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Full Length Research Paper

Hepatoprotective role of Garcinia kola (Heckel) nut extract on methamphetamine: Induced neurotoxicity in mice

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The hepatoprotective effect of aqueous extract of Garcinia kola (AEGK) was studied in 60 mice of mixed sexes. The animals were divided into 6 groups of 10 mice each. Group I received normal saline, groups and III got 100 and 200 mg/kg AEGK (orally), respectively. Group IV received 10 mg/kg methamphetamine (MAM) (s.c.) only. Groups V and VI got 100 and 200 mg/kg of AEGK respectively, before 10 mg/kg methamphetamine which was used to induce neurotoxicity. The serum levels of AST, ALT, ALP, total bilirubin and its conjugated metabolite were used to assess liver damage. Fifty percent of the animals in group IV died. 30% died in group V and none in group VI after 10 - 30 min interval of MAM administration. The serum levels of some of the marker enzymes and bilirubin were decreased significantly in groups VI at 200 mg/kg of AEGK ($P < 0.05$). The Blood glucose level increased transiently in the MAM treated groups. There was a slight rise in serum WBC after an initial fall at 100 mg/kg AEGK. The results suggest a possible hepatoprotective potential of AEGK. This may justify their local use in the management of some hepatic dysfunction and stress-related conditions.

Key words: Garcinia kola extract, methamphetamine, neuroprotection, mice.

INTRODUCTION

Garcinia kola (Gultiferae) is an evergreen plant found in the equatorial forest of Sub-Saharan Africa. The plant grows wild and is also domesticated because of the wide medicinal values of the extract of its various components in folk medicine. The G. kola nut (GKN) is culturally and socially significant in some parts of South Eastern Nigeria (West Africa) where the yellow nut is served for traditional hospitality in private, social and cultural functions. It is commonly called bitter kola because of the bitter taste of the nut.

As a result of its wide spread consumption, especially among the Ibos of South Eastern Nigeria, some studies have been carried out on the extract of various components of the plant. The phytochemical studies shows that GKN contains phenolic compounds, steroids, xanthines, benzophenones (Etkin, 1981; Iwu, 1982), tannins, guttiferins and saponins (Etkin, 1981). Animal and human studies revealed that the extracts of GKN exhibit aphrodisiac effects on male subjects (Iwu, 1993; Orie and Okon, 1993) for which reason they are sometimes called “male kola in some parts of Nigeria. It is reported to suppress ovulation and delay fertility in female subjects (Iwu and Igboko, 1982). GKN extracts have been shown to possess antipyretic, anti-inflammatory, analgesic (Olaleye et al., 2000), antiviral, hepatoprotective (Iwu, 1985; Akintonwa and Essien, 1990), CNS stimulant (Orie and Okon 1993), antidepressant, antioxidant (Adaramoye et al., 2005), antidiabetic (Braide et al., 2003; Akpanta et al., 2005) activities. The registration of GKN formulation as a hop substitute in the brewing of beer and wine was under consideration by the Food and Drug Administration (FDA, 1999).

The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB) and conjugated bilirubin (CB) were used as hepatic markers (Mercer and Tainuno, 1982; Muragesh et al., 2005). The levels of these non - functional enzymes correlate hepatic damage. However, ALT is more specific for hepacellular injury than the

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other enzymes (Alagbonna and Onyeyilli, 2003).

Methamphetamine (MAM) is the d - isomer of the parent drug, amphetamine. It is a potent sympathomimetic agent with greater pressor effect when compared with the parent compound on equimolar basis, especially on the CNS. The induction of neurotoxicity is achieved at higher doses of about 10 - 40 mg/kg s.c. (Innis and Nickerson, 1975; Bloom, 1985; Imam and Ali, 2000). MAM is useful in the induction of experimental neurotoxicity in animal models, especially in mice (Imam et al., 2001). The ability of GKN extract to attenuate the raised serum levels of the liver marker enzymes is an indication of its hepatoprotective effect.

