The Effects of Bitter Kola Supplemented Diet on Hepatotoxicity of Mercury in Wistar Rats

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ABSTRACT: The effect of bitter kola on the hepatotoxicity following mercury poisoning (mercuric chloride solution of 10ppm) was investigated in rats for a duration of six weeks. Thirty (30) acclimatized Wistar rats were divided into five groups(n=6).Group I served as control and were fed on normal rat chow and clean water ad libitum, group II received normal chow and mercury contaminated water (10ppm), group III animals were given clean water and 5% w/w bitter kola supplemented rat chow, group IV rats received bitter kola supplemented rat chow and mercury contaminated water, group V animals were placed for the first week of the experiment on mercury contaminated water and normal rat chow before substituting with clean water and bitter kola supplemented rat chow. Two (2) animals from each group were sacrificed at the end of 2nd, 4th and 6th week and blood collected by cardiac puncture before the liver was harvested .Serum Alkaline phosphatase (ALP), Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and hepatic content of Mercury (Hg) were determined by standard laboratory methods. The results (Mean ± SEM) showed that G kola supplemented diet significantly lowered the hepatic mercury content as well as limited the hepatotoxic effects of the heavy metal indirectly assessed by measurement of the serum levels of alkaline phosphatase and the transaminases. @ JASEM

The element, mercury is considered a global pollutant (Fitzgerald et al, 1998; Jackson, 1997). Although exposure to mercury from therapeutics is now uncommon, occupational and environmental exposure continue to pose potentially serious treat to the living and unborn human populations (Klaasen,1999).Poisoning from environmental sources usually arise from contaminated drinking water as well as plant and animal sourced food products. The metal has been reported to be highly prone to bioaccumulate, leading to biomagnification along the food chain (EPA,1997). The absorption, distribution, metabolism, excretion and toxicodynamics of mercury have been reported to depend on the form and oxidation states (ATSDR, 1989). The forms of mercury important from a toxicological point of view are elemental (vapor), inorganic salts and organic salts of mercury. Ingestion of inorganic mercury salts such as mercuric chloride have been reported to cause mainly severe gastrointestinal irritation and renal failure (Klaasen,1999).The toxic effects of organic and elemental mercury have also been widely reported(Wikipedia,2009).Several epidemiologic studies had been conducted on the exposure of humans to mercury through fish and marine mammal consumption in different geographical areas (Bakir et al,1973).And about 25 food related compounds, including cysteine, fish protein, garlic, gluthation, r-Linolenic acid, phospholipids, and others have been reported to alter the metabolism of mercury.(Chapman and Chan,2000).Selenium ,Vitamin C and E as well as other essential minerals have been claimed to be effective against mercury toxicity in animal studies (Chowdhury and

Chandra,1987; Watanabe,2002) . On the contrary, mega doses of vitamin B_{12} with or without folic acid or folic acid alone have been reported to increase methyl mercury concentration in the liver of guinea pigs administered mercuric chloride subcutaneously(Zorn and Smith,1990).The liver is recognized as one of the soft tissues or organs that ingested mercury especially the organic mercurials distribute to leading to increased hepatic content (Klaasen 1999).Hepatic Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Alkaline Phosphatase have been reported to increase in domesticated rabbits that were treated with mercurous chloride salt (Anjum and Shakoori, 1994). In another study conducted on teleost fish exposed to LC_{50} (1.8 mg/L and sublethal (0.3mg/L) concentrations of mercuric chloride, it was also observed that GPT and GOT blood levels were elevated by 96hr for lethal exposure while for sublethal exposure resulted in elevated enzyme levels by the 15th and 30th day: Alkaline phosphatase level also increased following chronic exposure (Sastry and Sharma, 2000).

