3.14 [38.00–47.00]	8.09±0.82 [b] [6.60–9.00]	8.18±1.13 [b] [6.90–9.80]	13.90±0.43 [a b] [13.58–14.52]	14.30±0.89 [b] [12.65–15.26]
12	43.50±1.44 [42.00–46.00]	44.29±1.68 [42.00–47.00]	8.44±0.80 [b] [6.95–9.65]	7.91±0.36 [b] [7.40–8.35]	14.38±0.73 [b] [13.40–15.26]	14.88±0.81 [b] [13.77–16.00]
16	43.50±2.72 [39.00–46.50]	45.36±1.80 [43.00–48.00]	7.94±1.24 [b] [6.15–9.40]	7.75±1.07 [b] [6.55–9.80]	14.64±0.89 [b] [12.79–15.63]	14.78±1.00 [b] [12.65–15.63]
20	45.07±1.90 [42.50–47.50]	44.00±3.12 [38.00–45.50]	7.89±1.33 [b] [6.05–9.35]	7.88±1.31 [b] [6.05–9.70]	14.96±1.12 [b] [13.58–16.56]	14.65±0.68 [b] [13.21–15.26]
24	43.64±0.94 [42.00–45.00]	46.29±2.00 [43.50–49.00]	7.72±1.29 [b] [6.15–9.62]	8.08±1.14 [b] [6.28–9.75]	15.36±0.72 [b] [14.28–16.06]	15.83±0.92 [b] [15.61–17.85]
30	42.40±2.80 [39.00–46.00]	44.20±1.48 [42.00–46.00]	8.06±0.80 [b] [7.25–8.68]	7.64±0.51 [b] [7.15–8.18]	15.17±1.25 [b] [14.28–16.28]	15.39±0.80 [b] [14.28–16.28]
40	42.17±1.61 [41.00–44.00]	42.00±2.55 [39.00–45.00]	8.30±0.69 [b] [7.55–9.60]	7.97±0.98 [b] [6.60–9.10]	14.14±0.81 [b] [13.58–15.07]	14.37±1.07 [b] [13.40–16.00]

Results are presented as means ± standard deviation, with the minimum and maximum values in parentheses. [a b] Different superscripts in a column indicate significant difference between the designated means across the ages ($p < 0.05$); No significant differences between the sexes ($p > 0.05$).

Table 2: The mean erythrocyte corpuscular values of albino mice of varied ages and sexes

Age (weeks)	Mean corpuscular volume (fl)		Mean corpuscular haemoglobin (pg)		Mean corpuscular haemoglobin concentration (g/dl)	
	Males	Females	Males	Females	Males	Females
4	77.03±13.83[a] [63.08–98.82]	65.65±10.42[a] [52.32–93.02]	24.56±5.68[a] [18.12–30.83]	20.63±5.60 [16.75–29.02]	31.67±2.50[a] [27.42–35.49]	31.49±0.51[a] [30.70–32.03]
8	51.68±6.05[b] [45.56–55.70]	52.55±4.99[b] [43.88–57.89]	17.32±1.66[b] [15.37–20.58]	17.66±1.63 [15.73–19.35]	33.61±1.57[ab] [30.86–33.95]	33.62±0.93[ab] [32.47–34.63]
12	51.90±4.98[b] [44.56–61.15]	56.16±4.33[ab] [50.90–63.51]	17.19±2.09[b] [13.89–20.88]	18.78±1.21 [17.11–20.62]	33.06±1.24[ab] [31.16–34.63]	33.52±1.82[ab] [30.60–35.96]
16	56.03±9.65[ab] [45.88–68.70]	59.32±7.16[ab] [47.96–68.70]	18.87±3.34[ab] [15.05–23.90]	19.37±2.82 [15.24–22.15]	33.67±1.23[ab] [32.24–35.85]	32.58±1.81[ab] [29.08–35.05]
20	58.50±9.98[ab] [48.13–72.52]	56.89±7.75[ab] [43.81–67.67]	19.29±2.50[ab] [16.65–23.01]	18.97±2.68 [14.96–22.39]	33.21±2.15[ab] [30.52–36.00]	33.35±1.04[ab] [31.79–34.76]
24	58.06±9.70[ab] [43.66–70.73]	58.55±10.09[ab] [47.18–74.84]	20.30±2.83[ab] [15.77–23.70]	21.23±3.69 [17.85–27.71]	35.18±1.46[b] [32.83–36.50]	36.40±2.37[b] [31.86–38.67]
30	52.73±5.31[b] [46.71–60.69]	58.15±5.46[ab] [51.34–63.62]	18.89±2.34[ab] [16.30–22.46]	20.23±1.78 [17.46–22.21]	35.75±0.79[b] [34.90–37.00]	34.82±1.30[ab] [33.71–37.00]
40	50.44±4.19[b] [43.92–64.95]	53.56±9.20[b] [43.96–66.67]	16.92±2.66[b] [14.57–20.84]	18.33±3.29 [15.13–22.56]	33.52±0.64[ab] [32.04–34.75]	34.20±1.01[ab] [32.79–35.56]

