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Adaptogenic potentials of *Camellia sinensis* leaves, *Garcinia kola* and *Kola nitida* seeds

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Accepted 7 June, 2007

In this study, we screened *Garcinia kola* seed (GKS), *Kola nitida* seed (KNS) and *Camellia sinensis* Leaves (TEA) for adaptogenic potentials. The investigation was carried out in albino rats to determine the ability of these plant materials to increase non-specific resistance against physical, chemical and biological stressors. The results show that the three plant materials protected the animals from bacteria-induced mortality and morbidity and significantly reduced infection-induced leucocytosis in the rats. This confirms the ability of the herbs to protect the organisms from biological stress. However, while GKS was more effective than the other adaptogens in reducing monocytosis, KNS and TEA were effective in reducing neutropenia. Only KNS alleviated lymphocytosis. The effect of the adaptogens in alleviating physical stress was evaluated by the cold immobilization stress-induced ulcer models. The three adaptogens significantly ($P < 0.05$) protected the rats from cold-immobilization stress ulcers when compared to the control treatment. The ulcer protection property of these adaptogens is of the order: KNS > GKS > TEA. The result shows that *G. kola* seed (GKS) alleviated the hepatic degenerative changes associated with ciprofloxacin. *G. kola* seed, *K. nitida* seed, and *C. sinensis* leaves have shown remarkable ability to increase non-specific resistance to biologic, physical and chemical stressors and could therefore qualify as adaptogens.

Key words: adaptogens, *Camellia sinensis*, *Garcinia kola*, *kola nitida*, phytoadaptogens, stress.

INTRODUCTION

Stress is non-specific response of the body known to alter the physiological homeostasis of the organism resulting in various neuronal, endocrine and visceral dysfunctions (Shivakumar et al., 2006). The ability to develop and maintain resistance against a variety of stressors encountered in human life is crucial for survival. The desire to control the coping mechanisms has led to the origin of the science of adaptation.

Adaptogens are substances that help organisms to

adapt to unfavourable stressful conditions, which could be physical, chemical, biological or mental conditions (Brehkman, 1980; Dahanukar et al., 1997; Esimone and Nworu, 2007). Some pioneer researchers in this field put forth specific criteria that need to be fulfilled to qualify as an adaptogen, which include: ability to produce a non-specific response (i.e. increases resistance against multiple physical, chemical or biological stressors); brings any dysfunctioning body system back into balance and must be innocuous (i.e. must not influence normal body functions more than required) (Dahanukar et al., 1997; Brehkman and Dardymov, 1969).

Adaptogens could be synthetic or natural substances. However, most researches on adaptogens have focussed

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on natural substances (specifically, plants), and the term “phytoadaptogens” is now commonly used for adaptogens of plant origin (Esimone and Nworu, 2007). Certain plants like *Eleutherooccus senticosus*, *Panax ginseng*, *Raponticum carthamoides* and *Rhodiola rosea*, which are used as tonics in folk medicine, have been demonstrated to fulfil these criteria and therefore qualified as phytoadaptogens (Brekman and Dardymov, 1968).

G. kola Heckel (Guttiferae) is a large forest tree found throughout West and Central Africa (Hutchinson and Dalziel, 1954). *G. kola* seed (GKS) extracts have been shown to possess anti-inflammatory (Iwu and Igboke, 1982; Adegoke et al., 1998) antidiabetic (Iwu et al., 1990), antimicrobial (Hussain et al., 1982; Iwu and Igboke, 1982), antihepatotoxic (Iwu et al., 1987; Braide, 1991), inhibition of drug metabolism (Braide, 1991; Braide, 1990) and molluscicidal activity (Okunji and Iwu, 1991). Most of these pharmacological activities are believed to be related to its antioxidative activity and to its ability to increase immunity in general (Okunji et al., 1999). The seed has long been used as a traditional medicine in sub-Saharan Africa for a variety of indications including hepatitis and other viral infections such as those caused by influenza and Ebola viruses, and as antidote for ingested poison and for oral hygiene (Iwu, 1999).

