UNDER PEER REVIEW

Original Research Article

Effect of *Costus afer* Methanolic Stem Extract on Serum Lipid Profiles and Total Bilirubin Level as Markers for Hepatocyte Damage in Ethanol Induced liver Cirrhosis in Rats.

Abstract

AIM: The study is to investigate the impact of *Costus afer* methanolic stem extract on lipid metabolic profiles and bilirubin levels in alcohol-induced liver cirrhosis progression in rats.
MATERIALS AND METHODS: Male wistar strain albino rats were randomly divided into five groups: Control (normal feed and water only), *Costus afer* control (CA), Ethanol control (Ethanol), ethanol + *Costus afer* (60mg) treated rats (CA + ETHANOL) and ethanol + *Costus afer* (120mg) treated rats (CACA + ETHANOL). Serum levels of lipids and bilirubin were measured.
RESULTS: The result showed that ethanol only administration caused triglyceride, cholesterol, low density lipoprotein and bilirubin to increase by 46.1%, 64.3%, 10.71% and 0.18% respectively compared to control rats ($p<0.05$). High density lipoprotein was shown to have decreased by 11.2% compared to the control.
CONCLUSION: Evidence suggests that oxidative stress is involved in the pathophysiology of complications and alcoholic diseases. Co-treatment with the extract modestly modulates the effect of ethanol to near normal.

Keywords : Costus afer, triglyceride, cholesterol, low density lipoprotein, high density lipoproteins, bilirubin.

1. INTRODUCTION

Cirrhosis of the liver results in gross distortion in liver architecture. Lipids are one of the necessary components which control cellular functions and homeostasis, liver is an important site for lipid metabolism. Owing to reduced liver biosynthetic capacity, low levels of triglycerides (TG) and cholesterol are usually observed in chronic liver disease. Portal hypertension, hepatic encephalopathy, hepatorenal syndrome, spontaneous bacterial peritonitis and esophageal variceal bleeding are the major complications of diabetes mellitus, renal failure, and acutecirrhosis[1].Alcoholic cirrhosis is the end spectrum of alcoholic liver disease (ALD), which includes fatty liver or simple steatosis, alcoholic hepatitis, fibrosis, cirrhosis and superimposed hepatocellular carcinoma [2]. Fatty liver is the most common form of ALD, which develops in more than 90% of heavy drinkers. But, only about 30% of heavy drinkers develop a more severe form of ALD, such as fibrosis and cirrhosis [2]. Cirrhosis is the final result of chronic liver damage, which is characterized by parenchymal injury leading to extensive fibrosis and nodular regeneration. As about 30% of the heavy drinkers develop cirrhosis, there are many other factors that are involved in the development of alcoholic cirrhosis, which include sex, obesity, drinking patterns, dietary factors, non-sex-linked genetic factors and cigarette smoking [3, 4].

The molecular pathogenesis of ALD involves alcohol metabolism and secondary mechanisms such as oxidative stress, endotoxin, cytokines, and immune regulators [5]. The final and

UNDER PEER REVIEW

irreversible form of ALD is alcoholic cirrhosis, which usually develops slowly and insidiously. Fibrosis that occurs in alcoholic cirrhosis is a wound-healing response that occurs virtually in all forms of chronic liver injury, and is characterized by excessive accumulation of collagen and other extracellular matrix proteins [2]. Activated hepatic stellate cells are the major source of the increased production of extracellular matrix proteins, along with portal fibroblasts and bone marrow-derived myofibroblasts [2].

The liver plays a key role in the metabolism of plasma lipids and lipoproteins [6]. As majority of endogenous cholesterol is synthesized in the hepatic microsomes, synthesis and metabolism of cholesterol is impaired in chronic liver disease resulting in a decrease in plasma levels [7]. Severe metabolic impairment in cirrhosis can produce a worsening of the serum lipoprotein pattern. High-density lipoprotein (HDL) cholesterol and its major apolipoproteins have been shown to be reduced in cirrhosis, as also the serum levels of low-density lipoprotein (LDL) cholesterol [7].