However, there is paucity of data on the neurological effect of AEGK. This study evaluates the possible neuroprotective effects of the aqueous extract of Garcinia kola (AEGK) using liver and kidney functions and some blood parameters as indices. The study aims at providing some information on the possible neuroprotective role of AEGK. It also aims at providing some scientific basis for the use of the GKN in folk medicine to treat hepatitis and diabetes - related conditions.

MATERIALS AND METHODS

Plants materials

The nuts of Garcinia kola were purchased from Owerri Municipal Markets, Owerri, Imo State, Nigeria. It was authenticated by a plant taxonomist, Dr. C. Okeke, Department of Plant Science and Biotechnology, Imo State University, Owerri. A voucher specimen is deposited in the University Herbarium.

Extraction

The nuts were dehusked and chopped into bits, sun-dried to constant weight and pulverized using a mechanical grinder (Thomas Contact Mill, Pye Unicam, Cambridge, England). 200 g of the power was obtained. This was soaked in distilled water in a soxhlet apparatus and extracted after 24 h. The solvent was evaporated using oven (Acumex, India) (50°C) and rotatory evaporator (Laborato 400, China). The dry residue (1.24 g) was constituted in normal saline (NS) (100 mg/ml) and used for the experiment.

Animals

The mice (BALD strain), weighing 20 - 35 g of mixed sexes were obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt. They were housed in stainless steel cages and allowed to acclimatize for two weeks in the Animal House of the College of Medicine, Imo State University, Owerri, under 12 h light/ dark cycle before commencement of the experiment. The animals had access to standard feed (Guinea Feeds, Ltd, Ewu, Edo State, Nigeria) and water *ad libitium*.

Drugs and chemicals

The methamphetamine (Desoxgn) used was the product of Glaxosmith Kline, England.

Experimental design

The Animals were divided into 6 groups of 10 mice each. Group 1 received normal saline plus feed and water for two weeks and served as the negative control. Groups ii and iii received 100 and 200 mg/kg of AEGK, respectively for 2 weeks only. Group iv received normal saline for 6 weeks and also served as negative control, while Groups v and vi received 100 and 200 mg/kg AEGK for 6 weeks. All the 6 weeks segment groups additionally received 10 mg/kg methamphetamine (s.c.) on the 6th week. The AEGK were administered to the mice by oral intubation between 9 - 10:00 am daily. They also had feed and water *ad libitium*.

Biochemical studies

The animals were starved 24 h prior to the collection of blood samples. The samples were drawn by cardiac puncture into marked sample bottles and allowed to clot for 45 min at room temperature. The serum was obtained by centrifugation at 2500 rpm at 30°C for 5 min using Wisperfuge centrifuge (model 1384, Samson, Holland).

The serum was separated using Pasteur pipette into sterile serum sample tubes from where they were drawn for the biochemical assay. The method of Reithman and Frankel (1957) was adopted for the ALT and AST assay. ALP was estimated using the method of King and King (1954) as adapted by Cheesbrough (2000). Bilirubin and glucose were determined using the methods of Malloy et al. (1937) as modified by Tietz (1996). Serum sodium and potassium were estimated using reagent titrimetric method. Serum chloride was determined by the method of Schales and Schales (1941).

Histological studies

At the end of the experiment, the mice were sacrificed under chloroform anaesthesia .The livers were dissected out and immediately fixed in 10% formal saline. Using a standard tissue processor, the tissues were then dehydrated in ascending grades of alcohol: 70, 95% and absolute alcohol in 2 changes each. After which clearing was done with xylene/absolute alcohol [50:50 v/v ratio]. This was followed by infiltration in molten paraffin wax at 60°C in 2 changes. They were further processed for staining with haematoxyline and eosin (H&A) as described by John et al. (1990). Photomicrographs of the slides were taken for histological examination.

Statistics

The results were analyzed using Duncan multiple range test. Data were expressed as mean ± standard deviations. Differences between means were considered at 95% confidence limit and probability level of 0.05 was taken as significant.