Results are presented as means ± standard deviation, with the minimum and maximum values in parentheses. [a b] Different superscripts in a column indicate significant difference between the designated means across the ages ($p < 0.05$); No significant differences between the sexes ($p > 0.05$).

Table 3: The total leukocyte counts and absolute lymphocyte and neutrophil counts of albino mice of varied ages and sexes

Age (weeks)	Total leukocyte counts (10^3/µl) Males	Total leukocyte counts (10^3/µl) Females	Absolute lymphocyte counts (10^3/µl) Males	Absolute lymphocyte counts (10^3/µl) Females	Absolute neutrophil counts (10^3/µl) Males	Absolute neutrophil counts (10^3/µl) Females
4	*5.57±1.89 [a] [3.45–8.70]	*4.29±1.12 [a] [3.10–6.45]	3.34±1.04 [a] [2.23–4.96]	2.63±0.86 [a] [1.52–4.19]	*2.15±0.91 [1.14–3.74]	*1.55±0.38 [1.22–2.09]
8	6.04±1.49 [a] [4.20–8.30]	5.26±1.86 [a] [3.30–8.15]	4.03±0.95 [a] [2.52–5.23]	3.22±1.16 [a b] [2.08–5.46]	1.90±0.60 [1.24–2.82]	1.88±0.73 [1.22–3.34]
12	5.97±0.46 [a] [4.30–6.60]	5.38±1.03 [a] [3.35–6.40]	3.72±0.32 [a] [2.32–4.28]	3.43±0.66 [b] [2.21–4.46]	2.13±0.30 [1.50–2.66]	1.94±0.39 [1.12–2.64]
16	7.76±2.11 [b] [4.65–10.55]	7.69±2.67 [b] [4.85–11.80]	5.34±1.58 [b] [2.79–7.39]	4.67±1.54 [b] [3.29–7.04]	2.30±0.63 [1.35–3.09]	2.85±1.34 [1.31–5.07]
20	7.25±2.52 [b] [4.50–11.30]	5.76±1.68 [a b] [3.70–7.35]	4.98±1.81 [a b] [3.11–7.80]	4.14±1.11 [b] [2.59–5.29]	*2.20±0.72 [1.35–3.39]	*1.52±0.53 [0.97–2.32]
24	7.77±1.27 [b] [5.50–9.45]	5.74±1.81 [a b] [3.70–8.80]	*5.47±0.87 [b] [4.18–6.62]	*3.90±1.30 [b] [2.46–6.16]	*2.21±0.51 [1.32–2.82]	*1.71±0.56 [1.00–2.55]
30	5.97±1.52 [a] [3.75–7.80]	6.80±2.49 [a b] [4.05–9.90]	3.55±1.03 [a] [2.29–5.07]	4.17±1.45 [b] [2.79–6.02]	2.38±0.58 [1.43–2.90]	2.45±1.26 [1.21–4.16]
40	*4.83±0.75 [c] [3.20–5.15]	*7.00±1.63 [b] [4.20–8.70]	2.45±0.08 [c] [1.87–2.82]	3.43±1.37 [a b] [1.62–5.31]	2.33±0.80 [1.15–3.20]	3.53±1.66 [2.10–6.48]

