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A-21 Day Safety Evaluation Of Methanol Extract Of Stem Bark of *Artocarpus Altilis* (Parkinson) Fosberg (Moraceae) in Wistar Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Background: Medicinal plants have been used as therapeutic agents since prehistoric era. *Artocarpus altilis* (Breadfruit) is used in African traditional medicine to treat hypertension with scanty information on its safety profile in animals.
Objectives: This study was designed to evaluate the toxicological effects of oral administration of methanol extract of *Artocarpus altilis* (MEAA) in rats.
Materials and Methods: Thirty male Wistar rats were divided into 6 groups of 5 animals each and were treated orally with corn oil (control), 100, 250, 500, 1000 and 2000 mg/kg of MEAA for twenty one days.
Results: MEAA caused insignificant ($p>0.05$) changes in the activities of serum alanine and aspartate aminotransferases (ALT and AST) and alkaline phosphatase (ALP) relative to the control. Cardiac and hepatic AST (114.8±4.8 and (111.0±1.0) serum urea (1.1±0.2), creatinine (0.3±0.1), lactate dehydrogenase (17.3±5.8) and creatinine kinase (15.5±4.4) were significantly decreased ($p<0.05$) in rats treated with 2000 mg/kg of MEAA when compared to control [(134.8±5.8 and 129.7±5.0), 2.94±0.3, 0.4±0.1, 38.5±13.3 and 41.3±2.9]. The MEAA significantly decreased ($p<0.05$) serum total cholesterol and triglyceride while high density lipoprotein- cholesterol (HDL-c) level was increased. Histopathological examination of liver, kidney and aorta slides from MEAA- treated rats showed little alteration from the control.
Conclusions: The MEAA could be safe when used over a long period for therapeutic purposes.
Keywords: *Artocarpus altilis*, biochemical indices, lipid parameters, toxicity profile.

INTRODUCTION

Herbal medicine or phytomedicine is recognized as the most common form of alternative medicine especially in countries with low income. It is used by about 60% of the world population in developing and developed countries (Rickert *et al*. 1999; Ogbonnia *et al*. 2008). Today, medicinal plants are increasingly being used in most parts of the world as agents for the treatment of skin diseases, wounds and as antimicrobial, hypoglycemic, hypolipidemic, contraceptive, abortifacients, emmenagogues or oxytocic and antihypertensive (Araya *et al*. 2015; Bala *et al*. 2015; Ernst *et al*. 2015 and Maione *et al*. 2015 and). It is a common belief that herbs are non-toxic, safer and cheaper when compared to modern or synthetic drugs in the treatment of diseases. It is now known that severe side effects may occur if some herbs are taken over a long period of time. Medicinal plants such as Ginseng (*Panax species*), Madenhair tree (*Ginkgo biloba*) and Liquorice (*Glycyrrhiza glabra*) (Becker, 1996; Rowin and Lewis 1996; Dhom 2010) have been linked with adverse effects. Hence, the safety evaluation of commonly used herbs is warranted.

Artocarpus altilis (Parkinson (Fosberg) Moraceae) is a flowering tree in the mulberry family. The fruit can be eaten cooked or further processed into a variety of other foods. It is an excellent source of fibre, calcium, copper, iron, magnesium, potassium, thiamine, niacin, carbohydrates and vitamins and very low in fat (Rincon *et al*. 2007). In traditional medicine, leaves of this plant are used for the treatment of liver disorders, hypertension and diabetes (Zerega *et al*. 2005; Lans, 2006). *In vitro* studies by Nwokocha *et al*. (2012) supported the folkloric use of this plant as antihypertensive remedy . The leaves of this plant are also excellent sources of antioxidants such as beta-sitosterol and other flavonoids (Wang *et al*. 2006). Studies from our laboratories confirmed the free radical scavenging and anti-atherogenic potentials of extracts from *Artocarpus altilis in vitro* and *in vivo* (Adaramoye and Akanni, 2014; Akanni *et al*. 2014). Furthermore, Delaisse *et al*. (1980) and Patil *et al*. (2002) showed that

Artocarpus altilis has cathespsin k inhibition activity and may prevent bone resorption and/ osteoporosis. Also, *Artocarpus altilis* showed inhibitory effects on 5α-reductase and may be useful in the selective treatment of benign prostate hyperplasia and prostate cancer (Shimizu *et al*. 2000). From the aforementioned, *in vivo* toxicity profile of *Artocarpus altilis* will give further insights into the health benefits of this unique herb. Therefore, this study was designed to evaluate the toxicity of methanol extract of the stem bark of *Artocarpus altilis* in male Wistar rats.

MATERIALS AND METHODS

Plant Material and Extraction

The stem bark of *Artocarpus altilis* was collected in Ibadan (Oyo State) and authenticated at the Forestry Research Institute of Nigeria, with the herbarium number 109796. The stem bark of *Artocarpus altilis* was air-dried and crushed into fine powder. The powdered part was defatted with n-hexane and, then extracted with methanol using soxhlet apparatus. The extract was concentrated in vacuum at 40^0C with rotary evaporator to dryness.

Study Design

Thirty male albino rats (Wistar strain) ranging from 140-150g obtained from Department of Veterinary Physiology, University of Ibadan, Nigeria. The animals were kept in well ventilated cages at room temperature (28–30^0C) and under controlled light cycles (12-h light/12-h dark). They were maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. Rat handling and treatments confirm to the guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use. These animals were distributed into six groups of five animals each and were given a period of two weeks for acclimatization before the experiment. The first group served as the control and was given corn oil (Vehicle for the extract). The second, third, fourth, fifth and sixth groups received the methanol extract of *Artocarpus altilis* (MEAA) at a dose of 100, 250, 500, 1000 and 2000 mg/kg body weight/ day, respectively. The MEAA was prepared with corn oil and given daily to the animals by oral gavage for twenty-one consecutive days.