The plant, *Costus afer* Ker Gawl (Family: Zingiberaceae) is a tall perennial herbaceous, unbranched medicinal plant with creeping rhizome, commonly found in moist or shady forest and river banks of tropical West Africa [8; 9]. *C. afer* is commonly called gingerlily or bush ca [10]. It is known as "Okpete" or "Okpoto" in Igboland,"Kakizawa" in Hausa "irekeomode" in Yoruba and "Mbritem" in Efik all in Nigeria, in Ijaw it is called " Ogbodou", Anglophone Cameroon calls it "Monkey sugar cane" [11]. It is commonly used as a medicinal herb, especially the leaf, stem, seeds and rhizomes which are harvested from the wild [12]. It has been used in folkloric medicine to treat ailments such as inflammation, rheumatism, arthritis, cough, hepatic disorders, helminthic, miscarriages, epileptic attack, haemorrhoids, as laxative, diuretics, and also had served as an antidote for poison [13; 14]. The plant stem extracts had been studied in our laboratory and shown to possess potent antioxidants *invitro* [15]. Other studies include: *in vitro* and *in vivo* pharmacological activities of methanol leaf extract [14], leaf essential oil [16], and topical anti-inflammatory activity [17].

It is also known that long-term ingestion of alcohol causes serum lipid profile abnormality [18]. There is rarely any study on the effects of plant extracts on lipid profile abnormality in alcoholic cirrhosis in rats. Viewed from this angle, the present study was undertaken with the following aims and objectives:

I. To assess the degree of alteration of serum lipid levels in alcoholic cirrhos and
II. To detect the modulatory effect of *Costus afer* methanolic stem extract on the lipid profile of alcohol induced liver cirrhosis in rats.

2. MATERIALS AND METHODS

2.1 Plant Collection

The whole plant *Costus afer* was obtained from a farm land at Yenagoa, Bayelsa State, Nigeria and identified in the Plant Science/Biotechnology department of the University of Port-Harcourt.

UNDER PEER REVIEW

2.2 Plant processing and extraction

The stem were cut off from the leaves, air dried under room temperature and pulverized mechanically. The pulverized stem sample 500 g were weighed and soaked in 2500 ml of ethanol and for 72 h. The extract was filtered using Watman No. 1 filter paper and subsequently concentrated at 45°C using rotary evaporator (Buchi Rotavapor RE-3; Switzerland) under reduced pressure. The extracts derived were kept at 4°C until further use.

2.3 Methanol and Assay kits

Methanol and ethanol was purchased from Effective Medical Laboratories, Yenagoa, Bayelsa State, Nigeria. Total cholesterol (TC), triacylglycerol (TG) and high-density lipoprotein cholesterol (HDL-C) were determined using commercial kits (Randox Laboratory Ltd., UK). All other reagents used were of analytical grade.

2.4 Animals

Male albino rats (Wister strain) weighing between 150– 250 g, total of 50 were obtained from the Preclinical Animal House, Physiology Department, Niger Delta University, Nigeria. The animals were acclimatized for two weeks at the Departmental Animal House, Niger Delta University. The animals were maintained and cared for following the National Institute of Health (NIH) animal care guideline.

2.5 Preparation of Blood for Biochemical Analyses

Blood specimen was collected by venipuncture from fasting subjects using 5.0ml capacity disposable syringes. Blood samples were transferred into plain bottles to allow for coagulation. The coagulated blood samples were centrifuged at 3000rpm for 10mins, the serum transferred into Bijou bottle and stored frozen until required for biochemical analysis [19].

Blood sera were used for determination of various lipoproteins patterns like total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and Total Bilirubin

2.6 Ethanol induced liver damage: Male albino rats (Wister strain) (150-250g) were used. All the animals were divided into the six groups each group consists of 7 animals and they received the treatment as follows.