Garcinia kola (Syn Bitter kola) belongs to the Crusiaceace family. It is native to Asia, South Africa and Polynesia, it is also found in West and Central Africa.The genus has about 50 species of ever green trees and shrubs. The seed is chewed as masticatory, stimulant and for its bitter taste in traditional hospitality cultural and social ceremonies (Olaleye et al, 2000). The plant has also been reported to possess anti-ulcerogenic and gastric acid lowering effects (Okunji and Iwu,1991; Ibironke et al,1997); anti-fungal effects (Olojede et

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al,1993); antiviral, anti diabetic(Iwu,1986) and anti hepatotoxic activities (Akintowa and Essien,1990; Farombi et al,2000).

Given the possibility that some persons exposed to mercury may indulge in any of the different uses of bitter kola, there is need to evaluate the possible effects of this nutritional factor on the toxicokinetic and toxicodynamics of mercury. This study is an investigation into the effects of bitter kola supplemented diet on mercury induced hepatotoxicity in Wistar rats.

MATERIAL AND METHOD

Procurement and care of animals: 36 wistar rats were procured from the breeding colony of Edo state University Ekpoma and housed in plastic cages in the animal facility of Maddona University Okija. They were acclimatized to their new environment for 2 weeks during which time they were fed with clean drinking water and rat chow ad libitum.

Preparation of the mercury poisoned water: The molecular weight of Mercuric chloride ($HgCl_2$) was divided by the molecular weight of Mercury (271.5/201) to obtain 1.35g as the weight of 1 part of Mercury (Hg) in $HgCl_2$.1.35g of mercury chloride was dissolved in 1 litre of water to give a concentration of 1000parts per million.200mls of this solution was mixed with 20 litres of distilled water to obtain 20 litres of water containing mercury at a concentration of 10 parts per million.

Preparation of the bitter kola supplemented feeds: The bitter kola seeds were oven-dried to a constant weight and then pauverized with a blender (NIPL). The powder was initially made into a thick paste by adding some water to it to facilitate its adherence to the rat chow, increasing the likelihood of being ingested. The rat feed was mixed with the powdered bitter kola in the ration of 1:20.

Animal experiment: The animals were divided into 5 groups (n=5).
Group I rats were fed normal rat chow and clean water ad libitum for six weeks

Group II rats were treated with normal rat chow and mercury contaminated water (10ppm) ad libitum for six weeks
Group III rats were fed bitter kola supplemented rat chow and clean water ad libitum for six weeks
Group IV rats were fed bitter kola supplemented rat chow and mercury contaminated water (10ppm) ad libitum for six weeks
Group V rats received normal rat chow and mercury contaminated water (10ppm) in the first week before changing over to bitter kola supplemented feed and clean water ad libitum for the rest 5 weeks.

Two rats were randomly selected from each group at the end of the 2nd, 4th and 6th week and sacrificed by decapitation. Blood samples (5mls) were collected from the juglar veins into labeled EDTA bottles after which the animals were dissected to harvest their livers which were also kept in EDTA bottles.

Analyses of specimen: Serum alkaline phosphatase was determined using CE Assay kits (ID Labs Biotechnology Inc,2009). Serum Glutamate Oxaloacetate Transaminase was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenylhydrazine while serum pyruvate transaminase was measured by quantifying the concentration of pyruvate hydrazine formed along with the 2,4-dinitrophenyl hydrazine (Reitman and Frankel,1957; Schmidt and Schmidt, 1963).

The Mercury content of the liver homogenate was determined by atomic absorption mass spectrophotometry (Chun et al, 1997)

Statistical analysis: The results were expressed as Mean ± SEM which were fed into the data sheet of Microsoft Excel 2003 software with which statistical analysis using the Student's t-Test was carried out. Within any given sacrifice date results in one group was compared with that of other groups: Group I vs II, I vs III, I vs IV,I vs V: Groups II vs III, II vs IV, II vs V: Group III vs IV, III vs V: Group IV vs V.P-values less than 0.05 were considered to be statistically significant