Preparation of Serum

Blood was collected from the heart of the animals into plain centrifuge tubes and was allowed to stand for 1 hour. Serum was prepared by centrifugation at 3,000 *g* for 15 minutes in a Beckman bench centrifuge. The clear supernatant was used for the estimation of urea, creatinine, triglycerides, total cholesterol, high density lipoprotein-cholesterol and enzymes.

Preparation of tissues

Rats were fasted overnight and sacrificed via cervical dislocation 24 hours after the last dose of extract. Liver, heart and kidney were quickly removed and washed in ice-cold 1.15% KCl solution, dried and weighed. A section of liver, aorta and kidney were fixed in 10% formalin for histopathological examination. Other parts were homogenized in 4 volumes of 50 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 *g* for 15 minutes to obtain post-mitochondrial supernatant fraction (PMF).

Biochemical assays

Protein contents of the samples were assayed by the method of Lowry *et al*. (1951) using bovine serum albumin as standard. Serum urea level was determined by the method of Talke and Schubert (1965). Serum Creatinine level was determined by the method of Jaffe (1886). The activities of alanine and aspartate aminotransferases of samples (ALT and AST) were assayed by the combined methods of Mohun and Cook (1957) and Reitman and Frankel (1957).

The estimation of serum alkaline phosphatase (ALP) activity was based on the method of Williamson (1972). ALP activity was measured spectrophotometrically by monitoring the concentration of phenol formed when ALP reacts with disodium phenyl phosphate at 680 nm.

Serum total cholesterol level was assayed by the method of Richmond *et al*. (1973). The method involved enzymatic hydrolysis and oxidation of cholesterol with the formation of quinoneimine (an indicator) from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxide.

The serum level of triglyceride was determined by the methods of Jacob and Van Denmark (1960) and Koditshech *et al*. (1969). These methods were based on the hydrolytic oxidation of triglycerides with the formation of glycerol which is substrate for other enzymes with the subsequent formation of hydrogen peroxide. This then reacts with 4-aminophenazone and 4-chlorophenol in the presence of peroxidase to give quinoneimine which is measured spectrophotometrically at 500 nm. The lipoproteins (measured using the enzymatic colorimetric method), very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were precipitated by the addition of phosphotungstic acid and magnesium chloride. After centrifugation at 3,000 *g* for 10 minutes at 25^0C, the clear supernatant contained HDL fraction was assayed for cholesterol. The activity of serum lactate dehydrogenase (LDH) was determined by the method of Zimmerman and Weinstein (1956)

Creatinine kinase (CK-NAC) in the serum was also determined by the method of Stein (1985).

Histology of tissues

The tissues fixed in 10% formalin were dehydrated in 95% ethanol and then cleared in xylene before embedding in paraffin. Micro sections (4 μm) were prepared and stained with haematoxylin and eosin (H&E) dye (Llewellyn 2009) and were examined under a light microscope by a Histopathologist who was ignorant of the treatment groups.

Statistical analysis

All values were expressed as the mean ± S.D. of five animals per group. Data were analyzed using one-way

ANOVA followed by the post-hoc Duncan multiple range test for analysis of biochemical data using SPSS (20.0). Values were considered statistically significant at $p< 0.05$.

RESULTS

There were insignificant ($p>0.05$) differences in weight-gain of MEAA-treated rats relative to the control (Table 1) . In addition, weight and relative weight of organs (liver, kidney and heart) in MEAA-treated rats were statistical similar ($p>0.05$) to the control. Administration of MEAA at all doses caused insignificant ($p>0.05$) effects on the activities of serum alanine and aspartate aminotransferases (ALT and AST) and alkaline phosphatase (ALP) when compared to the control (Table 2). Furthermore, MEAA at 100, 250, 500, 1000 and 2000 mg/kg significantly ($p<0.05$) increased the levels of HDL-c by 41, 45, 54, 56 and 77%, respectively when compared to the control.There were no significant ($p>0.05$) differences in the levels of serum creatinine and urea in rats treated with MEAA (1000 and 2000 mg/kg) (Figure 1). Strikingly, MEAA at 100, 250 and 500 mg/kg significantly ($p<0.05$) decreased the levels of urea while MEAA at 100 and 250 mg/kg decreased creatinine levels in the rats (Figure 1). Administration of MEAA produced insignificant ($p>0.05$) differences in the activities of cardiac and hepatic ALT relative to the control (Figure 2 and 3). However, MEAA at 500, 1000 and 2000 mg/kg significantly ($p<0.05$) decreased the activities of cardiac and hepatic AST in the rats (Figures 2 and 3). The MEAA at all doses significantly ($p<0.05$) decreased the levels of serum CK-NAC and triglyceride when compared to the control (Figures 4 and 5). The activities of serum LDH and the levels of total cholesterol were significantly ($p<0.05$) decreased in MEAA-treated rats, except MEAA at 250 mg/kg (for LDH) and, 1000 and 2000 mg/kg (for total cholesterol). In figures 6a, 6b and 6c, the control slides showed normal and intact cytoarchitecture of the liver, aorta and kidney with little or no visible lesions. The slides from MEAA-treated rats (at low doses; 100, 250 and 500 mg/kg) were very similar to controls. At higher doses of MEAA (1000 and 2000 mg/kg), slide from liver of the rats showed mild necrosis.