CONTROL: Normal, treated with feed and water only
CA: *Costus afer* control (60mg/kg p.o.) for 6 weeks
ETHANOL : Ethanol control 40% (2 ml/100 g p.o) for 6 weeks
CA + ETHANOL: *Costus afer* (60 mg/kg p.o.) + 40% Ethanol for 6 weeks
CACA + ETHANOL: *Costus afer* (120 mg/kg p.o.) + 40% Ethanol for 6 weeks

24 hours after the final doses were administered, rats were sacrificed by cervical dislocation and serum was processed for biochemical assays.

2.7 Lipid profile assays: Total cholesterol (TC), triacylglycerol (TG) and high-density lipoprotein cholesterol (HDL-C) were determined using commercial kits (Randox Laboratory Ltd., UK). Low-density lipoprotein cholesterol (LDL-C) concentration was determined by

UNDER PEER REVIEW

difference according to the formula described by Friedewald et al., (1972)[20], as reported by Oluba, et al., (2012)[21].

2.8 Bilirubin assay: Serum bilirubin was measured by the method as described by Enemor et al., (2005) [22]

2.9 *Statistical Analysis*

Statistical analysis was performed using the Graphpad Prism for Windows statistical package, version 6.0 (Graphpad Software Inc).

Data were expressed as means ± S.D. The effects of treatments were evaluated statistically using the one-way analysis of variance (one-way ANOVA) followed by the Turkey's post-hoc test to correct for multiple comparison treatments. Statistical significance was set at the $p < 0.05$ level.

3. RESULTS AND DISCUSSION

An overview of Table 1 showed that serum lipid profile and bilirubin levels of non-cirrhotic rats (control) were generally within reference intervals. The lipid parameters were relatively higher in ethanol alone administered rats as compared to the other groups ($p<0.05$).

Table1. Effect of various treatments on the serum lipid profile and bilirubin level

Experimental Groups	Triglycerides (TG) mg/dl	Sig	Total cholesterol (TC)mg/dl	Sig	HDL mg/dl	Sig	LDL mg/dl	Sig	Total Bilirubin (µmol/l)	sig
Control	***53.13 ± 0.69***	*a,b	***65.84± 0.41***	*a, b	**40.76 ± 0.32**	*a	**21.25 ± 0.41**	*a	**2.47 ± 0.50**	*a
C A	***52.24*± 0.71**	*a	***61.91*± 0.19**	*a	**38.44 ± 0.47**		**17.78 ± 0.40**	#b	**1.98 ± 1.14**	
Ethanol	**87.25 ± 0.54**		**97.79± 0.61**		**28.43 ± 0.28**	#b	**51.29 ± 0.47**		**7.25 ± 0.71**	
C A + Ethanol	**56.66 ± 0.63**		**67.08± 0.67**		**39.26 ± 0.46**		**19.22 ± 0.48**		**3.11 ± 0.53**	#b
CACA +Ethanol	**57.04 ± 0.17**	*a	**66.27± 0.75**	*a	**30.37 ± 0.45**	$d	**18.83 ± 0.53**	$ d	**2.26 ± 0.45**	

UNDER PEER REVIEW

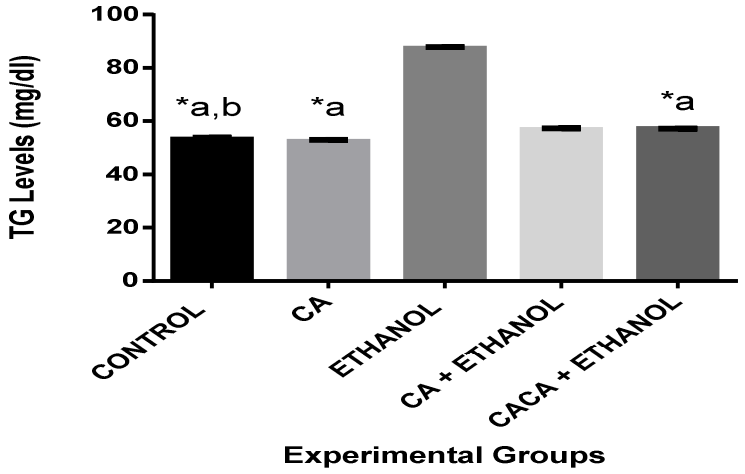

Figure 1: Effect of different treatments on the Triglyceride levels in the Serum of rats.