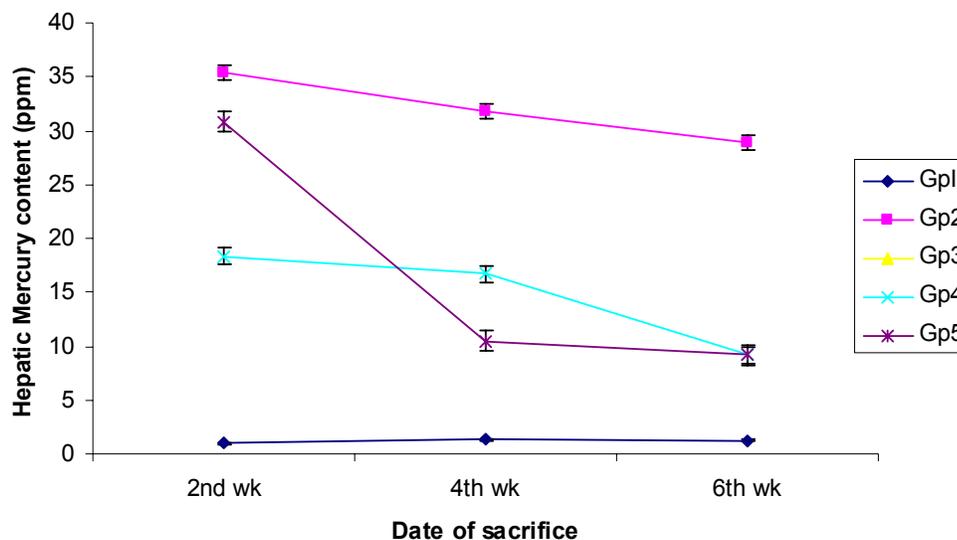* Corresponding author: *2Ejebe D.E.

RESULTS AND DISCUSSION

Figure 1: Effects of Bitter Kola Supplemented Diet on Hepatic Mercury Content of Wistar Rats Exposed to Mercury Contaminated Water

n = 5,Mean ±SEM , wk (week); Group(Gp) I-normal rat chow + clean water;-Gp2 narmal rat chow + Mercury contaminated water:Gp3- bitter kola supplemented chow + clean drinking water: Gp4- bitter kola supplemented chow + mercury contaminated water: Gp5- normal rat chow + Mercury contaminated water (1 wk) followed by bitter kola supplemented diet + clean drinking water for 5weeks.

Effect on hepatic mercury content: Group I rats that had normal rat chow and clean water only were observed to have had very low hepatic content of mercury inspite of no contamination of their drinking water during the experiment(Figure 1). This may be suggestive of some form of previous exposure of the colony from which they had been procured to the heavy metal. Mercury had been previously reported to contaminate seeds of grain preserved by it and procurement of animal feeds prepared from such grains grown from such seeds have led to mercury poisoning with a gradual accumulation of the metal in organs (Bakir et al,1973; WHO 1976).

Group II rats that had normal rat chow as well as mercury contaminated water had the highest mean hepatic level of mercury;35,33 and 28 parts per million by the 2nd,4th and 6th week respectively. The marked elevation in the hepatic mercury content in these rats compared to those of group I strongly suggest that the ingestion of mercury in their drinking water resulted in further accumulation of the heavy metal in the liver of these rats. This increments were all statistically significantly different from those of other groups (P<0.05).

Group III rats that had bitter kola supplemented rat chow and clean drinking water had virtually no trace on the graph. This means that hepatic content of mercury was essentially negligible. This observation could suggest that the bitter kola in the diet may have enhanced the removal of Hg from the liver either by biliary or renal excretion. The

likelihood of this is increased by the observation in group I rats that had low levels of the heavy metal by the 2nd, 4th and 6th weeks even though they had not been exposed experimentally to mercury. This observation in Group III, rats point to the possible existence of an antidotal action of bitter kola in mercury poisoning.

In group IV rats, although the hepatic level of mercury were much higher than those of group I rats, the peak levels noticed in the animals sacrificed by the 2nd,4th and 6th weeks were lower than those of group II rats who received normal rat chow and mercury contaminated water. Again suggesting that bitter kola could reduce the potential to accumulate the metal by the liver possibly by an enhancement of its elimination from the organ.