*Results are presented as means ± standard deviation, with the minimum and maximum values in parentheses. [a b c] Different superscripts in a column indicate significant difference between the designated means across the ages ($p < 0.05$); * Asterisk superscript on the sexes indicates significant differences between them at the specified age ($p < 0.05$).*

Table 4: The absolute monocyte, eosinophil and basophil counts of albino mice of varied ages and sexes

Age (weeks)	Absolute monocyte counts (10^3/µl) Males	Females	Absolute eosinophil counts (10^3/µl) Males	Females	Absolute basophil counts (10^3/µl) Males	Females
4	0.03±0.03 [0.00–0.06]	0.07±0.04 [0.03–0.14]	0.02±0.04 [0.00–0.11]	0.02±0.04 [0.00–0.09]	0.01±0.02 [0.00–0.05]	0.01±0.02 [0.00–0.06]
8	0.03±0.06 [0.00–0.14]	0.06±0.07 [0.00–0.16]	0.04±0.05 [0.00–0.15]	0.06±0.07 [0.00–0.16]	0.00±0.00 [0.00–0.00]	0.01±0.02 [0.00–0.05]
12	0.06±0.05 [0.00–0.11]	0.04±0.03 [0.00–0.05]	0.05±0.05 [0.00–0.11]	0.04±0.03 [0.00–0.04]	0.01±0.02 [0.00–0.05]	0.00±0.00 [0.00–0.00]
16	0.09±0.08 [0.00–0.21]	0.13±0.09 [0.00–0.27]	0.04±0.09 [0.00–0.24]	0.04±0.08 [0.00–0.21]	0.00±0.00 [0.00–0.00]	0.00±0.00 [0.00–0.00]
20	0.05±0.07 [0.00–0.16]	0.03±0.06 [0.00–0.16]	0.02±0.03 [0.00–0.07]	0.06±0.08 [0.00–0.21]	0.00±0.00 [0.00–0.00]	0.00±0.00 [0.00–0.00]
24	0.08±0.05 [0.00–0.14]	0.05±0.03 [0.00–0.09]	0.02±0.04 [0.00–0.08]	0.04±0.08 [0.00–0.06]	0.01±0.03 [0.00–0.09]	0.02±0.03 [0.00–0.06]
30	0.02±0.02 [0.00–0.05]	0.06±0.09 [0.00–0.20]	0.03±0.02 [0.00–0.05]	0.08±0.04 [0.04–0.12]	0.01±0.03 [0.00–0.07]	0.04±0.04 [0.00–0.10]
40	0.03±0.05 [0.00–0.09]	0.04±0.06 [0.00–0.14]	0.02±0.02 [0.00–0.04]	0.01±0.02 [0.00–0.06]	0.00±0.00 [0.00–0.00]	0.00±0.00 [0.00–0.00]

Results are presented as means ± standard deviation, with the minimum and maximum values in parentheses. No significant differences between the sexes and also between the varied ages ($p > 0.05$).

The absolute monocytes, eosinophil and basophil counts did not significantly ($p > 0.05$) vary across the age sets studied for both males and females, and there were no significant differences ($p > 0.05$) between the sexes (males and females) all through the age sets studied (Table 4).

DISCUSSION

The overall minimum and maximum PCV recorded for the mice used in this study (38.0 – 49.0 %) lies within the minimum and maximum values of 37.6 – 51.0 % reported for various strains of mice (Bolliger and Everds, 2010). The relatively lower mean PCV recorded for the 4-week old mice was mainly due to their lower erythrocyte counts when compared to other age sets. The finding of relatively lower PCV at a younger age which increases as the mice grows into adulthood is similar to what obtains in rats, dogs and cats, the reverse of which occurs in cattle, sheep and goats (Raskin and Wardrop, 2010). The later decrease in mean PCV at an older age (30 and 40 weeks of age) is in agreement with the reports of Bolliger and Everds (2010), and was attributed to plasma volume expansion as the mice aged, rather than lowered erythrocyte mass. The lack of significant variation in mean PCV across the ages and between the sexes as recorded in this study makes it possible for a researcher assessing for PCV to use any age set of mice to conduct his/her studies without worrying about age and sex differences.