= P< 0.05, # = P< 0.01, $ = P < 0.001,
a = Control vs CA, Ethanol.
b = Ethanol vs CA, CA + Ethanol, CACA + Ethanol.
c = CA vs CA + Ethanol, CACA + Ethanol.
d = CA + Ethanol vs CACA + Ethanol.

3.1 Triglyceride Assay

Figure 1 showed that the extract had no effect on the serum triglyceride level. Ethanol- only treated group showed a modest increased level compared to other groups (p<0.05). There was no significant difference between the control and *Costus afer* extract treated groups at p<0.05 (***53.13 ± 0.69mg/dL and 52.24*± 0.71mg/dL respectively**), but there was a significant difference observed between the *Costus afer* treated group and the co-treated groups and also no significant difference in the co-treated groups (p<0.05) for the co-treated group had a modest increase.

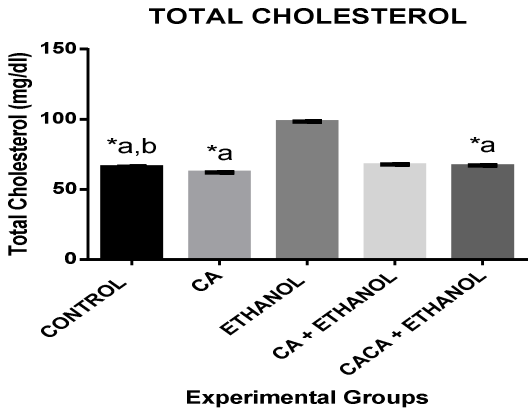

Figure 2: Effect of different treatments on the Total Cholesterol levels in the Serum of rats.

UNDER PEER REVIEW

= P< 0.05, # = P< 0.01, $ = P < 0.001,
a = Control vs CA, Ethanol.
b = Ethanol vs CA, CA + Ethanol, CACA + Ethanol.
c = CA vs CA + Ethanol, CACA + Ethanol.
d = CA + Ethanol vs CACA + Ethanol.

3.2 Total Cholesterol Assay

The extract reduces the serum total cholesterol levels significantly compared to other groups. There was a significant increase in the ethanol only treated group (**97.79± 0.61mg/dL**), the co-treated groups (**67.08± 0.67 and66.27± 0.75 respectively**) showed no significant difference (p<0.05).

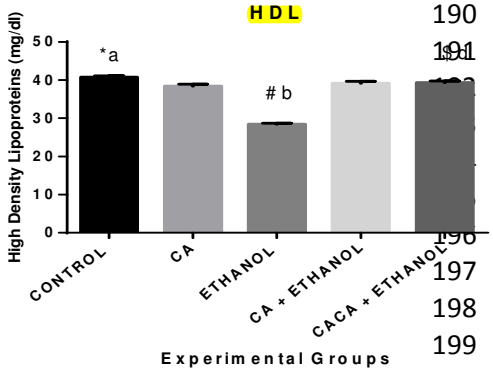

Figure 3: Effect of different treatments on the High Density Lipoproteins levels in the Serum of rats.

= P< 0.05, # = P< 0.01, $ = P < 0.001,
a = Control vs CA, Ethanol.
b = Ethanol vs CA, CA + Ethanol, CACA + Ethanol.
c = CA vs CA + Ethanol, CACA + Ethanol.
d = CA + Ethanol vs CACA + Ethanol.

3.3 HDL-Cholesterol Assay

High density lipoproteins (HDL) slightly reduced when treated with the extract, there was a significant reduction in the level of HDL in the serum of ethanol only treated group (p<0.05). the co-ttreated groups had no significant difference, but slightly reduced comparative to the control (p<0.05).