RESULTS

The results of the biochemical parameters are presented in Table 1. In the absence of the neurotoxin (MAM), the serum levels of ALT, ALP and TB were significantly reduced ($p < 0.05$) relative to group I control. Although the AST level decreased, it was non - significant ($p > 0.05$). The CB concentration was slightly raised against the control. This reduction did not follow a dose dependent pattern.

Table 1. The effect of AEGK on methamphetamine-induced hepatotoxicity in mice (mean ± SD) (n = 6 - 10).

Group	TB (mg/l)	CB (mg/l)	ALT mg/l	AST (mg/l)	ALP, (mg/l)	Na^+ (meq/l)	K^+ (meq/l)	Cl^- (meq/l)	Glucose (mg/l)	WBC Counts/mm^2
I (Control)	2.7± 0.8	0.2 ± 0.01	4.75 ± 0.3	5.67 ± 0.22	48.3 ± 0.5	120.7± 1.1	5.2 ± 0.7	121.3 ± 1.25	84.7 ± 1.64	2433.3 ± 115.4
II (100 mg/kg)	2.0 ± 0.26	0.38 ± 0.25	3.5 ± 0.13	5.17 ± 0.24	48.5 ± 0.79	121.7 ± 1.0	5.6 ± 0.4	107.3 ± 11.0	83.3 ± 0.53	2366.7 ± 59.7
III (200 mg/kg)	2.0 ± 0.57	0.4* ± 0.01	3.5 ± 0.5	4.3 ± 0.10	43.3 ± 0.76	121.0 ± 1.0	5.1 ± 0.4	104.7 ± 4.17	69.3 ± 1.53	2500 ± 0.00
IV Control	4.9 ± 0.7*	1.0 ± 0.015*	7.25 ± 0.84*	5.5 ± 0.16	66.0 ± 0.17*	121.7 ± 2.08	4.78 ± 0.06	122.4 ± 11.0	98.0 ± 1.14	1366.7 ± 115.4*
V (100 mg/kg)	4.16± 0.28	0.67 ± 0.56	7.10 ± 0.85	5.43 ± 0.54	48.27± 0.69	122.4 ± 2.5	5.03 ± 0.59	107.0 ± 0.57	106.8 ± 11.0	1500 ± 200
VI (200 mg/kg)	3.97± 0.59	0.62 ± 0.09	3.89 ± 0.84*	4.73 ± 0.28	44.33 ± 0.51*	121.8 ± 1.75	59.0 ± 0.40	107.0 ± 0.57	106.8 ± 11.0	1750 ±150

Key: TB = Total bilirubin, CB = conjugated bilirubin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, WBC = white blood cell, * = significantly different from control ($p < 0.05$).

The serum electrolyte levels were relatively unchanged, except for chloride ion which fell transiently. The blood glucose was reduced significantly ($p < 0.05$) in the absence of MAM at 200 mg/kg but the WBC count was slightly raised at 200 mg/kg of AEGK, relative to group I animals. In the presence of the hepatotoxin, the marker enzymes and metabolites were elevated significantly ($p < 0.05$) (Group I and IV) suggesting the induction of hepato-neurotoxicity, except for AST whose level remained relatively constant. The serum electrolytes were relatively unchanged. There was also significant decrease in the plasma WBC and transient rise in blood glucose when compared with the control. When 200 mg/kg AEGK was challenged with 10 mg/kg (s.c.) MAM, the serum AST, AST, ALT, TB and CB decreased relative to their respective controls in group IV. The decease was significant for ALT and ALP ($p < 0.05$). However the decrease at 100 mg/kg AEGK was transient and non - significant ($p > 0.05$). The histological studies (Figures 1 - 4) support this observation. The sodium level was not altered at both doses in the presence of the hepatotoxin, but the K+ concentration increased slightly ($p > 0.05$). Although there was a fall in the serum level of chloride ion, it was not significant ($p > 0.05$) at the dose levels of AEGK tested.