DISCUSSION

The current findings (both biochemical and histopathological data) have shown that methanol extract of *Artocarpus altilis* (MEAA) at low doses (100-500mg/kg) did not cause a significant damage to the tissues of male Wistar rats. The body weight-gain, weight and relative weight of organs in MEAA-treated rats and control were similar. Also, MEAA administration is not hepatotoxic nor nephrotoxic since serum ALT, AST, urea and creatinine of MEAA-treated rats were statistically similar to the control.

The liver, a vital organ involved in the maintenance of metabolic function and detoxification of drugs is extremely sensitive to toxic metabolites.

If its normal metabolic function is hampered due to hepatic damage, there will be elevation in serum levels of hepatic markers like ALT, AST, ALP, and bilirubin (Payasi *et al*. 2010). It has been established that AST can be found in the liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leukocytes and erythrocytes, whereas ALT is predominantly present in the liver (Rej 1997). The increased levels of serum enzymes such as AST and ALT indicate an increased permeability and damage and/or necrosis of hepatocytes (Goldberg and Watts 1965). Thus, the normal levels of serum, hepatic and cardiac ALT and AST observed in this study may be an indication that the extract was non-toxic.

Alkaline phosphatase is a hydrolase which hydrolyzes monophosphates at an alkaline pH. It is particularly present in the cells which line the biliary ducts of the liver and also found in other organs such as bones, placenta, kidney and intestine. The membrane bound enzymes like ALP and GGT are released into bloodstream depending on the pathological phenomenon (Sillanaukee 1996). Increase in serum ALP may be considered as an indicator of cholestasis in early stages or mild circumstances preceding other indicators e.g. hyperbilirubinemia (Adedapo *et al*. 2007). Our study revealed that MEAA-treated rats had serum ALP activities within the normal range and this further support the fact that the extract is non-toxic.

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass (Yuegang *et al*. 2008) while urea is a major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys, urea is filtered out of blood by glomerulli and is partially being reabsorbed with water (Corbett 2008). Creatinine and urea are markers of kidney function and, in particular urea is used in differential diagnosis of acute renal failure and pre-renal condition where blood urea nitrogen–creatinine ratio is monitored (Mitchell and Kline 2006). In the present study, administration of MEAA caused insignificant effects in the levels of urea and creatinine of the rats.

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phospho-creatine kinase is an enzyme expressed by various tissues and cell types (Hekimsoy *et al*. 2005). Creatinine kinase catalyses the reaction between creatine and adenosine triphosphate (ATP) to form phosphocreatine (PCr) and adenosine diphosphate (ADP) (Michael *et al*. 2004).

Table 1: Changes in the body weight and relative weight of organs of rats treated with Methanol Extract of *Artocarpus altilis* (MEAA) for twenty-one days.

Treatments

MEAA (mg/kg)	Body Weight (g)		Weight gain (g)	Weight of Organs (g)			Relative Weight of Organs (as % body weight)		
	Before	After	Liver	Kidney	Heart	Liver	kidney	Heart	
Control	148.0±10.84	179.00±22.36	31.0±3.06	5.36±0.79	1.06±0.18	0.56±0.15	2.92±0.47	0.58±0.10	0.30±0.07
100	150.0±00.00	175.00±18.71	25.0±4.12	5.28±0.53	1.1±0.07	0.55±0.05	3.14±0.45	0.65±0.07	0.32±0.04
250	147.0±05.47	175.00±17.68	28.0±5.03	5.56±0.33	1.18±0.10	0.61±0.06	3.21±0.39	0.67±0.07	0.35±0.06
500	150.0±00.00	181.25±12.50	31.3±2.70	5.29±0.37	1.12±0.22	0.61±0.13	2.93±0.29	0.62±0.14	0.34±0.07
1000	151.0±10.84	179.00±12.45	28.0±2.90	5.51±0.35	1.15±0.05	0.56±0.07	3.47±0.18	0.73±0.06	0.35±0.02
2000	147.0±00.0	173.67±05.77	26.7±3.41	5.24±1.08	1.36±0.14	0.63±0.04	3.17±0.46	0.69±0.05	0.34±0.01

Data are expressed as mean ± S.D of 5 animals per group
Control = Animals treated with corn oil alone.

Table 2: Changes in the levels of some biochemical indices in the serum of rats treated with Methanol Extract of *Artocarpus altilis* (MEAA) for twenty-one days.

Treatments

MEAA (mg/kg)	**AST (U/L)**	**ALT**	**ALP**	**HDL- C (mg/dL)**
Control	31.41±2.15	7.03±2.07	47.84±30.49	341.92±44.16
100	30.08±4.70	6.33±1.69	39.56±3.29	482.78±83.97*
250	28.41±4.73	6.51±1.01	42.32±7.45	493.66±69.11*
500	29.11±6.81	5.84±1.33	49.27±8.39	530.14±92.08*
1000	28.94±5.26	7.68±1.13	47.30±9.75	532.94±28.08*
2000	30.73±5.17	6.48 ±0.75	46.00±9.75	603.69±9.17*

Data are expressed as means ± S.D. of 5 animals per group *Significantly different from control ($p < 0.05$)