The trend of increase in erythrocyte counts and haemoglobin concentration from a lower level at a younger age (4 weeks) to higher levels recorded for adults in this study is in agreement with that reported by Moore (2000) and Bolliger and Everds (2010) for various strains of mice. The minimum and maximum erythrocyte counts (10^6/µl) recorded in this present study (4.25 – 9.80) is relatively lower than the 7.00 – 11.00 reported by Bolliger and Everds (2010) and Charles River (2012) for mouse strains in North America, but the minimum and maximum HbC (g/dl) recorded in this present study (10.42 – 17.50) was not far different from the 10.00 – 17.00 g/dl reported for North American mouse strains by Bolliger and Everds (2010). The lack of significant difference in erythrocyte count between the males and females as recorded in this study was however not in agreement with the reports of Bolliger and Everds (2010) of slightly lower erythrocyte counts in males than in females.

The relatively higher MCV and MCH recorded at a younger age (4 weeks) and the progressive decrease as the mice grew into young adults (8 – 12 weeks of age) and the reverse trend recorded for the MCHC is in agreement with the patterns reported by Bolliger and Everds (2010). The lack of significant difference between the sexes (males and females) in their MCV. MCH and MCHC was also in agreement with reports by Bolliger and Everds (2010) and Charles River (2012). It is however worthy of note that the MCV, MCH and MCHC recorded in this present study were relatively higher than those reported for North American mouse strains/colonies by Bolliger and Everds (2010) and Charles River (2012).

The minimum and maximum total leukocyte counts (10^3/µl) recorded for mice used in this study (3.10 – 11.80) were relatively higher than those reported by Bolliger and Everds (2010) for mice strains in North America (2.0 – 10.0), but were lower than that reported by Charles River (2012). The pattern of increase in TLC from a lower level in the 4-week old mice to significantly higher levels at adulthood as recorded in this study was in agreement with reports by Bolliger and Everds (2010). The significant differences recorded between males and females at week 4 of age were not reported for North American strains but the differences between the sexes at week 40 of age were in agreement with the reports of Bolliger and Everds (2010).

The predominance of lymphocytes followed by neutrophils and the negligible absolute numbers of monocytes, eosinophils and basophils recorded in the differential leukocyte count of this study is in agreement with reports on North American mice strains (Bolliger and Everds, 2010; Charles River, 2012). The very low absolute numbers of monocytes, eosinophils and basophils is a common finding in the blood of all animals, but

the predominance of lymphocytes over neutrophils recorded in this study has been reported to also occur in rats, guinea pigs, cattle, sheep and goats, while in dogs and cats, neutrophils predominate (Raskin and Wardrop, 2010). The age-related trend of increase in the ALC from a lower level at week 4 of age to a higher level at adulthood and later decrease at weeks 30 and 40 of age is also in agreement with the reports of Bolliger and Everds (2010). This trend could be correlated with the immunological functions of lymphocytes and the age-related changes in immunocompetence which is usually lowest/weakest in the very young and aged when compared to adults (MacKinney, 1978; Ihedioha, 2004; Stockham and Scott, 2008). The absence of significant age-related variations in the ANC of the mice used in this study is in contrast to the reports of increase in ANC from young age to adulthood in North American mice strains (Bolliger and Everds, 2010). However, the significantly higher ANC recorded for the male mice at weeks 4, 20 and 24 of age concur with the reports of Bolliger and Everds (2010) that ANC are higher in male than in female mice. The lack of significant variations with age and differences between the sexes in the very low absolute numbers of monocytes, eosinophils and basophils is in agreement with the reports of Bolliger and Everds (2010) and Charles River (2012).

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