Clinical observation

About 10 - 30 min after the administration of the neurotoxin, 50% of the animals in control Group IV died. 30% of those in Group V also died. No death was recorded for the animals in Group VI; though the animals were slightly debilitated, but finally recovered fully. The remaining animals in the neurotoxin treated groups subsequently recovered but the recovery in Group VI was faster and sustained.

Histological observation

Figure 1 show a section of the liver of the mice in Group 1 used as negative controls, having received only normal saline. Normal hepatocytes are seen radiating from the central vein.

However, in Figure 2 which shows a liver section from Group 4 mice (positive controls); there was gross distortion of the cell cords. There are vacoulations in the cytoplasm of the cell. This is evidence of cellular damage.

Figure 3 is a liver section from Group 5 mice which received 100 mg/kg of AEGK and methamphetamine. There was minimal distortion of the cell cords.

Figure 4 shows the section from Group 6 mice which received 200 mg/kg of AEGK and methamphetamine. The cell cords are fairly well preserved like those of the Group 1 mice. This is evidence of protection.

DISCUSSION

Methamphetamine (MAM) is a potent N-methyl d-analog of phenylethylamine. It belongs to the class of sympathomimetic CNS stimulants but acts as a neurotoxin at higher doses (10 - 40 mg/kg s.c.) with more profound action on the CNS, relative to the periphery (Shockley, 1991; Hoffman et al., 1996). Like other amphetamines, it blocks neuronal re-uptake of the catecholamine neurotransmitters, especially dopamine, at the nerve endings of sympathetic neuron (Hoffmann et al., 1996; Imam et al., 2001). This event

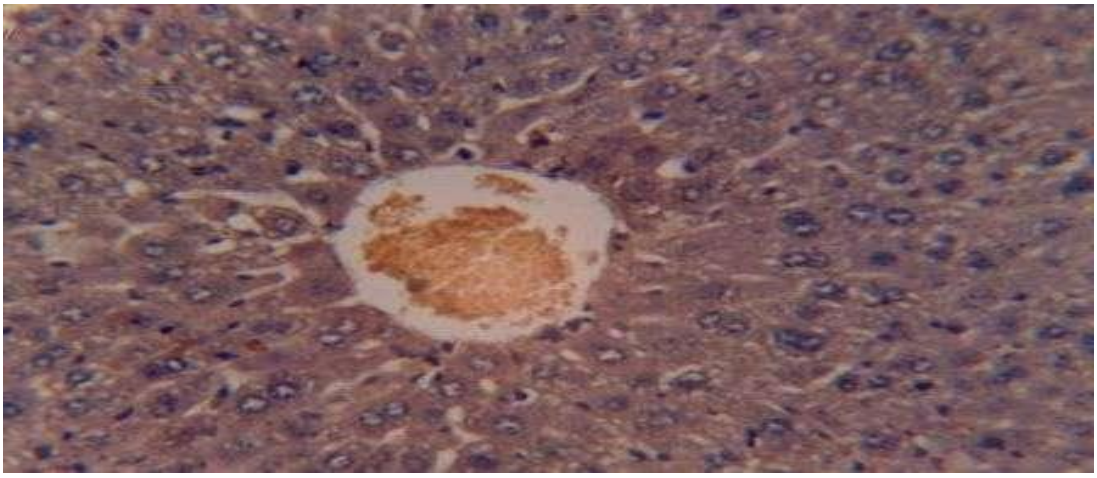

Figure 1. Section from the liver of mice which received normal saline and no methamphetamine. It shows normal hepatocytes and cell cords.