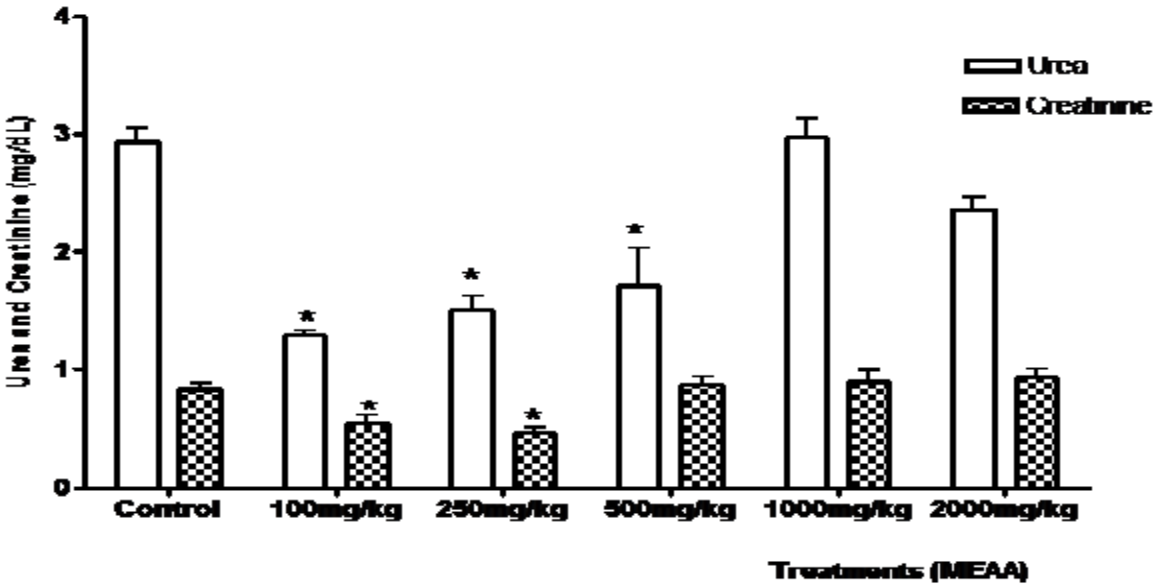

Figure 1. Changes in the levels of Urea and Creatinine in rats after twenty-one days of treatment with Methanol Extract of *Artocarpus altilis* (MEAA) at different concentrations.

* Significantly different from control ($p<0.05$)

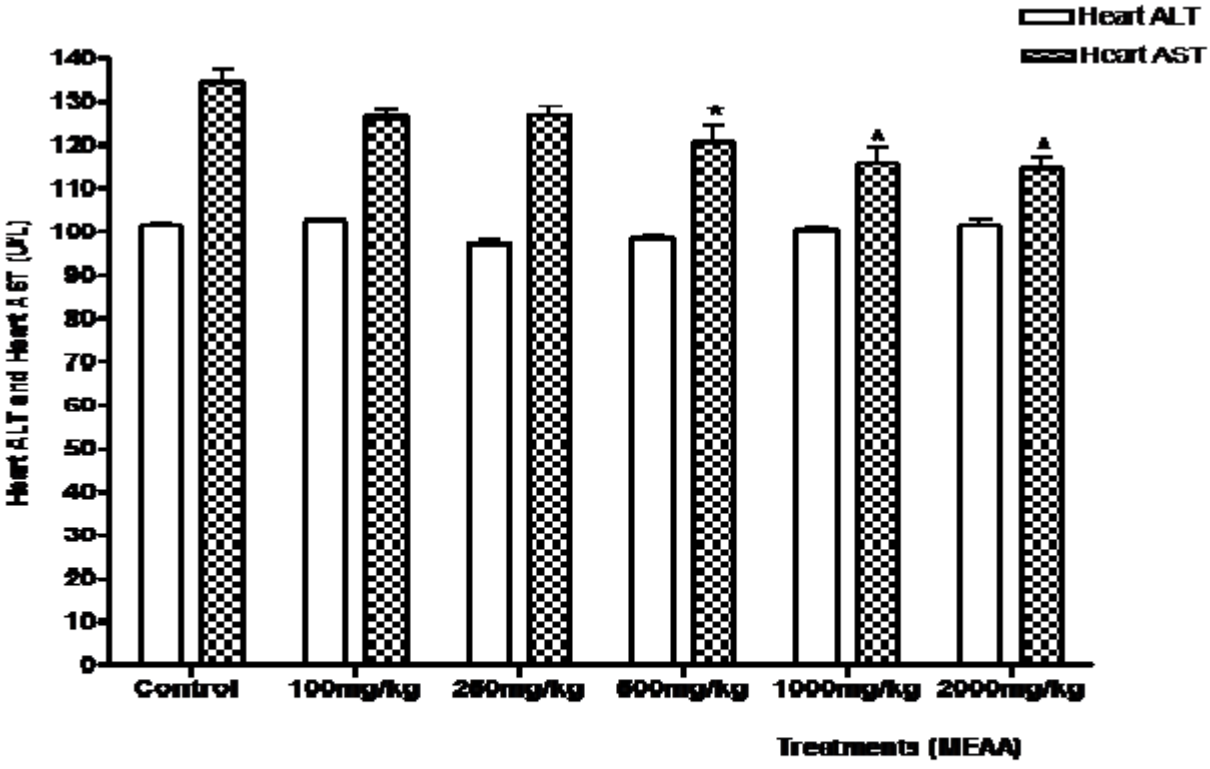

Figure 2. Changes in the activities of heart Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in rats after twenty-one days of treatment with Methanol Extract of *Artocarpus altilis* (MEAA) at different concentations.