K. nitida belongs to the genus cola and family Sterculiaceae. The *K. nitida* seeds (KNS) are usually up to 10 per carpel, each seed with only 2 cotyledons, rarely 3. Cola extracts have been used in the preparation of many non-alcoholic cola drinks. Cola extract is no longer incorporated because of its addictive properties, but kolanut extracts still remain an essential ingredient on the preparation of “coca cola” drink (Brekman, 1980). *K. nitida* is commonly used to dispel hunger, sleep and fatigue (Oladukun, 1989). Some of the active ingredients found in local kolanuts (e.g. theobromine and caffeine) are responsible for the physiological characteristics of *K. nitida* seeds. Caffeine is a known central nervous stimulant. In Russia, kolanuts administered as drugs were used to improve the light sensitivity of the eye and this was important especially in pilots who flew at high altitudes.

The tea plant (*C. sinensis*, family, Theaceae) originated in the South-west part of China and has been cultivated for more than 3000 years. *C. sinensis* (TEA) is a perennial evergreen plant that is a semitree or shrub depending on the environment. The mild stimulant effect and sense of well-being produced by tea have been attributed to its caffeine content (Mark, 1992). However, many of the pharmacological activities reported for tea extracts have been found to be due to the polyphenol content (Stagg and Millin, 1975; Ikeda et al., 1992, Sakagami et al., 1992; Xu et al., 1992). Some of the interesting medicinal effects of tea constituents include activation of leukocytes (Sakagami et al., 1992) antioxidant (Xu et al., 1992) and antimutagenic (Hayatsa et al., 1992) activities, lowering of plasma cholesterol levels (Ikeda et al., 1992),

protection from the effects of radiation (Uchida et al., 1992) and inhibition of angiotensin-converting enzyme (Hamilton-Miller, 1995). Tea extracts have been shown to have several useful antimicrobial effects.

In this study, we screened *Garcinia kola* seed, *Kola nitida* seed and *Camellia sinensis* leaves for adaptogenic potentials. These plant parts are used locally in some parts of West Africa as social masticatory agents and as tonics.

METHODS

Phytochemical tests

Phytochemical analysis was carried out on the crude GKS, KNS and TEA according to established methods (Evans, 1996, Harbourne, 1984).

Effects of GKS, KNS and TEA against biological stressor

The studies were conducted on healthy albino rats of either sex (130 – 200 g). They were housed at room temperature in metallic cages and fed commercial grower’s mash (Guinea Feed Brand) throughout the experiment. After a one week acclimatisation period, the animals were divided into four groups (n = 5). The first 3 groups received *G. kola* seed (100 mg/kg), *K. nitida* seed (100 mg/kg) and tea leaves extracts (10 mg/kg), respectively. The fourth group served as the control and was given distilled water (0.5 ml/animal). The various treatments were administered orally to the animals for a period of seven days. On days 8 and 9, the rats were infected intra-peritoneally with 0.1 ml and 0.15 ml of 1×10^9 cfu/ml glucose-enriched *Klebsiella pneumoniae*, respectively. Bodily changes, total white blood cells (WBC) and differential counts were monitored for 21 days.

Estimation of total WBC

Blood was collected before commencement of treatment (day zero) and on the 8th, 10th, 12th, 14th and 21st day post-treatment by tail snipping into sample tubes coated with EDTA. Blood samples from the tubes were drawn up to the 0.02 ml calibration on the WBC dilution capillary system and then mixed with 0.38 ml of leucocytes diluting fluid (3% acetic acid) and allowed for 10 min for the erythrocytes to lyse. Thereafter, the Neubauer counting chamber was filled with the diluted sample and the set up examined microscopically in oil-immersion under 100 x magnifications. The total numbers of white cells in the 4 large squares are counted and at the end, the total white cell count (WBC) is calculated from the relation: $WBC = n \times 20 \times 2.5$, where n is the number of cells counted. The dilution factor is 1:20 and the volume factor for the counting chamber is 1/0.4 (i.e. 2.5).

Estimation of differential count

The Leishman staining technique was used. A drop of blood was placed on one end of the glass slide using an applicator stick. The cover slip is used to make a smear of the blood on the glass slide using the push wedge technique. The stain is made to cover the blood film and then left to stand for 2 min. Thereafter, distilled water, two times the quantity of stain is used to flood the thin film of the blood; the setup is rocked gently for 2 min and then allowed to stand for 15 min before rinsing the stain. The slide was left to dry

Table 1. Phytochemical constituents of *Garcinia kola* seed (GKS), *Kola nitida* seed (KNS) and *Camellia sinensis* leaves (TEA).