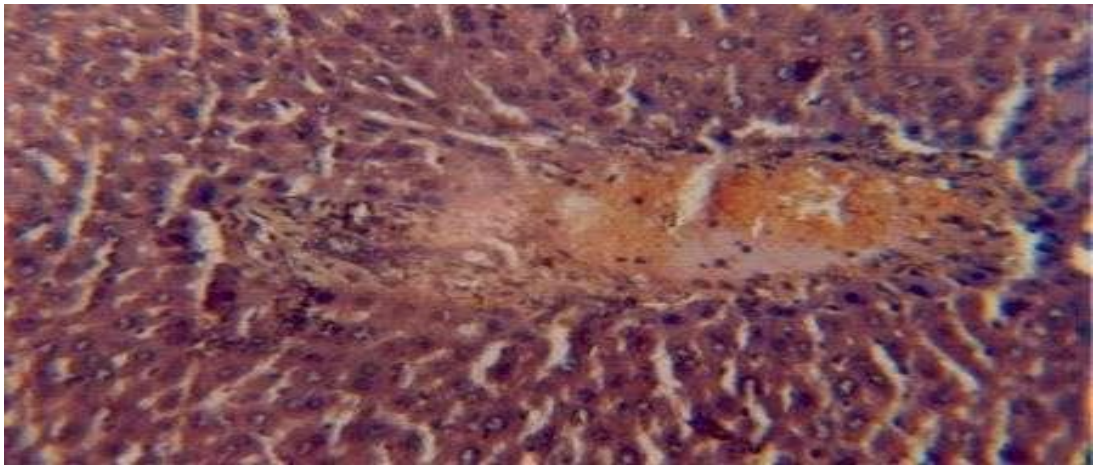

Figure 2. Section of the liver from the mice which received normal saline and methamphetamine (Positive control). It shows much vacuolation and distortion of the hepatic cell cords.

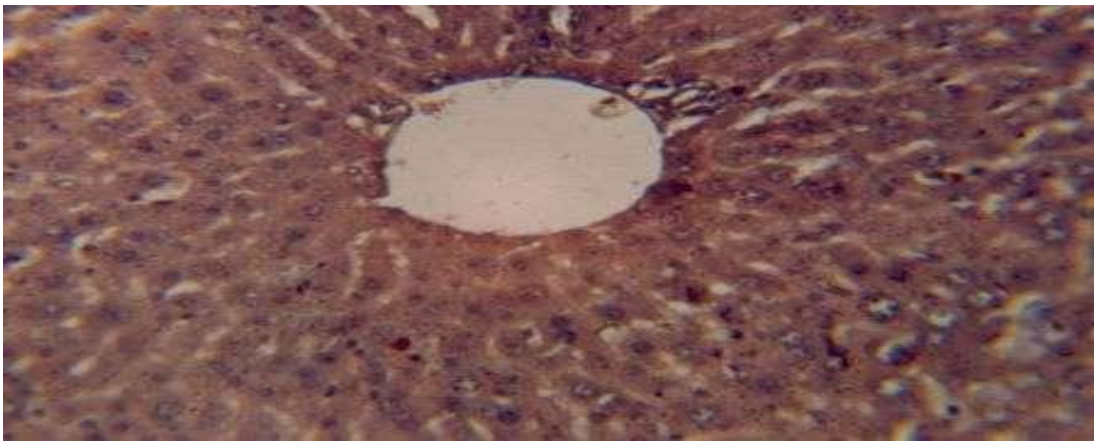

Figure 3. Section of liver from mice which received 100 mg/kg and methamphetamine. It shows slight vacuolation with minimal distortion of the cell cords.

