Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 10, Issue 1, 2022

Amlodipine Elicited Dose-dependent Benefit in Rats' Liver Histology and Some Seric Bioindicators Following Monosodium Glutamate Intoxication

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ABSTRACT

Amlodipine (AML), a calcium channel blocker based anti-hypertensive, and monosodium glutamate (MSG), a hepatotoxic flavouring could be taken simultaneously. The study evaluated the effect of concomitant exposure of MSG and AML in rats' liver histology, and some serum bioindicators. Rats groups (sample size = 6) A, B, C, D, E and F, respectively were daily-exposed *via* gavage for 14 days to water and feed, AML therapeutic-dose (TAML), AML overdose (HAML), MSG (8 mg/g), MSG plus TAML, and MSG plus HAML. Exposure to MSG significantly ($P \leq 0.05$) increased the level of alanine aminotransferase, ALT (19.50 ± 1.04 IU/L), aspartate aminotransferase, AST (169.25 ± 2.28 IU/L) and alkaline phosphatase, ALP (30.75 ± 0.85 IU/L) but decreased the computed AST:ALT ratio (8.51 ± 0.34) compared to others. Rats liver photomicrographs revealed normal parenchymal architecture (control)while alterations ranged from moderate (TAML, HAML and MSG plus TAML fed) to severe (MSG, and MSG plus HAML fed). Thus, amlodipine elicited beneficial response, and dose-dependent diminution of monosodium glutamate-related adversity, in the rats' liver histology and some seric biofunctional capacity.

Keywords: Alanine aminotransferase, Alkaline phosphatase, Amlodipine, Aspartate aminotransferase, Hepatoprotective, Therapeutic dose.

INTRODUCTION

Amlodipine, a synthetic and long acting dihydropyridine, is one of the most widely used class of calcium channel blockers with marked antihypertensive and antianginal properties compared to other classes of blood pressure lowering drugs [1,2]. Amlodipine, as other calcium channel blockers, enhances vascular smooth muscle and myocardial dilatation to increase blood flow and oxygen delivery to the myocardial tissue [3]. However, owing to its slow elimination rate or longer half life ($t_{1/2}$), amlodipine has higher bioavailability than other calcium channel blockers [4]. On discontinuation, amlodipine generally returns the blood pressure to baseline without any

adverse rebound elevations [2]. The unique pharmacokinetic profilesof amlodipine is fundamental to its clinical benefits [2,4] and probably to its wide and frequent use as choice antihypertensive among others. Hypertension or high blood pressure generally results from long duration of abnormally high pressure in the arteries [2]. It is a common ailment, and over the years, patients do resort to self-purchase and self-medication of antihypertensives, including amlodipine. Strictly, amlodipine is not an over the counter drug, but it could be procured on request from pharmaceutical outlets. Thus, with self-purchase *cum* self-medication tendency, possibility of its abuse exists with significant adversity in animals.

It is possible to take amlodipine together with foods flavoured with overdose of MSG. Concomitant intake of amlodipine and MSG may present unknown effects that could worsen MSG related adversity on the functional integrity of the liver which is the major xenobiotic metabolising organ. The study objectives were to determine alterations in the rats' liver histology and some seric enzymes activity (ALT, AST and ALP) following concomitant exposure to amlodipine with overdose of MSG. In previous studies, monosodium glutamate, the sodium salt of glutamic acid mostly used as packaged food flavouring elicited adverse effects, including on the liver function [5,6]. In another study, high MSG concentration (8000 mg *per* kg) adversely affected the liver [7]. Monosodium glutamate contained in condiment with varied brand names but without MSG content indication on the labels could be bought and consumed without restriction hence stands a high chance of inadvertent abuse with inherent MSG-intoxication adversity [8]. Thus, the study aimed to investigate changes in the liver histology and some seric biofunctional indicators of monosodium glutamate intoxicated rats treated together with amlodipine. ALT activity particularly served as a specific bioindicator of liver function status [9,10] and histopathologic assessment of organs substantiated seric chemistry observations [11].

