European Journal of Medicinal Plants
4(2): 158-170, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

An Evaluation of the Preventive Effect of the Methanol Extract of Thai Basil (*Ocimum basilicum L.*) on Ethanol-induced Hepatotoxicity in Rats

Saramma George[1*]

[1]*Department of Biomedical Sciences, School of Medicine, University of Botswana, Botswana.*

Author's contribution

The whole work was carried out by the author SG.

Original Research Article

Received 22nd August 2013
Accepted 5th November 2013
Published 3rd December 2013

ABSTRACT

Aim: The present study was investigated the preventive effect of methanol extract of *Ocimum bacilicum* (MOEB) on ethanol induced hepatotoxicity in rats.
Study Design: Male Wistar rats weighing 200–250 g were divided into six groups of six rats each as follows: the normal control rats we read ministered with distilled water(NC), the ethanol control was administered with ethanol (5 g/kg)(EC), extract treated group given only the extract (120 mg/ kg body)(ET) and experimental groups EX1 to EX3 were fed with ethanol (5 g/kg) plus MOEB in graded doses of 80 mg, 120 mg and 160 mg/kg body for 30 days.
Place and Duration of Study: This study was conducted at the Department of Biological Sciences (DBS) of University of Botswana between July 2009-June 2010.
Methodology: At the end of the experiment, rats were sacrificed; blood and liver tissue were collected to conduct the bioassays. From the blood collected, thiobarbituric acid-reactive substances (TBARS), reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were estimated together with the liver markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). From the liver tissue collected, about 10 small pieces were fixed in 10% formalin for histological preparations and the rest was used for bioassays such as TBARS, GSH, CAT and SOD.

**Corresponding author: E-mail: saramma.george@mopipi.ub.bw;*

Results: The results indicated that there were significant difference between the experimental rats (EX-groups) when compared with alcohol control (EC-group) in all the parameters checked and they were not significantly different from the normal control (NC-group). Again, the groups, treated with extracts only, did not show any negative effect, which clearly indicated that the extract had no toxic effects. A significant reduction in the levels of TBARS, ALT, and AST and a significant increase in the levels of GSH, CAT and SOD were noticed in EX groups when compared with the EC groups. Thus the results indicated a significant protection by these extracts against ethanol-induced hepatotoxicity ($P \leq 0.05$).
Conclusion: The present study showed that MEOB is a potent antioxidant agent in preventing and controlling the hepatotoxicity induced by ethanol and induce a protective effect by decreasing the oxidative stress and increasing the antioxidant status.

Keywords: MEOB; lipid peroxidation; oxidative stress; hepatotoxicity.

1. INTRODUCTION

Ethanol-induced tissue damage occurs in a variety of organs, including the liver, where ethanol is actively oxidized; extra hepatict issues in the rat also have been shown to exhibit oxidative damage following acute or chronic ethanol intoxication [1]. Oxidative stress plays a major role in various diseases such as atherosclerosis, alcoholic liver cirrhosis and cancer [2]. Oxidative stress occurs where there is an imbalance between the production of reactive oxygen species and the biological system's ability to readily detoxify the reactive intermediate or easily repair the resulting damage [3]. The liver, the largest internal organ of the body is a 1.3 Kg biochemical factory with an extraordinary variety of processes and products. It breaks a variety of foreign substances, some of which are converted into metabolic products thus playing a major role in detoxification processes [4]. The pathogenesis of liver injury caused by the abuse of alcoholic beverages, in spite of decades of research is, largely unknown. In recent years an increasing number of studies have shown that alcoholic patients and experimental animals exposed to ethanol display biochemical signs of oxidative damage suggesting the possible involvement of free radicals in causing some of the toxic effects of alcohol [5]. Ethanol induced tissue damage occurs in a variety of organs including the liver, because the ethanol metabolism is initiated in the liver tissue which produces acetaldehyde- a highly toxic molecule and then to acetate [6].

Oxidative stress is a key step in the pathogenesis of ethanol associated liver injury. Ethanol administration induces an increase in lipid peroxidation either by enhancing the production of oxygen reactive species or by decreasing the level of endogenous anti oxidants [7]. The reactive oxygen species (ROS) such as O_2^-, OH^- and H_2O_2, together with unstable intermediates in the peroxidation of lipids are well known inducers of cellular and tissue pathogenesis leading to numerous disease states including cardiovascular diseases [8] and age related degenerative conditions [9,10]. Neurodegenerative diseases such as Alzheimer's disease [11] and cancer [12] are also linked to damage from ROS as a result of an imbalance between the rate of generation of radicals and the scavenging of radicals. Formation of ROS takes place constantly in every cell during normal metabolic processes. Cellular sites for production of ROS include mitochondria, microsomes, and enzymes. The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against hepatic damage [13].

A number of plants have been shown to possess hepatoprotective property by improving the antioxidant status. Many plants with medicinal properties from different families investigated have the hepato protective properties [14]. The genus *Ocimum* of the family is Lamiaceae (Labiatae) is a group of approximately 150 aromatic plants found mainly in the tropical and subtropical regions of the world [15]. Many species of this genus are considered to be highly medicinal and have extensive applications in indigenous systems of medicine in many Asian, African, and South American countries. *O. basilicum* has been widely used in the Ayurvedic system of medicine for its various medicinal properties such as anticancer, antistress, hypoglycemic, hepato protective and its antifungal activities [16]. Nowadays there has been a revival of interest in plant derived drugs and there is a good scope for using herbal medicines possessing antioxidant property with adequate safety and efficacy as an alternative to synthetic drugs [17]. In this context, the present study has been designed to carry out to evaluate the hepato protective effects of *O. basilicum*on hepatotoxicity induced by ethanol intoxication.