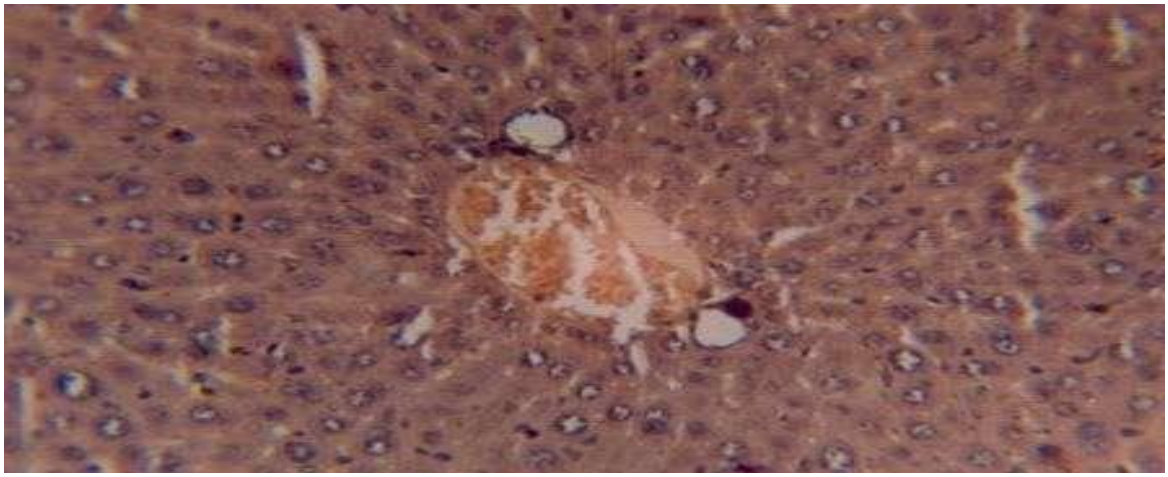

Figure 4. Section of liver from mice which received 200 mg/kg AEGK and the methamphetamine. It shows no vacuolation and has reasonable presentation of the cell cords. Looks almost like the tissue from normal saline control which received no toxin.

exacerbates the pharmacological actions of the affected neurotransmitters, exposing them to extensive metabolic degradation. Under this condition, there may be hypertensive crisis, cerebral hemorrhage, increased cardiac output and increased renal blood flow. Subsequently, the rate of enzymic degradation exceedsthe rate of neuronal biosynthesis, leading to depletion of the neurotransmitter, neuronal failure and possibly death.

Imam and Ali (2000) and Imam et al. (2001) have demonstrated that MAM neurotoxicity may be associated with the neuronal generation of free radicals such as peroxynitrites, super oxides and hydroxyl from dopamine metabolism, leading to oxidative damage and possibly death. They also showed that antioxidants such as selenium or glutathione could offer neuro-protection in these cases. Kampen et al. (2003) made similar observations for methylphenyltetrahydropyridine (MPTP), a potent neurotoxin.

In the assessment of liver damage, the serum concentrations of the hepatic marker enzymes, AST, ALT and ALP, are important as well as serum total bilirubin and its metabolites. The hepatocellular marker enzymes are non-functional, with negligible systemic concentrations in normal situations, but leak into general circulation when there is necrosis or damage to the hepatic cells; neuronal or skeleton muscle cells; consequently their serum concentrations rise above normal values (Murray et al., 2000). However, ALT is more specific for the liver tissue because the other enzymes and metabolites could be released as a result of damage to other organs.

Damage to hepatocytes and blood cells exacerbate therelease and metabolism of haem and its metabolites including bilirubin and its excretory products. Thus, the serum level of bilirubin and its conjugate metabolites would be expected to rise with a corresponding decrease in blood cell count due to mild haemolysis. This observation was made by Uko et al. (2001) in rats and agrees with the outcome of the present study (Table 1).

The renal functional status could be assessed using the serum levels of electrolytes and proteins. The sodium and chloride levels are complementary and may be altered in nephrotoxicity (Murtey et al., 2000; Nwanjo et al., 2006). The plasma potassium concentration inversely relates to the sodium concentration.

The liver is innervated by the x^{th} cranial nerve and the ciliac ganglia and is therefore subject to neuronal control. The sympathetic stimulation of the adrenal medulla causes the release of adrenaline into systemic circulation. As a hormone, adrenatine regulates blood glucose via the glycogen metabolic pathway in the liver (Glew et al., 1989). In neuronal damage involving the liver, the glycogen metabolic pathway would be affected adversely. A complementary immuno-response to cellular damage may lead to transient compensatory increase in blood glucose concentration.