* Significantly different from control ($p<0.05$)

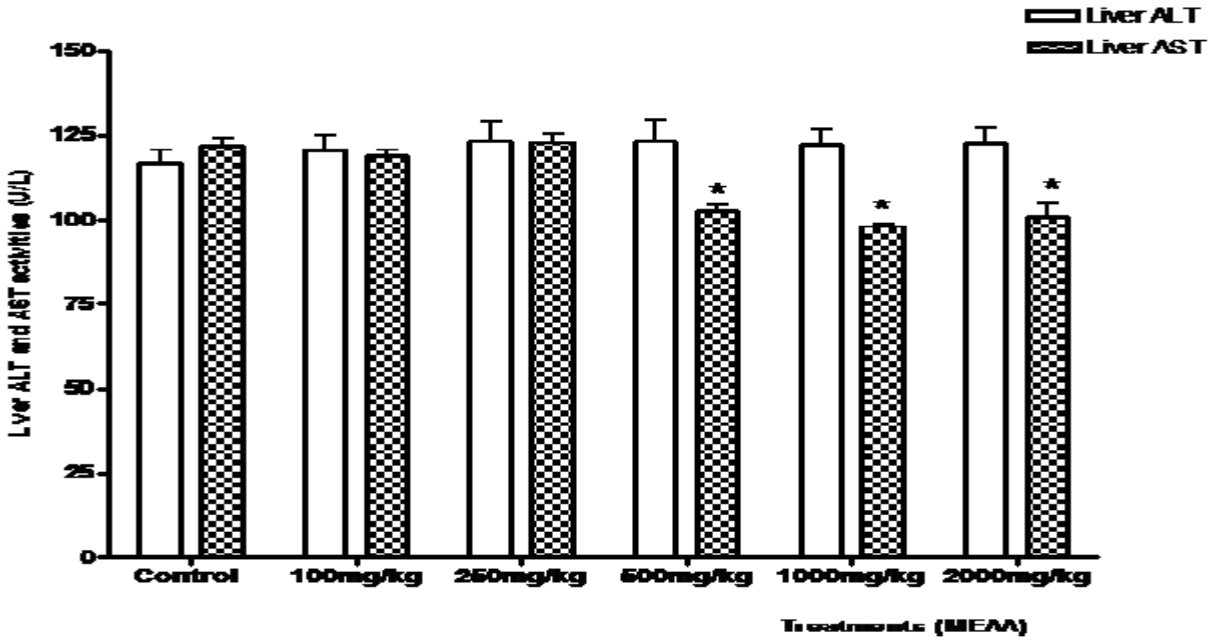

Figure 3. Changes in the activities of liver Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in rats after twenty-one days of treatment with Methanol Extract of *Artocarpus altilis* (MEAA) at different concentations.

*** Significantly different from control ($p<0.05$)**

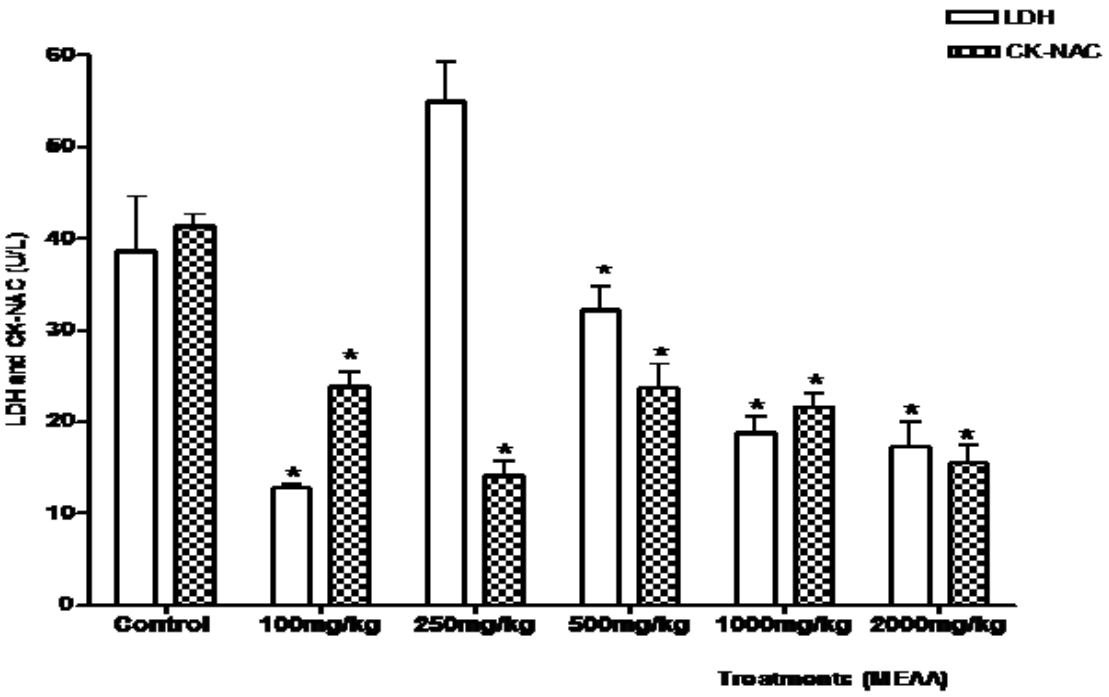

Figure 4. Changes in the activities of Lactose Dehydrogenase (LDH) and Creatinine kinase (CK-NAC) in rats after twenty-one days of treatment with Methanol Extract of *Artocarpus altilis* (MEAA) at different concentations.