The observation made in group V rats which had normal rat chow and mercury contaminated water for the first week before they were switched over to bitter kola supplemented diet and clean water for the remaining 5weeks revealed that the mean hepatic concentration of mercury in the 2nd week rose to near that of group II rats. However by the 4th and 6th week the rats in this group showed marked decrease in their hepatic mercury content compared to corresponding rats in group II (Figure 1). This further supports the possibility that bitter kola can attenuate the accumulation of mercury in the liver in poison situations. And since the administration of the bitter kola was after the exposure to the metal the mechanism of this

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antidotal action is not likely to be through interference with absorption from the gastrointestinal tract. Analysis of the data for hepatic mercury content revealed significant differences except for group IV vs V in the 6th week (P> 0.05). Secondary plant metabolites in Garcinia kola   present in all varieties of the plant have been reported to be chelators of divalent metal, protein binders as well as anti-nutrients (Mahanato et al, 1982; Chaudel and Rastogi1980; Duncan et al 2000). Oxalates and tanins contained in G kola have also been reported to have formed chelates with divalent metals at certain concentrations (Aremu, 1989; Abara et al 2000; Mahanato et al 1982). The observation in this study that G. kola supplemented rat diet significantly lowered the hepatic mercury content may be related to the chelating activities of some of these phytochemical constituents. Specific antidotal therapies for most heavy metal poisonings are

known to be largely based on the use of chelating agents (Klaasen 1999). Several studies have shown that approved therapeutic heavy metal chelators produce their useful antidotal actions by mobilization and enhancing the excretion of metallic cations (Grazino et al, 1992; Cantilena and Klaassen, 1982)

Effect on serum level of hepatic enzymes: Serum levels of hepatic enzymes such as Alkaline Phosphatase, Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) have been used as surrogate markers for hepatic injury (Liz,2003, Svetlovet et al,2006). Elevated alkaline phosphatase have been strongly correlated with some form of damage to the hepatobiliary system while injury to the hepatic parenchymal tissue has been correlated with elevation in the GPT and GOT levels in the serum (Wendy and Brickwell, 2007).
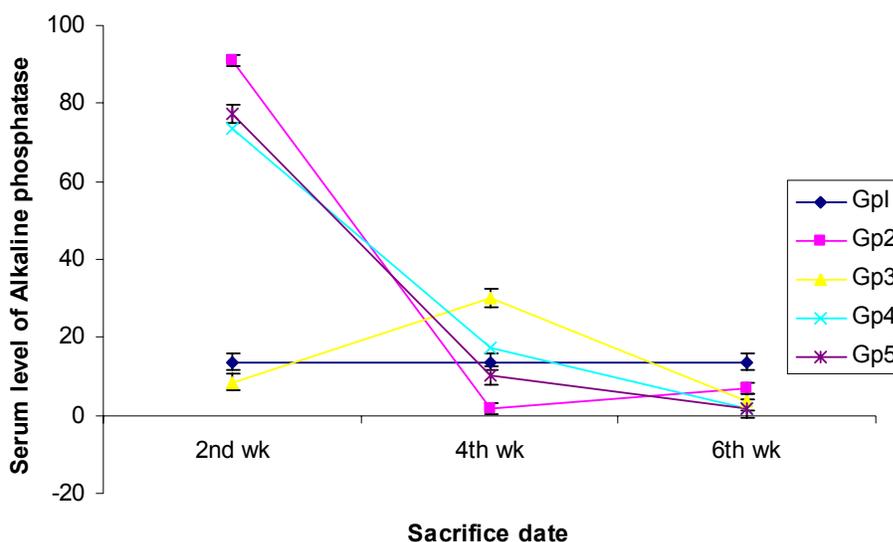

Figure 2: Effects of Bitter Kola Supplemented Diet on Serum Level of Alkaline Phosphatase (IU/L)in Wistar Rats Exposed to Mercury Contaminated Water
 n=5, Mean ± SEM ; wk (Week);Gp I-normal rat chow + clean water;Gp2- narmal rat chow + Mercury contaminated water: Gp3- bitter kola supplemented chow + clean drinking water: Gp4- bitter kola supplemented chow + mercury contaminated water: Gp5- normal rat chow + Mercury contaminated water (1 wk) followed by bitter kola supplemented diet + clean drinking water for 5weeks.