MATERIALS AND METHODS

Sample procurement and preparation

Monosodium glutamate (99%) and Amlodipine tablets (10 mg) were bought from a reputable Pharmacy shop at Umuahia, Abia state, Nigeria. Other chemicals used in the study wereanalytical grade products of Randox Ltd and were used without further purification. Male Wistar rats (thirty-six; 80 g-165g) were obtained from Ogive Farm, Aba, Abia state, Nigeria. The

animals were kept for one week to acclimatize in the animal house of the study institution. The rats were maintained under laboratory conditions in stainless steel cages.

Ethical adherence and experimental design

The study adhered strictly to the ethical guidelines on animal use [12]. The rats were randomly grouped into six (n = 6) and respectively exposed to feed (Top feed products, Nigeria) and water (group A), AML therapeutic dose (group B), AML overdose (group C), MSG (group D), AML therapeutic dose + MSG (group E) and AML overdose + MSG (group F). The exposure was oral through a gavage. The administration of amlodipine was once each day at a dose of 10 mg/kg body weight [13] and after 24 hours interval, in line with prescription format of its dosage for a 70kg-man given in weight *per* volume (w/v). The actual therapeutic concentration was prepared by dissolving adult dosage (1 tablet) of amlodipine in 15 ml of distilled water corresponding to the rats' weight according to manufacturer's instructions with slight modification as described in recent report [14]. Amlodipine overdose was calculated as therapeutic dose for 70 kg man multiplied by 5. Rats' intoxication with MSG was achieved at 8 mg/g body weight (bw) and by daily exposure according to Mariyamma *et al.* [15] with slight modification (for 14 instead of 20 days) and as supported by other studies [10,16]. After 14 days, the rats were sacrificed after overnight fast. The animals were sacrificed by cervical dislocation. The blood sample of the respective rats was collected through cardiac puncture into a clean non anticoagulated polystyrene tube. After centrifugation at 3000 rpm for 5 minutes of clotted blood sample, the serum was collected and stored in a refrigerator for biochemical analysis.

Determination of the study bioindicators

ALT and AST activity in the rats' serum were determined by the method of Reitman and Frankel [17]. ALP activity in the rats' serum was estimated by the end point colorimetric method [18]. The serum AST:ALT ratio was calculated from the results of the serum AST and ALT activities as obtained from this study while the calculation of change relative to any group was with Cemaluk relative change formula [14].

$$Change\ relative\ to\ K\ (\%) = \frac{(V - K)}{K} \times 100$$

The constant group value is represented by K whereas the variable group value is represented by V [14].

Liver tissues from the sacrificed rats were preserved in 10% phosphate-buffered formalin for 48 hours and thereafter dehydrated in graded concentrations of absolute alcohol, cleared in xylene and embedded in paraffin wax at 60 °C. The paraffin-embedded tissues were sectioned on a microtome at 5 µm, mounted on clean glass slides and dried in an oven. The sections on the slides were routinely stained with haematoxylin and eosin for histopathological examination with a Motic™ compound light microscope. The photomicrographs were taken using a Motic™ 9.0 megapixels microscope camera at × 100 and × 400 magnifications.

Statistical analysis

The numeric data were subjected to one-way analysis of variance (ANOVA) using statistical package for social sciences (SPSS) version 20.0. Results were expressed as mean ± standard error of mean (SEM). Difference was accepted as significant at $P \leq 0.05$ and by relative change of up to ten (10) percent and above as calculated with Cemaluk relative change formula [14].