UNDER PEER REVIEW

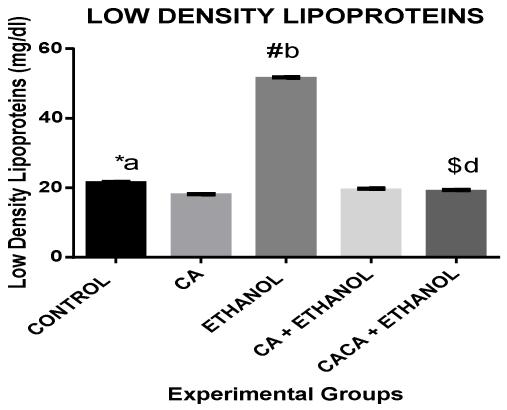

Figure 4: Effect of different treatments on the Low Density lipoproteins levels in the Serum of rats.

= P< 0.05, # = P< 0.01, $ = P < 0.001,
a = Control vs CA, Ethanol.
b = Ethanol vs CA, CA + Ethanol, CACA + Ethanol.
c = CA vs CA + Ethanol, CACA + Ethanol.
d = CA + Ethanol vs CACA + Ethanol.

3.4 LDL-Cholesterol

Treatment with CA significantly reduced the level of LDL, but co-treatment slightly increased the level of LDL comparative to the CA treated group. The ethanol only treated showed a significant increase in the LDL levels comparative to other groups (p<0.05).

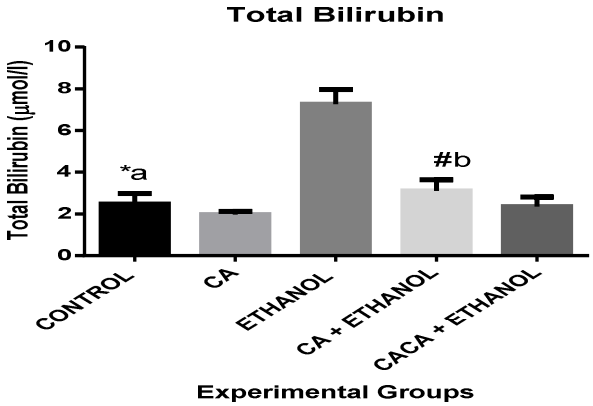

Figure 5: Effect of different treatments on the Total Bilirubin levels in the Serum of rats.

= P< 0.05, # = P< 0.01, $ = P < 0.001,
a = Control vs CA, Ethanol.
b = Ethanol vs CA, CA + Ethanol, CACA + Ethanol.
c = CA vs CA + Ethanol, CACA + Ethanol.
d = CA + Ethanol vs CACA + Ethanol.

UNDER PEER REVIEW

3.5 Bilirubin Assay

Bilirubin, which tests the amount of bile pigment in the blood, figure 5 showed that treatment with extract significantly reduced the bilirubin level comparative to control group ($p<0.05$). Treatment with the toxicant increased the level significantly ($p<0.05$), It was also observed that the dose - effect relationship is dependent on the increase in the concentration of the extract. There was a significant difference between the co-treated groups ($p<0.01$)

The oxidation of ethanol via the alcohol dehydrogenase (ADH) pathway results in the production of acetaldehyde and the reduced form of nicotinamide adenine dinucleotide (NADH). A major contribution to the adverse effects of ethanol arises from the changes in the redox status, due to the increase in NADH and decrease in nicotinamide adenine dinucleotide (NAD+) concentration. The generation of a large amount of reducing equivalents in liver, via ADH pathway, has been correlated with a number of metabolic disorders, including hyperlipidemia [23].