Phytoconstituent	<i>Garcinia kola</i> seed	<i>Kola nitida</i> seed	<i>Camellia sinensis</i> leaves
Alkaloid	-	++	-
Saponins	+	++	++
Tannins	+	+	++
Flavonoids	++	+	-
Protein	+	+	-
Glycoside	+	+	+
a. Cyanogenetic glycoside	-	-	-
b. Cardiac glycoside	+	+	+
c. Anthracene glycoside	+	-	-
d. Steroidal glycoside	+	+	ND
Carbohydrate			
Reducing sugar	+	+	+
Starch	+	+	-
Sterols and triterpenoids	+	ND	-

+ = Present in low concentration; ++ = present in abundance; ND = not determined; - = absent.

and then examined on the microscope using the oil immersion objective lens of 100x magnification. The cells were counted and differentiated using a tally counter.

Effects of GKS, KNS and TEA against physical stressor

Albino rats of either sex (170 - 220 g) divided into four groups of 5 rats each were respectively given GKS (100 mg/kg), KNS (100 mg/kg), TEA (10 mg/kg) and water (0.5 ml/animal) orally twice daily for 5 days. Animals were allowed free access to food (Grower's mash, Guinea Feed brand) and clean water. Thereafter, the rats were fasted for 18 h and then immobilized individually inside specially improvised plastic containers at 4°C (in a refrigerator) for another 18 h. At the end of the period, the rats were sacrificed by using over dose of chloroform anaesthesia. The stomach was removed and opened along the greater curvature, rinsed under a stream of water and pinned on a flat corkboard and observed visually using a 10x magnification hand lens. Erosions formed on the glandular portion of the stomach were counted and each given a severity rating on a 1 - 3 scale based on the diameter of the ulcer (viz: 1 = ulcers ≤ 1 mm; 2 = ulcers > 1 mm ≤ 2 mm; 3 = ulcers ≥ 2 mm). The total score for each stomach was designated as the ulcer index (UI) for that stomach (Main and Whittle, 1975).

Effects of GKS, KNS and TEA against chemical stressor

This was assessed by evaluating the effect of the adaptogens on fluoroquinolone-induced histopathological changes in albino rat livers. Male albino rats (100 - 170 g) were divided into 8 groups of 4 animals each. Group 1 - 3 received each of the adaptogen and ciprofloxacin (CIP); group 4 - 6 received the adaptogens alone; group 7 received ciprofloxacin alone while group 8, which served as the control, received only water, the 3 adaptogens were administered orally at a dose of 20 mg/kg while the ciprofloxacin was given at 10 mg/kg. The various treatments were administered to the animals twice daily for five days with free access to food and water. On the sixth day, all the animals were sacrificed and slices of the liver fixed in 10% normal saline for 24 h. The specimens were dehydrated subsequently in different grades of alcohol (70, 80, 90, 95 and

100%) and mounted in an auto-processor, each phase requiring about 3 h. The alcohol was removed by passing the specimen through dishes containing xylene and then through containers of paraffin wax. The specimens were then transferred into a vacuum-embedder which expels entrapped air in the tissues while at the same time embedding paraffin into the spaces.

Thereafter, the specimens were embedded into molten wax which was allowed to solidify by placing on ice. A microtome was used to cut ribbons of the specimen of about 5 μ thick. De-waxing of the cut section was done by passing it through xylene, absolute alcohol, 80 and 70% alcohol. The sections were stained with haematoxylin and eosin and examined by light microscopy.

RESULTS AND DISCUSSION

The Phytochemical constituents of *G. kola* seed (GKS), *K. nitida* seed (KNS) and *C. sinensis* leaves (TEA) are presented in Table 1. This preliminary phytochemical test reveals that the major (most abundant) phytoconstituent in GKS, KNS and TEA are flavonoids, alkaloids and tannins, respectively. Flavonoids (specifically, biflavonoids) have been found to be the most abundant phytoconstituents of GKS (Iwu and Igboko, 1982). Example of typical biflavonoids isolated from *G. kola* seed includes kolaviron, amentoflavonone, kolaflavanone, GB-1, GB-2 and GB-1a. These biflavonoids have been implicated for most of the properties of *G. kola* seed. (Iwu and Igboko, 1982, Adegoke et al., 1998; Iwu et al., 1987; Iwu et al., 1990; Braide, 1991).