In this study, 10 mg/kg (s.c.) MAM was used to induce hepato-neurotoxicity in the mice (Table 1, Groups I and IV). This is shown by the significant ($p < 0.05$) elevation of serum levels of the liver marker enzymes and metabolites. The induction of nephrotoxicity could not be ascertained from the current observations due to the marginal alterations in the level of the electrolytes in the presence of the neurotoxin.

The study evaluated the hepato-protective role of AEGK in MAM - induced neurotoxicity in mice. During the course of treatment with MAM, it observed that 50% of the mice in Group IV died 10 - 30 min after the administration of the neurotoxin. Thirty percent of the mice in Group V also died, while none died in Group VI. The animals may have died either due to neuronal exhaustion or oxidative damage or both, while the animals in Group VI survived possibly due to a neuroprotective action

offered by the 200 mg/kg AEGK.

The 100 mg/kg AEGK caused a decline in the serum levels of the marker enzymes, especially in the cases of conjugated bilirubin and ALT, where the fall were significant ($p < 0.05$), indicating a possible hepatocellular protective effect as reported by Muragesh et al. (2005). A similar pattern of attenuation for ALT, TB, CB ALP and AST were seen in the presence of the neurotoxin at 200 mg/kg of the AEGK. The results tend to suggest a possible hepatoprotection by AEGK in mice. This line of reasoning is also supported by attenuated serum levels of TB and CB which followed similar trend as the hepatocellular marker-enzymes in the presence of the neurotoxin. Muragesh et al. (2005) noted that the return of the marker enzymes towards normal levels in the presence of the extract is an indication of membrane stabilization as well as repair of tissue damage via rapid regeneration of parenchyma cells. They also noted that decrease in ALP and TB serum levels may be indications of restoration of secretary mechanisms of the hepatocytes. The above observations were made in the current study (Table 1).

The mechanism by which the AEGK could cause hepatoprotection in the presence of the neurotoxin is not known. It may be related to the ability of the AEGK to cause membrane stabilization via reduced neuronal firing and arrest of free radical reaction. It is important to note that Adaramoye et al. (2005) had demonstrated the antioxidant potential of AEGK. The histopathological studies (Figures 1 - 4) showed that the AEGK reversed the damage of hepatocytes associated with the hepatotoxin especially at the dose of 200 mg/kg in 6th weeks.

It is not possible to draw similar conclusion for the renal function because there seemed to be no induction of nephrotoxicity. The serum levels of the electrolytes remained relatively unchanged in the absence and in the presence of the neurotoxin. Although there was a decrease in the chloride level in both phases, and at 100 and 200 mg/kg, this was marginal and may not make meaningful impact on the functional status of the kidney. Gidado and Umar (2001) obtained similar results on renal parameters for Dutura strumonium (Jimson weed) in rats.

The blood glucose was reduced in the absence and presence of MAM. This agrees with the work of Braide and Grill (1990) who reported the antidiabetic activity of the aqueous extract of GKN in rats. The blood sugar rose slightly in the presence of MAM relative to the control ($p > 0.05$). The rise at 100 mg/kg was greater than for 200 mg/kg AEGK; which tends to suggest an initial rise and subsequent decline in the sugar level as the dose of AEGK increased. The initial rise in blood glucose in the presence of the neurotoxin was expected because of the hyperglycemic action of the neuro-endocrine hormone (adrenaline) whose release is stimulated by the neurotoxin.

These outcomes lend more support to the possible hepato-protective effect of AEGK. Iwu (1985) and Akintonwa and Essien (1990) previously reported the hepatoprotective potentials of AEGK. Moreover, Iwu et al. (1990) and Adaramoye et al. (2005) reported the hypoglycemic effect of AEGK in rats. It is concluded that the AEGK could play a hepatoneuroprotective role in mice. This tends to justify the use of the aqueous extract in folk medicine as antidiabetic, antihepatitis and possible mood stabilizing agent.

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