*** Significantly different from control ($p<0.05$)**

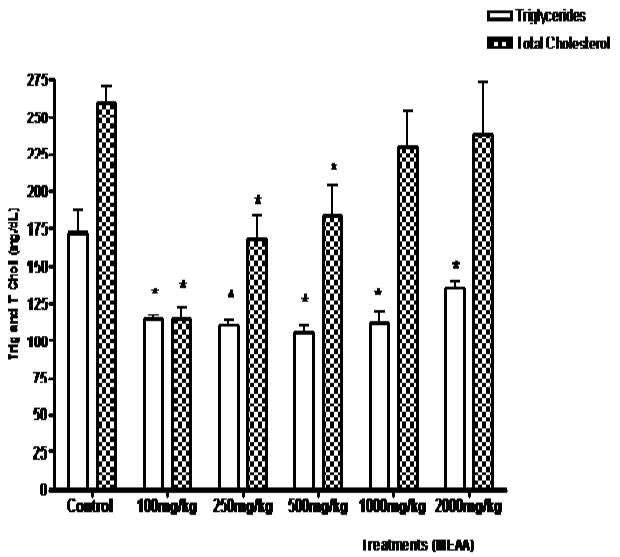

Figure 5. Changes in the levels of Triglycerides and Total Cholesterol in rats after twenty-one days of treatment with Methanol Extract of Artocarpus altilis (MEAA) at different concentrations.

* Significantly different from control ($p<0.05$)

This is a reversible reaction in that ATP can be generated from phosphocreatinine and ADP (Bong *et al.* 2008). In tissues and cells that consume ATP rapidly, especially skeletal muscle, brain, photoreceptor cells of the retina, spermatozoa and smooth muscle, PCr serves as an energy reservoir for the rapid buffering and regeneration of ATP *in situ*, as well as for intracellular energy transport by the PCr shuttle or circuit (Wallimann *et al*. 1992). Clinically, creatine kinase is assayed in blood tests as a marker of myocardial infarction, muscular dystrophy and acute renal failure (Schlattner *et al*. 2006). The activities of CK-NAC were significantly decreased in rats given MEAA, suggesting the possible protective role of MEAA. The enzyme, lactate dehydrogenase (LDH) catalyzes the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD^+.

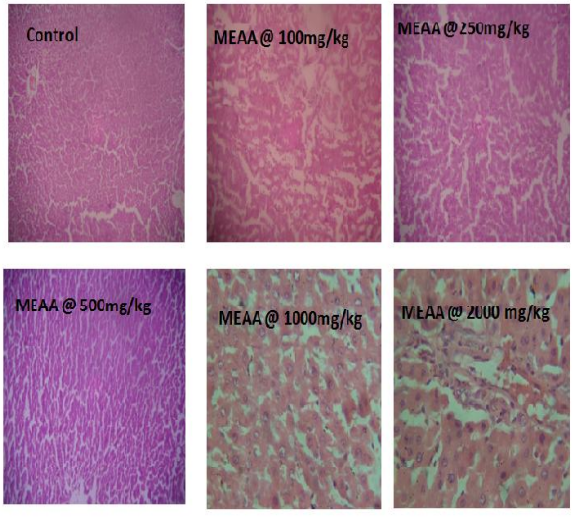

Figure 6a. Changes in histology of liver samples of rats treated with different doses of Methanol Extract of *Artocarpus altilis* (MEAA) for twenty-one days (X 200)

It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver (Joseph *et al*. 2002). At high concentration of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased (Joseph *et al*. 2001). It is often used as a marker of tissue breakdown since it is abundant in red blood cells and can also function as a marker for hemolysis (Butt *et al*. 2002). LDH can also be used as a marker of myocardial infarction (Selwood and Jaffe 2011). Therefore, the observed decrease in the activities of both CK-NAC and LDH in MEAA-treated rats is an indication of cardioprotective ability of the extract.

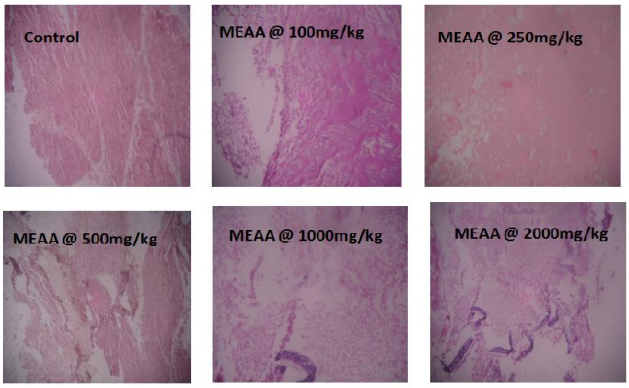

Figure 6b. Changes in histology of aorta in rats treated with different doses of Methanol Extract of *Artocarpus altilis* (MEAA) for twenty-one days (X 200).

High serum triglyceride level has been reported to be an important cardiovascular diseases risk factor (Harnafi *et al*. 2009). In this study, MEAA caused a significant decrease in serum total cholesterol and triglyceride coupled with concomitant increase in HDL-cholesterol level. These findings revealed the presence of hypolipidemic agents in the extract and also confirmed that the extract may reduce CVD risk factors. The results are in consonance with the study of Adaramoye and Akanni (2014) which reported the protective effects of extract of *Artocarpus altilis* against dietary cholesterol-induced hypercholesterolemia in rats. The histology of aorta, kidney and liver slides from rats given MEAA (at low doses; 100, 250 and 500 mg/kg) revealed normal and intact cytoarchitecture. However, at higher doses of MEAA, only the liver slides showed mild necrosis with cellular infiltration.