Dietary exposures to mercury and bitter kola individually led to an acute rise in the blood level of alkaline phosphatase (Figure 2). However, while the Hg effect peaked by the 2nd week bitter kola induced elevated ALP had a slower onset with delayed peak by the 4th week. However the blood levels of this enzyme in both group I and III rats returned essentially to normal by the 6th week. This suggests that both of these substances may have some injurious effect on the hepatobiliary cells from the onset of administration that is not sustained chronically. The lowering of the peak

level of this enzyme attained in group IV rats co-administered Hg contaminated water and bitter kola by the 2nd and 4th week compared to those observed for group II and III is suggestive of mutual antagonism of their individual noxious effect on the hepatobiliary cells of the liver. This possibility supports the existence of antidotal effect of bitter kola on mercury poisoning with respect to hepatotoxicity. This effect is also suggested by the rapid drop of the serum ALP in group V rats from the peak level attained by the 2nd week to near control blood levels by the 4th and 6th week.
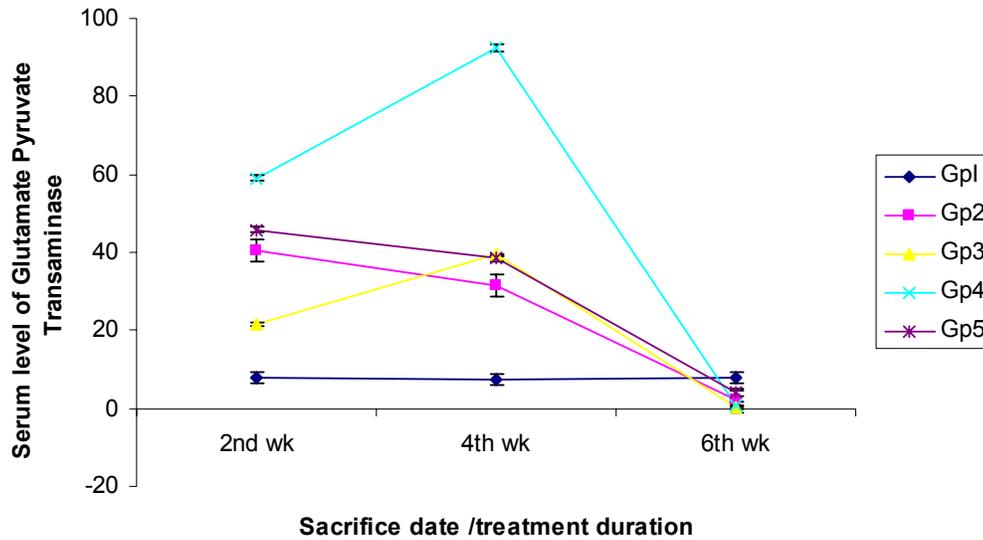
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Figure 3: Effects of Bitter Kola Supplemented Diet on Serum Level of Glutamate Pyruvate Transaminase (IU/L) in Wistar Rats Exposed to Mercury

n=5, Mean ± SEM ; wk (Week);Group I-normal rat chow + clean water;II- narmal rat chow + Mercury contaminated water: III- bitter kola supplemented chow + clean drinking water: IV- bitter kola supplemented chow + mercury contaminated water: V- normal rat chow + Mercury contaminated water (1 wk) followed by bitter kola supplemented diet + clean drinking water for 5weeks.