RESULTS AND DISCUSSION

Adversity on the liver, that usually results when the dose of the toxicant exceeds safe hepatic detoxification pathways, affects metabolic homeostasis [19] necessitating this study which by standard protocols, assessed the concomitant exposure effect of MSG and AML on the liver histology, and some serum bio-functional indicators, of rats. ALT enzyme, localized primarily in the hepatocytes cytosol, offers useful evaluation of the extent of damage sustained by the liver [9,10]. It (ALT) is a specific marker for liver damage compared to other marker enzymes, including AST and ALP. Result showed that exposure to MSG significantly ($P \leq 0.05$) increased the level of ALT (19.50 ± 1.04 IU/L) compared to others. This implies that compared to MSG fed, the decreased level of ALT in the other treatment groups was significant ($P \leq 0.05$) (Table 1). This seemingly demonstrated overriding MSG related adversity on the rats' liver and apparent capacity of amlodipine (either at therapeutic or overdose concentration) to mitigate MSG related adversity in rats' liver. Amlodipine safety [2], calcium channel blockers related hepatoprotective activities [20,21] and in particular, AML related reduction in ALT activity and even mitigation of agent induced liver damage [13] have been reported. The report herein of

mitigation capacity of AML on MSG related adversity in the liver is novel and warrants follow-up in humans.

Table 1: Influence of varied doses of AML on the activity (IU/L) of ALT and AST in MSG assaulted rats' serum

Group	ALT (IU/L)	Change relative to control and (MSG) groups (%)	AST (IU/L)	Change relative to control and (MSG) groups (%)
Control	13.83 ± 0.83^{a}	0.00 (– 29.07*)	160.00 ± 3.11^{d}	0.00 (– 5.45)
TAML	15.00 ± 0.36^{d}	8.45 (– 23.07*)	134.00 ± 1.29^{a}	– 16.25* (– 20.79*)
HAML	14.83 ± 0.70^{c}	7.23 (– 23.94*)	142.00 ± 5.43^{b}	– 11.25* (– 16.07*)
MSG	19.50 ± 1.04^{f}	40.99* (0.00)	169.25 ± 2.28^{f}	5.78 (0.00)
MSG + TAML	15.60 ± 0.91^{e}	12.79* (– 20.00*)	156.40 ± 2.51^{c}	2.25 (– 7.58)
MSG + HAML	14.75 ± 0.79^{b}	6.65 (–24. 35*)	165.00 ± 2.58^{e}	3.12 (– 2.50)

Values with different superscript letters indicated statistically significant difference at ($P \leq 0.05$). Values marked * indicated relative change of 10 percent and above. Results expressed as mean ± standard error of mean, SEM

AST enzyme is located in the cytoplasm and mitochondria of many high metabolic organs, including the heart, skeletal muscles, liver, kidney and erythrocytes hence unlike the ALT is a nonspecific marker for liver damage [22]. Thus, an increase in AST activity could be indicating injury to any of the high metabolic organs including the liver but when accompanied by an increase in ALT activity could be a supportive indicator of liver injury [10]. Result showed that exposure to MSG significantly ($P \leq 0.05$) increased the level of AST (169.25 ± 2.28 IU/L) compared to others (Table 1). The observation supports MSG related adversity on any of the high metabolic organs, particularly the liver, in the MSG fed rats. Increased AST activity with commensurate increase in ALT activity as seen in the MSG fed rats group compared favourably with earlier report of induction of liver damage in rats following intake of high dose of MSG [23]. Generally, increase in ALP, activity could, aside to its increased *de novo* synthesis, be due to obstructed billirubin transport to the liver duct. Thus, ALP enzyme activity is a marker for endoplasmic reticulum and plasma membrane integrity but, as AST, could serves as a non-

specific indicator of liver function integrity. Result in Table 2 showed that exposure to MSG significantly ($P \leq 0.05$) increased the level of ALP (30.75 ± 0.85 IU/L) compared to others. Thus, the significant ($P \leq 0.05$) reduction in AST and ALP activity in the TAML fed and HAML fed rats compared to control and relative to MSG fed rats suggested non adverse effect of amlodipine intake irrespective of dose on the liver. This is in line with the observation in this study of non-significant influence of tested doses of amlodipine on ALT activity, and on the suggested non adversity on the rats' liver. Also, the observation is in line with the reported general safety of AML [2] and AML related reduction in AST activity [13]. Also, a significant ($P \leq 0.05$) increase in the ALP activity in the MSG group when compared to the control group suggested compromised liver integrity and functional capacity [10].