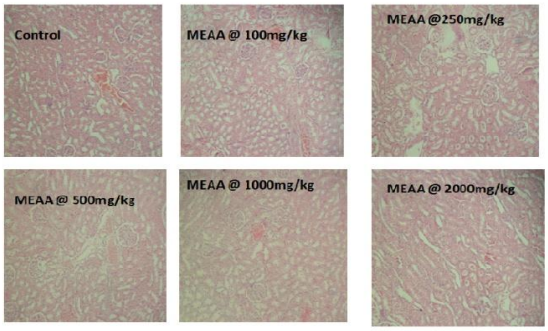

Figure 6c. Changes in histology of kidney in rats treated with different doses of Methanol Extract of *Artocarpus altilis* (MEAA) for twenty-one days (X 200).

CONCLUSION

In conclusion, there were no major changes in body weight, weight and relative weight of organs of MEAA-treated rats. Likewise, biochemical indices and histological examination revealed that the tissues of MEAA-treated rats and control were identical. This study therefore suggests that the methanol extract of stem bark of *Artocarpus altilis* could be safe when used for 21-days for therapeutic purposes.

REFERENCES

Adaramoye, O.A., Akanni, O.O., (2014). Effects of Methanol Extract of Breadfruit (*Artocarpus altilis*) on Atherogenic Indices and Redox Status of Cellular System of Hypercholesterolemic Male Rats. Adv. Pharmacol. Sci. 605425. doi: 10.1155/2014/605425

Adedapo, A.A., Abatan, M.O., Olorunsogo, O.O., (2007). Effects of some plants of the spurge family on the haematological and biochemical parameters of rats. Vet. Arhiv. 77 (1): 29-38.

Akanni, O.O., Owumi, S.E., Adaramoye, O.A., (2014). *In vitro* studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from *Artocarpus altilis*, *Ficus exasperate* and *Kigelia africana*. Asian Pac. J. Trop. Biomed. 4 (1): S492-S499.

Araya, S., Abera, B., Giday, M., (2015). Study of plants traditionally used in public and animal health management in Seharti Samre District, Southern Tigray, Ethiopia. J. Ethnobiol. Ethnomed. 11: 22.

Bala, M., Pratap, K., Verma, P.K., Singh, B., Padwad, Y., (2015). Validation of ethnomedicinal potential of *Tinospora cordifolia* for anticancer and immunomodulatory activities and quantification of bioactive molecules by HPTLC. J. Ethnopharmacol, doi: 10.1016/j.jep.2015.08.001.

Becker, B.N., (1996). Ginseng -induced diuretic resistance. JAMA. 276-607.

Bong, S., Moon, J., Nam, K., Lee, K., Chi, Y., Hwang, K., (2008). Structural studies of human brain-type creatine kinase complexed with the ADP–Mg^{2+}–NO_3−creatine transition-state analogue complex. FEBS Letters 582 (28): 3959–3965.

Butt, A.A., Michaels, S., Greer, D., Clark, R., Kissinger, P., Martin, D.H., (2002). Serum LDH level as a clue to the diagnosis of histoplasmosis. AIDS Read. 12 (7): 317–21.

Corbett, J.V., (2008). Laboratory tests and diagnostic procedures with nursing diagnoses. 7th Ed. pp. 90–107.

Delaisse, J.M., Eackhout, Y., Vaes, G., (1980). Inhibition of bone resorption in culture by inhibitors of thiol proteinases. Biochem J. 192 (1): 365-368.

Dhom J. C.., (2010). The Importance of Liquorice in the Management of Auto-Immune Disorders Cited from: http://www.regenerativenutrition.com/liquorice-auto-immune-multiple sclerosis-ibs.asp.

Ernst, M., Grace, O.M., Haris Saslis-Lagoudakis, C., Nilsson, N., Simonsen, H.T., Rønsted, N., (2015). Global medicinal uses of Euphorbia L. (Euphorbiaceae). J. Ethnopharmacol. S0378-8741 (15): 30184-307.

Goldberg, D.M., Watts, C., (1965). Serum enzyme changes as evidence of liver reaction to oral alcohol. Gastroenterol. 49: 256-261.

Harnafi, H., Aziz, M., Amrani, S., (2009). Sweet Basil (*Ocimum basilicum L.*) improves lipid metabolism in hypercholesterolemic rats. E Spen Eur E J. Clin. Nutr. Metab. 4: 181–186.

Hekimsoy, Z., Oktem, I.K., (2005). Serum creatinine kinase levels in overt and subclinical hypothyroidism. Endoc Res. 31 (3): 171–5.

Jacobs, N.J., Van Denmark, P.J., (1960). Enzymatic determination of serum triglycerides. Biochem. Biophys. 88: 250-55.

Jaffe, M., (1886). Ueber den Neiderschlag, welchen Pikrinsäure im normalen harn Erzeught und übereine neue Reaction des Kreatinins. Z. Physiol. Chem. 10: 391–400.

Joseph, J., Badrinath, P., Basran, G.S., Sahn, S.A., (2001). Is the pleural fluid transudate or exudate? A revisit of the diagnostic criteria. Thorax 56 (11): 867–70.

Joseph, J., Badrinath, P., Basran, G.S., Sahn, S.A., (2002). Is albumin gradient or fluid to serum albumin ratio better than the pleural fluid lactate dehydroginase in the diagnostic of separation of pleural effusion? BMC Pulmo. Med. 2: 1

Koditschek, L.K., Umbreit, W.W., (1969). Alpha-glycerophosphate oxidase in *Streptococcus faecium* F 24. J. Bacteriol. 98 (3): 1063-1068.