The major phytoconstituent of *K. nitida* seed has been shown to be xanthine alkaloids of which caffeine is the most common (Evans, 1996, 1983). Sugar has also been shown to be slightly abundant in *K. nitida* seed (Oladukun, 1989; Obika et al., 1996). In fact, due to the high content sugar in *K. nitida* seed, it is commonly used by people to dispel hunger (Obika et al., 1996) for the same

Table 2. Effect of the adaptogens on total and differential leucocyte counts of infected albino rats.

Adaptogen		Cell count/ml of blood					
		Day 0	Day 8	Day 10	Day 12	Day 14	Day 21
<i>Garcinia kola</i> seed	TLC	12550 ± 4360	14825 ± 3093	11162 ± 2410	16562 ± 5355	17313 ± 5135	16700 ± 7422
	Neutrophils	2887 ± 946	3771 ± 698	2408 ± 805	2750 ± 1959	1533 ± 650	3593 ± 1942
	Lymphocytes	6937 ± 2414	7824 ± 1657	6562 ± 1695	12113 ± 3867	14937 ± 4567	10582 ± 5160
	Monocytes	2490 ± 1309	2490 ± 1063	2116 ± 795	1700 ± 108	846 ± 339	2337 ± 804
<i>Kola nitida</i> seed	TLC	13775 ± 3627	16088 ± 1849	12375 ± 2546	14525 ± 5116	14463 ± 4101	16525 ± 5007
	Neutrophils	3601 ± 2164	3338 ± 1016	3350 ± 2061	1241 ± 964	2251 ± 639	2781 ± 810
	Lymphocytes	7373 ± 1277	7966 ± 1935	6712 ± 1469	11765 ± 3873	11355 ± 3641	9634 ± 4590
	Monocytes	2232 ± 668	2531 ± 1005	2098 ± 242	1769 ± 764	891 ± 401	2870 ± 1069
<i>Camellia sinensis</i> leaves	TLC	13388 ± 4746	14875 ± 3459	11988 ± 3966	16788 ± 2482	18138 ± 3020	16500 ± 5720
	Neutrophils	3905 ± 1158	3538 ± 1286	2748 ± 1267	2636 ± 1824	1904 ± 1183	2793 ± 1148
	Lymphocytes	7201 ± 3127	8632 ± 2988	6956 ± 2978	13793 ± 2114	15445 ± 3582	10613 ± 3455
	Monocytes	1459 ± 927	2732 ± 378	2040 ± 729	1359 ± 830	841 ± 407	2902 ± 1461
Control	TLC	13025 ± 3232	16825 ± 1539	13300 ± 1994	16500 ± 4253	18625 ± 3671	18725 ± 1024
	Neutrophils	2783 ± 651	3155 ± 416	2545 ± 1259	2612 ± 1612	1573 ± 432	4591 ± 976
	Lymphocytes	7244 ± 1759	10404 ± 1245	8190 ± 1382	11971 ± 2642	15910 ± 3130	11046 ± 830
	Monocytes	2443 ± 554	2616 ± 595	1764 ± 218	1917 ± 677	1082 ± 740	2859 ± 520

reason, powdered “kolanut” are used in the preparation of beverages (Oladukun, 1989). Tea polyphenols have been consistently shown to be the major constituents of tea leaves (Hamilton-Miller, 1995; Mark, 1992; Stagg, 1975; Ikeda et al., 1992; Sakagami et al., 1992). However, the xanthine alkaloids, caffeine is also significantly present in tea and is said to be responsible for the mild stimulant effect and sense of well being produced by tea (Hamilton-Miller, 1995; Mark, 1992).