Table 2: Influence of varied doses of AML on the activity (IU/L) of ALP and activity ratio of AST to ALT in MSG assaulted rats' serum

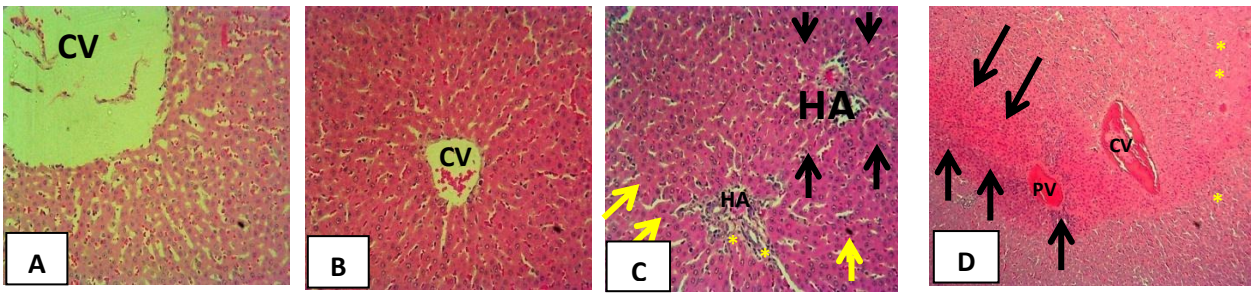
Group	ALP (IU/L)	Change relative to control and (MSG) groups (%)	AST:ALT Ratio	Change relative to control and (MSG) groups (%)
Control	27.00 ± 0.81^{b}	0.00 (–12.18*)	11.79 ± 0.79^{f}	0.00 (38.54*)
TAML	25.60 ± 0.55^{a}	–5.18 (–16.73*)	8.95 ± 0.23^{b}	–24.08* (5.17)
HAML	30.00 ± 1.31^{c}	11.11* (–2.43)	9.74 ± 0.78^{c}	–17.38* (14.45*)
MSG	30.75 ± 0.85^{f}	13.88* (0.00)	8.51 ± 0.34^{a}	–27.82* (0.00)
MSG + TAML	30.20 ± 1.01^{d}	11.85* (–1.78)	10.17 ± 0.45^{d}	–13.74* (19.50*)
MSG + HAML	30.50 ± 0.95^{e}	12.96* (– 0.81)	11.63 ± 0.74^{e}	–1.35 (36.66*)

Values with different superscript letters indicated statistically significant difference at ($P \leq 0.05$). Values marked * indicated relative change of 10 percent and above. Results expressed as mean ± standard error of mean, SEM

The possible combination ratio of the studied bioindicators of liver functions including AST and ALT, could also be diagnostic. Result showed that exposure to MSG significantly ($P \leq 0.05$) decreased the computed AST;ALT ratio (8.51 ± 0.34) compared to others. This implies that compared to MSG fed, the decreased level of AST;ALT ratio in the other treatment groups was

significant ($P \leq 0.05$) (Table 2). This further demonstrated MSG related adversity on the rats' liver. Intriguingly, the reduced AST:ALT ratio in TAML fed rats was significant ($P \leq 0.05$) whereas the ALT activity in, notably, the simultaneously exposed (MSG + TAML and MSG + HAML) groups relative to MSG fed, rats was lower and significant ($P \leq 0.05$). This may imply the overriding capacity of AML either at therapeutic or overdose concentration to mitigate MSG related adversity on the liver amid possible, albeit negligible, adversity on other high metabolic organs following synergistic interaction of MSG with AML. AML related adversity particularly at high dose and prolonged intake could not be ruled out. It is usually dosed on a once daily basis because of its long halflife that is related to its slow rate of elimination for over 40–60 hours [4].