Llewellyn, B. D., (2009). Nuclear staining with alum-hematoxylin. Biotech. Histochem. 84: 159-177.

Lans, C.A., (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J Ethnob and Ethnomed. 2: 45-56.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

Maione, F., Russo, R., Khan, H., Mascolo, N., (2015). Medicinal plants with anti-inflammatory activities. Nat Prod Res. 29: 1-10.

Michael, L., Bishop Edward, P., Fody., Larry, E., Schoeff, ed. (2004). Clinical chemistry: principles, procedures, correlations. Philadelphia: Lippincott Williams & Wilkins. p. 243. ISBN 978-0-7817-4611-3. OCLC 56446391.

Mitchell, H.R., Kline, W., (2006). Core curriculum in nephrology, Renal Function Testing. Am. J. Kidney. Dis. 47: 174–183.

Mohun, A.F., Cook, L.J., (1957). Simple method for measuring serum level of glutamate-oxaloacetate and glutamate-pyruvate transaminases in laboratories. J. Clin. Pathol. 10: 394–399.

Nwokocha, C.R., Owu, D.U., McLaren, M., (2012). Possible mechanisms of action of the aqueous extract of *Artocarpus altilis* (breadfruit) leaves in producing hypotension in normotensive Sprague-Dawley rats. Pharm. Biol. 50 (9): 1096-1102.

Ogbonnia, S., Adekunle, A.A., Bosa, M.K., Enwuru, V.N., (2008). Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr. J. Biotech. 7: 701-705.

Patil, A.D., Freyer, A.J., Killmer, L., Offen, P., Taylor, P.B., Votta, B.J., Johnson, R.K., (2002). A new dimericdihydrochalcone and a new prenylated flavone from the bud cover of *Artocarpus altilis*: Potent inhibitors of cathepsin K. J Nat Prod. 65 (4): 624-627.

Payasi, A., Chaudhary, M., Singd, B.M., Gupta, A., Sehgal, R., (2010). Sub-acute toxicity studies of paracetamol infusion in albino Wistar rats. Inter. J. Pharm. Sci. and Drug Res. 2 (2): 142–145.

Reitman, S., Frankel, S., (1957). A colorimetric method for the determination of serum level of glutamate-oxaloacetate and pyruvate transaminases. Am. J. Clin. Pathol. 28: 56–63.

Rej, R., (1997). Liver diseases and the clinical laboratory--the Twentieth Arnold O. Beckman Conference in Clinical Chemistry. Clin Chem. 43 (8): 1473-1475.

Richmond, W., (1973). Preparation and properties of a cholesterol oxidase from *Nocardia sp*. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem. 19 (12): 1350-1356.

Rickert, K., Martinez, R.R., Martinez, T.T., (1999). Pharmacist knowledge of common herbal preparations. Proc. West. Pharmacol. Soc. 42: 1-2.

Rincón, A.M. Bou Rached, L. Aragoza, L.E. Padilla, F. (2007). Effect of acetylation and oxidation on some properties of breadfruit (*Artocarpus altilis*) seed starch. Archivos. Latinoamericanos. Nutricion. 57 (3): 287 – 294.

Rowin, J., Lewis, S.L., (1996). Spontaneous bilateral subdural hematomas associated with chronic Ginkgo biloba ingestion. Neurology. 46: 1775–1776.

Schlattner, U., Tokarska-Schlattner, M., Wallimann, T., (2006). Mitochondrial creatine kinase in human health and disease. Biochimica et Biophysica. Acta. 1762 (2): 164–80.

Selwood, T., Jaffe, E.K., (2011). Dynamic dissociating homo-oligomers and the control of protein function. Arch. Biochem. Biophys. 519 (2): 131–43.

Shimizu, K., Kondo, R., Sakai, K., Buabarn, S., Dilokkunanant, U., (2000). 5α-Reductase inhibitory component from leaves of *Artocarpus altilis.* J Wood Sci. 46 (5): 385-389.

Sillanaukee, P., (1996). Laboratory markers of alcohol abuse. Alcohol 31: 613-616.

Stein, W., (1985). Creatinine Kinase Determination. Med. Welt. 36: 572.

Talke, H., Schubert, G.E., (1965). Enzymatische Harnstoff bestimmung in Blut andserum in Optischen Test nach Warburg. Klin.Wochschr. 43: 174.

Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., Eppenberger, H.M., **(**1992). Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem. J. 281 (1): 21–40.

Wang, MY., Dhingra, K., Hittelman, WN., Liehr, JG., deAndrade,M, and Li, DH. (2006). Lipid peroxidation-induced putative malondialdehyde–DNA adducts in human breast tissues. Cancer Epidemiol. Biomark Prev. 5: 705–710.

Williamson, T., (1972). A comparison between the phosphastrate and phenyl phosphate methods of alkaline phosphatase assay. Med. Lab. Technol. 29 (2): 182–187.

Yuegang, Z., Chengjun, W., (2008). Simultaneous Determination of Creatinine and Uric Acid in Human Urine by High Performance Liquid Chromatography. Anal. Sci. 24: 1589–1592.

Zerega, N.Y.C., Ragone, D., Motley, T.J., (2005). Systematics and species limits of breadfruit (*Artocarpus moraceae*) Systematic Botany, 30: 603 – 615.

Zimmerman, H.J., Weinstein, B.S., (1965). Lactic dehydrogenase activity in human serum. J. Lab. & Clin. Med. 48 (2): 607-609.

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