The effects of the adaptogens on total leucocyte counts, absolute neutrophil, monocyte and lymphocyte counts of biologically stressed (bacteria-infected) albino rats are presented in Table 2. One of the rats in the control group (the group not pre-treated with the adaptogens) died in the course of the experiment. The rest of the animals in this group were very weak by the 21st day prior to their being sacrificed. All the animals in the adaptogen pre-treated groups survived and were very active throughout the course of experiment. Our results show that by the 21st day post-treatment (and 14th day post-infection); the total leucocyte count was significantly reduced by all three adaptogens compared to the control (Table 2). The neutrophil counts were reduced by the adaptogens in the order: KNS or TEA > GKS. Only GKS significantly reduced the monocyte count; there was no significant difference between the effect of either KNS or TEA and the control. Lymphocyte counts were only significantly reduced by KNS. The reduction by GKS and TEA was not significantly ($P > 0.05$) different from control. In the assessment of adaptogenic potentials of a substance, it is currently desirable that the agent should be able to demonstrate/confer an increase in non-specific resistance against biological, physical and chemical stre-

ssors (Brehkman, 1980; Dahanukar et al., 1997).

Table 3. Effect of adaptogens on cold-immobilisation stress-induced ulcer in rats.

Adaptogen	Ulcer index	% Protection
<i>Garcinia kola</i> seed	6.33 ± 2.51*	61.24
<i>Kola nitida</i> seed	3.0 ± 1.73**	81.63
<i>Camellia sinensis</i> leaves	6.66 ± 1.52*	59.22
Control	16.33 ± 7.23	-

N = 5; * $P < 0.05$; ** $P < 0.01$.

Biological stressors like *Escherichia coli*, *Staphylococcus aureus*, *K. pneumoniae*, *Candida albicans*, etc. have been used for such studies (Dahanukar et al., 1997). In this case, stress manifestations are assessed by noting the mortality/morbidity of test animals, culturing the organisms from the blood and/or evaluating the effect of treatments with adaptogens on leucocyte counts (Dahanukar et al., 1997).

Our results show that all the adaptogens protected the animals from bacteria-induced mortality and morbidity. All the adaptogens significantly reduced infection-induced leucocytosis in the rats. This confirms the ability of the herbs to protect the organisms from biological stress, and hence their adaptogenic potentials. However, while GKS was more effective than the other adaptogens in reducing monocytosis, KNS and TEA were effective in reducing neutropenia; only KNS alleviated lymphocytosis. The apparent efficiency of KNS and TEA in alleviating monocytosis is a direct consequence of the normal physiolo-

gical effect of polyphenols. Usually, plant polyphenols are known to stimulate monocytes (Sakagami et al., 1992).

The effect of the adaptogens in alleviating physical stress was evaluated by the cold immobilization stress induced ulcer models (Bhattacharya and Ghosal, 2000). The ulcer indices of animals pre-treated with the adaptogens (compared with the control) are presented in Table 3. The adaptogens significantly ($P < 0.05$) protected the rats from cold-immobilisation stress ulcers when compared to the control treatment. The ulcer protection property of these adaptogens is of the order: KNS > GKS > TEA.

Gastric ulcerations induced by a large number of stressors, is presently the most widely used paradigm to evaluate anti-stress/adaptogenic activity (Dahanukar et al., 1997; Bhattacharya and Ghosal, 2000). Our results therefore, clearly show that all three adaptogens offered protection against cold immobilization stress. *K. nitida* seed (KNS) offered the best protection and is thus judged to be the best amongst the three tested adaptogens. *G. kola* seed (GKS) and TEA have identical/equivalent activities since there was no significant difference between their activities. The adaptogenic potentials of kolanuts is discernable from its social and medicinal uses. In Russia, kolanuts administrated as drugs were used to improve the light sensitivity of the eye and this was important especially in pilots who flew at high anti-inflammatory altitu-

Table 4. Effects of *Garcinia kola* seed on ciprofloxacin-induced hepatotoxicity in rats.

Treatment	Observation
Ciprofloxacin	There was severe degeneration of hepatocytes around the major central vein (centrilobular degeneration of hepatocytes)
Ciprofloxacin + <i>Garcinia kola</i> seed	There was a slight degeneration of hepatocytes at the periphery and at the centrilobular axes.

des. Under normal conditions, administration of kolanuts increased light sensitivity of the eye during 30 – 40 min and the action persisted for 4 – 5 h. *K. nitida* is socially used by people to dispel hunger, sleep and fatigue (Obika et al., 1996). Moreover, the popular coca cola drink described by Brehkman as having adaptogenic properties (Brehkman, 1980) is principally prepared from kola extracts.