The current investigation showed that exposure of rats to the tested dose of MSG compromised rats' liver integrity as evidenced by significant increases in, particularly the serum ALT activity [13,24] while the concurrent administration of AML with MSG attenuated the hepatotoxicity induced by MSG as revealed by decreased serum ALT activity and AST:ALT ratio. This could imply that AML protected the liver against MSG induced hepatotoxicity evidenced by significant decrease in, particularly the serum ALT activity of the group of rats simultaneously exposed to AML and MSG. The biochemical findings from this study were strongly supported by the results of histological evaluation. Histological changes served as definitive indicator of organ damage [11]. From this study, the photomicrographs of the liver sections revealed normal parenchymal architecture in the control rats and varied alterations which were moderate in TAML, HAML and MSG plus TAML fed rat groups, but severe in MSG and MSG plus HAML fed rat groups. This may be confirming the serum chemistry result that indicated MSG and MSG plus HAML related adversity on the functional integrity of the rats' liver. The moderate histological alteration in TAML and HAML treated rats observed tallied with that reported earlier [13].



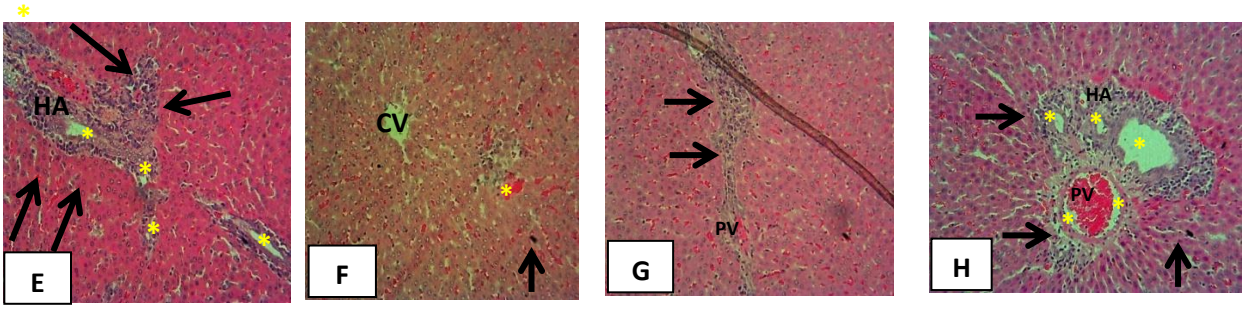


Figure 1: Photomicrograph of the liver section of rats in control group (A), TAMLfed group (B), HAML fed group (C), MSG fed group (D, E), MSG plus TAML fed group (F, G) and MSG plus HAML fed group (H). Hematoxylin and Eosin (H&E) stained, ×400 (except D, H&E stained, ×100)

Notes:
A: Showing normal parenchymal architecture of the central vein (CV).
B: Showing a moderate widespread hepatocellular vacuolar degeneration of the central vein (CV).
C: Showing an inflammatory cellular infiltration in the portal area (black arrows) with an apparent destruction of bile ductules in the portal area, and also multifocal karyomegaly (yellow arrows). Branch of the hepatic artery (HA); Bile ductules (asterisks).
D: Showing a zone of severe compression of hepatocytes and obliteration of sinusoidal spaces (delineated by black arrows). The portal and central veins are severely congested, and there is a locally extensive area of hepatocellular coagulative necrosis (asterisks). Portal vein (PV); Central vein (CV).
E: Showing severe inflammatory cellular infiltration in the portal area (arrows), accompanied by severe bile ductular hyperplasia (yellow asterisks). Branch of the hepatic artery (HA).
F: Showing moderate centrilobular hepatocellular degeneration, and a focus of hepatocellular necrosis admixed with inflammatory cells (arrow). There is also an area of haemorrhage (asterisk). Central vein (CV).
G: Showing moderate inflammatory cellular infiltration in the portal area (arrows), accompanied by moderate multifocal inflammatory cellular infiltration. Portal vein (PV).
H: Showing severe inflammatory cellular infiltration in the portal area (arrows). Hepatic artery (HA); Bile ductules (*).