Our results show changes in the level of triglyceride and cholesterol in the rat serum, a significant increase being registered after 6 weeks of ethanol administration. Alcohol feeding is known to increase the biosynthesis and to decrease the catabolism of fatty acid and cholesterol, resulting in hypertriglyceridemia and hypercholesterolemia [24-26]. The high levels of triglyceride after ethanol administration may be due to a number of factors such as the increased availability of the fatty acids for esterification [27]. This finding may be related with an increase in 3-hydroxy-3- methyl-glutaryl CoA reductase activity by ethanol, taking into account that this enzyme catalyze is the rate limiting step in cholesterol biosynthesis [28]. Phospholipids, the major component of biomembranes, are the primary targets of peroxidation process, and they can be altered by ethanol consumption [29]. The decrease in high density lipoproteins(HDL) concentration may be due to an accelerated degradation, and can result in the modification of composition, structure and stability of biomembranes thus leading to cellular dysfunction. Earlier reports also have shown that the administration of ethanol has resulted in the decrease of the HDL content in both kidney and liver of rats [27, 30]. A number of studies have shown that ethanol administration enhances the lipid peroxidation in the liver, and the peroxidation of liver lipids is a factor in the pathogenesis of ethanol-induced liver injury [23, 31, 32]. Bilirubin is produced by the liver and its level can increase when the liver becomes cirrhotic.

From the previous studies, it was found that *Costus afer* has strong antioxidants like total phenolic compounds, tannins, flavonoids etc. The presence of potent phytochemicals in the extract may be responsible for the hepatoprotective activity. The present studies using ethanol-induced ratsas models have shown TG, TC, LDL, and TB values were significantly reduced and HDL increased when co-treated with the extract indicating a greater level of protection in these models.

4. CONCLUSION: In conclusion, the results of this study demonstrate that *Costus afer* stem extract was effective in the modulation of hyperlipdemia in Alcohol-induced liver cirrhosis in rats. Our results show that the hepatoprotective effects of *Costus afer* extract may be due to both an increase in the activity of the inhibition of lipid peroxidation. However, the protective, curative and antioxidant qualities of *Costus afer* need to be confirmed by characterizing the active ingredient(s) of this plant as well as its mechanism(s) of action.

UNDER PEER REVIEW

REFERENCES

[1]. Studenik P. Lipid disorders in liver diseases. VnitrLek 2000;46:547-8.

[2]. Gao B, Bataller R. Alcoholic liver disease: Pathogenesis and new therapeutic targets. Gastroenterology.2011;141:1572–85

[3]. O'shea RS, Dasarathy S, McCullough AJ. Alcoholic liver disease.Hepatology.2010;51:307–28.

[4]. de Alwis Wilfred NM, Day CP. Genetics of alcoholic liver disease and nonalcoholic fatty liver disease. Semin Liver Dis. 2007;27:44–54

[5]. Seth D, Haber PS, Syn WK, Diehl AM, Day CP. Pathogenesis of alcohol-induced liver disease: Classical concepts and recent advances. J GastroenterolHepatol.2011;26:1089–105.

[6]. Canbay A, Bechmann L, Gerken G. Lipid metabolism in the liver. Z GGasteroenterol.2007;45:35–41.

[7]. Jarike AE, Momoh JA. Plasma total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol levels in liver cirrhosis in Nigerians. NigQt J Hosp Med. 1996;6:157–9.

[8]. Iwu MM, Anyanwu BN. Phytotherapeutic profile of Nigerian herbs 1: anti inflammatory and anti-arthritic agents. J Ethnopharmacol 1982; 6(3): 263-274.

[9]. Iwu MM. Handbook of African medicinal plants. CRC Press, Boca Raton, Florida, United States.464 pp, 1993.

[11]. Costusafer. Ker Gawl. Record from Protabase. In: PROTA (Plant Resources of Tropical Africa) Wageningen, Netherlands. Available via http://database.prota.org/search.html (Accessed on 13 January 2013).

[12]. Anaga AO, Njoku CJ, Ekejiuba, ES, Esiaka MN, Asuzu IU. 2004. Investigations of the methanolic leaf extract of Costusafer Ker-Gawl for pharmacological activities in vitro and in vivo. Phytomedicine 2004;11(2–3): 242–248.

[13]. Anyasor GN, Ogunwenmo KO, Oyelana A, Akpofunure BE. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costusafer*Ker Gawl. (Costaceae).Afri J Biotechnol 2010; 9 (31), 4880- 4884.