Caffeine which is a major constituent of both TEA and KNS is a nervous system (CNS) stimulant. This phyto-constituent is known to elevate mood, decrease fatigue and increase capacity for work (Rall, 1990). It may appear therefore, that the adaptogenic properties of KNS and TEA are due to their caffeine content. However, caffeine is also known to stimulate gastric secretions and has

been incriminated in exacerbating duodenal ulcers (Cohen and Booth, 1975). This fact is not in consonance with our observation that KNS and TEA (which contains caffeine) significantly protected cold-stressed rats from developing stomach ulcers. Alternatively, the kola catechins with their characteristic anti-inflammatory, anti-oxidant and astringent properties could be responsible for this observation.

TEA is refreshing, mildly stimulating and produces a general sense of well being (Hamilton-Miller, 1995; Mark, 1992). While these activities could be attributed to its caffeine content, two other important physiological activities of TEA (namely, anti-oxidant and anti-inflammatory) that authenticate their adaptogenic potentials are attributed to the polyphenol content of TEA (Hamilton-Miller, 1995; Stagg and Millin, 1975). During stress, prostaglandin and anti-oxidant systems of target organs serve as natural defence mechanisms to counteract the deleterious effect of stress-induced lipid peroxidation (Dahanukar et al., 1997; Avery, 1995). Lipid peroxidation leads to generation of oxygen free radicals, which are responsible for the stress manifestations observed. If depletion of anti-oxidants or prostaglandins occurs, it leads to stress manifestations. Adaptogens with anti-oxidant and anti-inflammatory properties (such as TEA and KNS) are able to thwart these effects by protecting cellular membranes from the adverse effects of free radical build up (Avery, 1995). Also consistent with the (specifically, antitumour) properties of several first line phytoadaptogens (Bespalov et al., 1992; Udinstev et al., 1992), tea polyphenols have been shown to be tumouricidal (Xu et al., 1992; Hayatsa et al., 1992). Therefore, it could be concluded that the major adaptogenic constituent of TEA is most likely the polyphenols, with only minor contributions from caffeine.

The result obtained with GKS (i.e. the reduction in ulcer index) is not surprising since flavonoids (which are the major constituents of *G. kola* seeds) have been shown to be the most important phytoconstituent associated with antiulcer activity (Pifferi, 1992; Pamer and Ghosh, 1976).

The Histopathology result is shown in Table 4. The result shows that *G. kola* seed (GKS) alleviated the hepatic degenerative changes associated with ciprofloxacin. Even though ciprofloxacin is not a standard hepatotoxin, the ability of GKS to alleviate hepatotoxicity resulting from this drug is a demonstration of its adaptogenic potentials. Hepatoprotection exhibited by certain phytoadaptogens is believed to be a major mechanism for their adaptogenic properties (Dahanukar et al., 1997; Avery, 1995; Udinstev et al., 1992).

The hepatoprotection exhibited by adaptogens is generally ascribed to the presence of constituents with anti-oxidant properties. *G. kola* seed (GKS) is ethnomedically employed as a hepatoprotective and anti-inflammatory agent (Iwu and Igboko, 1982; Adegoke et al., 1998; Iwu et al., 1987; Iwu et al., 1990; Braide, 1991). These pharmacological properties, attributed principally to its biflavo-

noids constituents (Iwu and Igboko, 1982; Braide, 1991), are believed to be related to its anti-oxidative activity and the ability to increase immunity in general (Okunji et al., 1999). Thus just like TEA and KNS, the various ethnopharmacological applications of GKS suggests that it is an adaptogen. Perhaps, that is why some researchers have variously described this herb as “adaptogen” without any empirical experiment to authenticate this claim. (Iwu, 1999; Meserole, 1999; Duncan, 1999).

The result of our experiment has however confirmed the adaptogenic property of *G. kola* seeds (GKS). It is possible that the anti-oxidant, anti-inflammatory, and immunostimulatory properties of the flavonoids constituents of this herb are responsible for the adaptogenic effects.

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