Amlodipine related hepatoprotective potential in carbon tetrachloride animal model of hepatotoxicity has been reported [25]. However, report herein of amlodipine related hepatoprotection against MSG hepatotoxicity is novel and deserves follow up as it could provide insight on the mechanism of MSG intoxication in animals. For instance, calcium channel blockers, including amlodipine, are particularly hepatoprotective against hepatotoxicity mediated

by disturbed calcium homeostasis and consequent calcium regulatory activity [21], suggesting calcium channel blockade as a hepatoprotective strategy by amlodipine against MSG related liver damage. Thus, calcium influx leading to the progression of hepatotoxicity may be a plausible mechanism of MSG related hepatotoxicity. This may provide insight to MSG related hepatotoxic mechanisms, warranting followup.

CONCLUSION

Amlodipine elicited beneficial response, and caused dose dependent diminution of monosodium glutamate related adversity, in the rats' liver histology and some seric biofunctional capacity. The apparent dose independent, albeit negligible, adversity on other high metabolic organs of the rats' following possible synergistic interaction of MSG with notably overdose of amlodipine may be significant on prolonged or higher dose use. This provokes followup studies which are recommended.

REFERENCES

1. Mukete, B.N., Cassidy, M., Ferdinand, K.C. & LeJemtel, T.H. (2015). Long-term anti-hypertensive therapy and stroke prevention: A meta-analysis. *American Journal of Cardiovascular Drugs,*15(4), 243-57.
2. Fares, H., Dinicolantonio, J.J., O'Keefe, J.H. & Lavie, C.J. (2016). Amlodipine in hypertension: a first-line agent with efficacy for improving blood pressure and patient outcomes. *Open Heart,* 3(2), e000473.
3. Derosa, G. & Maffioli, P. (2011). Drug safety evaluation of amlodipine. *Expert Opinion on Drug Safety,* 10(5), 795-804.
4. Mustafa, S.K., Agata, B., Michał, S.S.& Selma, Ş. (2011). Amlodipine.*FABAD Journal of Pharmaceutical Science,* 36, 207-222.
5. Mustafa, Z., Ashraf, S., Tauheed, S.F. & Ali, S. (2017). Monosodium glutamate, commercial production, positive and negative effects on human body and remedies – a review. *International Journal of Scientific Research in Science and Technology,* 3, 425-435.
6. Niaz, K., Zaplatic, E. & Spoor, J. (2018). Extensive use of monosodium glutamate: A threat to public health? *Experimental and Clinical Science Journal*,17, 273-278.

7. Singh, K. & Ahluwali, P. (2012). Effect of monosodium glutamate on lipid peroxidation and certain antioxidant enzyme in cardiac tissue of alcoholic adult male mice. *Journal of Cardiovascular Disease Research,* 3(1), 12-18.
8. Ogbuagu, O.E., Nweke, I.N. & Unekwe, P.C. (2015). Organ toxicity of monosodium glutamate in adult albino Wistar rats. *Journal of Medical Investigation and Practice,* 10(1), 1-7
9. Farah, D.P., Hazilawati, H., Rosly, S.M., Shanmugavelu, S. & Noordin, M.M. (2011). Expression of circulating CD146 associated with endovascular dysfunction in adenine-induced chronic renal failure in rats using an EvaGreen real-time RT-PCR assay. *Pertanika Journal of Tropical Agricultural Science,*34(2), 381-391
10. Obidike, I.J. & Egbuonu, A.C.C. (2019). Effects of ethanol extract of cocoa (*Theobroma cacao*) pod on normal and monosodium glutamate-intoxicated rats' hepatic histo-morphology, serum bio-functional parameters and serum antioxidant activities. *International Journal of Recent Research and Applied Studies,*11(1), 1-13
11. Egbuonu, A.C.C., Ogbu, A.E. & Ezeanyika, L.U.S. (2015). Sub- chronic oral esculetin (6, 7-dihydroxy-coumarin) exposure in male Wistar rats: Effect on some serum functions and organ histology. *Asian Journal of Biochemistry,* 10(2), 67-77.
12. National Research Council, NRC, USA (2011). Guide for the Care and Use of Laboratory Animals. 8th Edition, Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington DC, USA: National Research Council, National Academies Press.
13. Mohammed, N.E.M. & Abosaif, A.A. (2016). Effect of amlodipine, lisinopril and allopurinol on acetaminophen-induced hepatoxicity in rats. *Saudi Pharmaceutical Journal,* 24(6), 635-644.
14. Obi, E. & Egbuonu, A.C.C. (2019). Changes in the liver histomorphology, catalase and glutathione peroxidase activity in the serum and liver homogenate of normal and monosodium glutamate-intoxicated rats co-treated with artemether-lumefantrine. *International Journal of Molecular Biology Open Access,*4(2), 67-73.
15. Mariyamma, T., Sujatha, K.S. & George, S. (2009). Protective effect of *Piper longum* Linn on monosodium glutamate-induced oxidative stress in rats. *Indian Journal of Experimental Biology,* 47(3), 186-192.