Although both group II and group III rats had higher mean serum levels of GPT by the 2nd and 4th weeks when compared with that of Group I rats, these had decreased towards control level by the 6th week (Figure 3). The observation that Group IV rats had the highest serum levels of GPT by the 2nd and 4th week could suggest that the coadministration of mercury contaminated water and bitter kola resulted in some form of combined toxicity on the parenchymal cells of the liver an effect that also did not persist into the 6th week. All the statistically analysed data were significant except for group I vs III by the 6th week (P>0.05)
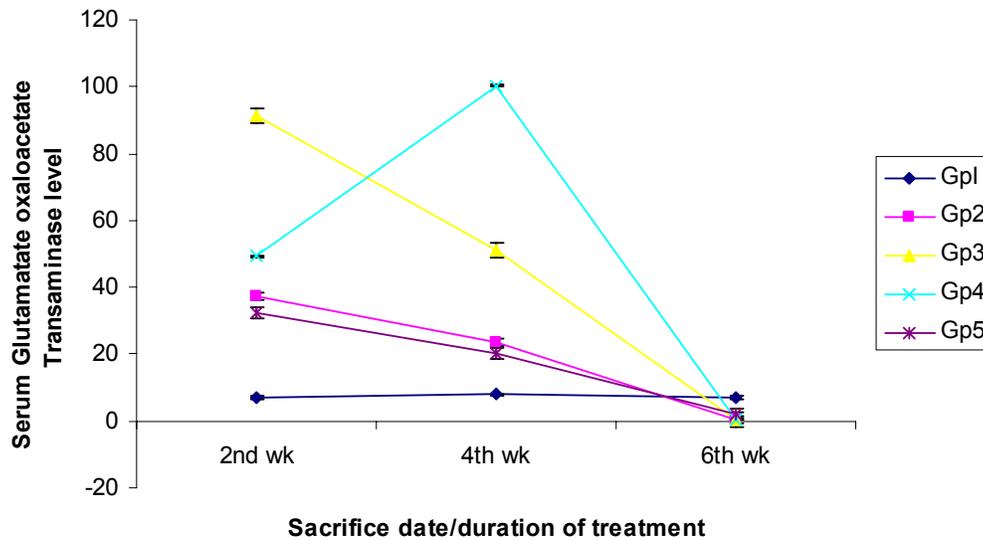

Figure 4: Effects of Bitter Kola Supplemented Diet on Serum Level of Glutamate Oxaloacetate Transaminase (IU/L) in Wistar Rats Exposed to Mercury

n=5, Mean ± SEM ; wk (Week);Group I-normal rat chow + clean water;II- narmal rat chow + Mercury contaminated water: III- bitter kola supplemented chow + clean drinking water: IV- bitter kola supplemented chow + mercury contaminated water: V- normal rat chow + Mercury contaminated water (1 wk) followed by bitter kola supplemented diet + clean drinking water for 5weeks.

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Higher sera GOT levels compared to control group were observed in Gp II and III rats by the 2nd week with bitter kola exposure appearing to have the more toxic effect. Group IV rats that were coadministered Hg and bitter kola showed delayed peak serum GOT level by the 4th week which was higher than the level attained in group II and III rats exposed to only Hg and bitter kola respectively. All the changes in serum GOT level the statistically analysed data were significant (P<0.05).

The ability of some chemical constituents of G kola to bind to heavy metals and proteins suggest that these could compete with mercury for its binding sites in tissues such as thiol, nitrogen and oxygen containing radical groups (Cantilena and Klassen,1982; Grazzino et al,1992).This kind of antagonism has long been recognized as a possible basis of poison antidotal action and may explain how the hepatotoxity of mercury measured indirectly by the level of hepatic enzymes could be attenuated by co-administered G.kola supplemented diet.

Conclusion: G kola supplemented diet significantly lowered the hepatic mercury content as well as limited the hepatotioxic effects of the heavy metal assessed indirectly by measurement of the serum levels of alkaline phosphatase and the transaminases

Recommendation: Further attempt to elucidate the exact mechanism underlying the observation in this study could entail the evaluation the effects of G kola on mercury excretion as well as the contribution of the different constituents of G kola recognized to possess chelatory activity to this observed antidotal-like action.

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