[14].Taiwo AO, Bolanle AA. The leaf essential oil of custosaferkerGawl from Nigeria.Flavour Frag J 2003; 18:309- 311.

[15]. Moody, JO, Okwagbe KE. Topical anti-inflammatory activity of *Custosafer*.Topical anti-inflammatory activity of Costusafer.Nig J Nat Prod Med. 2003; 7:46-48.

UNDER PEER REVIEW

[16].Mistra HP, Fridovich I. The role of Superoxide anion in the autoxidation of epinephrine and a simple assay of Superoxide Dismutase. J BiolChem 1972; 247:3170- 3175

[17]. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-394

[18]. Vaswani M, Hemraj P, Desai NG, Tripathi BM. Lipid profile in alcohol dependence. Indian J Psychiatry.1997;39:24–8.

[19]. Onyesome I, Lamprou D, Sygellou L, Owusu-Ware S,Antonijevic M, Chowdhry B. Sirolimus encapsulated liposomes for cancer therapy: physicochemical and mechanical characterization of sirolimus distribution within liposome bilayers. Molecular Pharmaceutics. 2013;10(11):4281-4293.

[20] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;**18**:499-502.

[21] Oluba, O.M., Olusola, A.O., Eidangbe, G.O., Babatola, L.J. and Onyeneke, E.C. Modulation of lipoprotein cholesterol levels in Plasmodium berghei malarial infection by crude aqueous extract of *Ganoderma lucidum. Cholesterol. 2012,* [Pubmed] *Article ID 536396, 6 pages doi:10.1155/2012/536396.*

[22] Enemor, V.H.A., Anosike, J.C., Nwoke, B.E.B. and Chikezie, P.C. Serum aminotransferases and bilirubin levels in malaria patients. *International Journal of Natural and Applied Sciences.* 2005, *1(2), 85-89.*

[23]. C.S. Lieber, Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. ***Mt. Sinai J. Med.,* 2000, *67*, 84-94.**

[24]. R.S. Kumar, M. Ponmozhi, V. Periyasamy and N. Namasivayam, *Asia Pac. J. Clin. Nutr.,***2002***, 11*, 157- 163.

[25]. G. Pushpakiran, K. Mahalakshmi, P. Viswanathan and C.V. Anuradha, Ethanol-Induced Changes of The Lipid Profile in Rat. Kidney*Pharmacol. Rep.,* **2005**, *57*, 576-587.

[26]. K.R. Shanmugam, C.H. Ramakrishma, K. Mallikarjuna and K. Sathyavelu Reddy, Perturbation in kidney lipid metabolic profiles in diabetic rats with reference to alcoholic oxidative stress. *Indian J. Nephrol.,* 2009, *19*, 101-106.

[27]. K. Aruna, R. Rukkumani, P. Sureshvarma and V.P. Menon, Role of an aminothiazole derivative on ethanol- and thermally oxidized sunflower oil-induced toxicity.*Pol. J. Pharmacol. Pharm.,* 2004, *56*, 233-240.

UNDER PEER REVIEW

[28]. L. Ashakumari, P.L. Vijayammal and V.P. Menon, *Indian J. Exp. Biol.,* **1995**,.*31*, 270-274.

[29]. S. Yamada, R.M. Mark and C.S. Lieber, Pathogenesis, Diagnosis, and Treatment of Alcoholic Liver Disease. ***Gastroenterology,* 1985, *88*, 1799-1806.**

[30]. D.S. Jaya, J. Augustine and V.P. Menon., Protective Role of N-acetylcysteine Against Alcohol and paracetamol Induced Toxicity. *Indian J. Clin.Biochem.,***1994**, *9*, 64-71.

[31]. S.K. Das and D.M. Vasudevan, Effects Of Chronic Ethanol Exposure On Renal Function Tests AndOxidative Stress In Kidney*Indian J. Clin. Biochem.,***2008**, *23*, 4-9.

[32]. K. Husain, B.R. Scott, S.K. Reddy and S.M. Somani., Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. *Alcohol*, 2001, *25*, 89-97.