16. Egbuonu, A.C.C. & Ejike, G.E. (2017). Effect of pulverized *Mangifera indica* (mango) seed kernel on monosodium glutamate-intoxicated rats' serum antioxidant capacity, brain function and histology. *EC Pharmacology and Toxicology*, 4(6), 228-243.
17. Reitman, S. & Frankel, S. (1957). A colorimetric method for the determination of serum glutamate oxaloacetate and pyruvate transaminase. *American Journal of Clinical Pathology*, 28, 56-63.
18. Englehardt, V.A. (1970). Measurement of alkaline phosphatase. *Aerztl Labour*,16, 42.
19. Eesha, B.R., Mohanbabu Amberkar, V., Meena Kumari, K., Sarath, B., Vijay, M., Lalit, M. & Rajput, R. (2011). Hepatoprotective activity of *Terminalia paniculata* against paracetamol induced hepatocellular damage in Wistar albino rats. *Asian Pacific Journal of Tropical Medicine*, 4, 466-469.
20. Liu, H.M., Yan, L.H., Luo, Z., Sun, X.M., Cui, R.B., Li, X.H. & Yan, M. (2013). Role of store-operated Ca^{2+} channels in ethanol-induced intracellular Ca^{2+} increase inHepG2 cells. *Zhonghua Gan Zang. Bing ZaZhi (Chinese Journal of Hepatology)*, 21,949-954.
21. Kheradpezhouh, E., Ma, L., Morphett, A., Barritt, G.J. & Rychkov, G.Y. (2014). TRPM2channels mediate acetaminophen-induced liver damage. *Proceedings of National Academy of Science. USA*, 111, 3176-3181.
22. Aniagu, S.O., Nwinyi, F.C., Olanubi, B., Akumka, D.D., Ajoku, G.A., Izebe, K.S., Agala, P., Agbani, E.O., Enwerem, N.M., Iheagwara, C.&Gamaniel, K.S. *etal.*(2004).Is Berlina grandi flora (Leguminosae) toxic in rats? *Phytomedicine,*11, 352-360.
23. Egbuonu, A.C.C., Opara, C.I., Akachukwu, D. & Onyedikachi, U.B. (2018). Effect of ethanolic extract of avocado pear (*Persea americana*) seed on normal and monosodiumglutamate-compromised rats' hepatic histo-morphology and serum bio-functional parameters. *Research Journal of Environmental Science,*12, 53-62
24. Alshubaily, F.A. & Almotairi, E.S. (2020). The hepatoprotective effect of *Moringa oleifera* leaf aqueous extract against cadmium-induced toxicity on malerats. *Journal of Biochemical Technology*, 11(1), 101-107.
25. Abdel, S.O.M.E., Hassan, N.S., Baiuomy, A.R. & Karam, S.H. (2007). The effect of amlodipine, diltiazem and enalapril on hepatic injury caused in rats by the administration of CCl4. *Journal of Pharmacology and Toxicology*, 2, 610-620.