2. MATERIALS AND METHODS

2.1 Plant Material

Tender parts of *O. basilicum* were collected locally from Botswana and the identification of the plant was done by Dr. M. P. Setshogo at the University of Botswana Herbarium (UCBA). The voucher specimen was submitted in the Herbarium and Voucher No. was given as (2006/B, A01) for *O. basilicum.*

2.2 Preparation of Extract

The plant was cut into small pieces and dried in the shade, then coarsely powered and soaked in 70% methanol for three days at room temperature. The extract was filtered and made solvent free by using Buchi type rotary evaporator at 65ºC and the yield ratio was 7.8% (W/W).

2.3 Animals

Male albino rats of Wistar strain of body weight ranging 200-250 grams were housed in colony cages at ambient temperatures of 25± 2ºC and 50-55% relative humidity with 12 hours light and dark cycle. They had water and food *ad libitum.* Experiment was conducted as per the internationally accepted principles for laboratory animal care unit of University of Botswana.

2.4 Chemicals

All the chemicals used were of analytical grade and bought from the Sigma-Aldrich Chemical Company, (St. Louis, MO) USA.

2.5 Experimental Design

Animals were divided into the following groups and all the groups had six rats each and the animals were grouped as follows:

Group 1: NC-Normal Control (rats received distilled water)

Group 2: EC-Ethanol Control (rats received ethanol 5gms/kg body weight.)

Group 3: ET-Extract treated ((rats received MEOB 120mg/kg body weight.)

Group 4: EX-1 Experimental rats (received ethanol 5gms/kg body weight + MEOB 80mg/kg body weight)

Group 5: EX-2 Experimental rats (received ethanol 5gms/kg body weight + MEOB 120mg/kg body weight)

Group 6: EX-2 Experimental rats (received ethanol 5gms/kg body weight + MEOB 160mg/kg body weight)

Treatment was carried out for 30 days and at the end of the experimental period the rats were sacrificed to collect the blood and liver tissue for histopathological observations and biochemical estimations.

2.6 Preparation of the Liver Homogenate

Rats were dissected and the liver was perfused with phosphate buffer saline through the hepatic portal vein. The lobes of the liver were collected and dried between filter papers to remove the excess blood. It was then cut with a heavy duty blade into small pieces and transferred to a glass Teflon homogenizing tube to prepare the homogenate (1 g, w/v) in phosphate buffer saline (pH7.4) in 4ºC. Then the liver tissue was centrifuged at 2000 rpm for ten minutes and the supernatant was taken for further use. About 10 pieces of each liver sample were kept in the fixative for histological preparations.

2.7 Biochemical Analysis

TBARS in plasma was estimated by the method described by Niehaus and Samuelsson [18] and the reduced glutathione was estimated by the method of Ellman [19].Superoxide dismutase was assayed by the method of Kakkar et al. [20].The assay of SOD activity was based on the principle of inhibitory effects of SOD on reduction of nitro blue tetrazolium dye by superoxide radicals. A single unit of enzyme was expressed as 50% inhibition of NBT (Nitro blue tetrazolium) reduction /min/mg protein. Catalase was estimated by the method of Hans Bisswagner [21]. AST ALT and ALP were estimated by using the kits from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA and the guidelines of the company were strictly followed. Protein was determined by the method of Lowry et al., [22] using Bovine Serum as standard, at 660 nm.

2.8 Histological Examination

The liver tissue was dissected out and fixed in 10% formalin and preserved. The tissue was processed after three days by dehydrating in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with Hematoxylin and eosin (H–E) dye for photo microscopic observations including cell necrosis, fatty deposit change, cell membrane degeneration, ballooning, and cytoplasmic degeneration.

2.9 Statistical Analysis

All data were expressed as the mean ±S.E. mean of n=6. Analysis of variance was performed by one way ANOVA and the significant difference between the means were determined by Holm-Sidak method. *p* value =0.05 was regarded as significant. In all these cases, Statistical Software Sigma stat, 3.1 was used to analyze the data.

3. RESULTS AND DISCUSSION

3.1 Blood Parameters

Table 1. Levels of lipid peroxidative indices

No	Groups	Parametres			
		Tbars (nmol/L) Plasma	AST (U/L) Plasma	ALT (U/L) Plasma	ALP (U/L) Plasma
1	NC	1.28 ± 0.08	40.16 ± 0.23	38.98 ±0.09	45.76 ± 0.04
2	EC	6.87 ±0.18 *	78.95 ± 0.41*	76.88 ±0.03*	88.56 ±0.17*
3	ET	1.27 ± 0.98	41.01 ± 0.53	37.82 ± 0.66	44.88 ±0.04
4	EX-1	1.32 ± 0.54	44.88 ± 0.23	41.23 ± 0.06	46.67 ±0.73
5	EX-2	1.28 ±0.23	40.43 ±1.01	38.88 ± 0.83	45.42 ±0.49
6	EX-3	1.27 ±0.41	39.85 ±0.03	37.98 ±0.09	43.87 ±0.18

n= 6in each group, shows significant difference and the results were given as mean ±SEM. P ≤0.05. NC-Normal Control rats; EC- Ethanol Control rats; ET-Extract treated rats; EX-1 Experimental rats ; EX-2 Experimental rats; EX-3 Experimental rats.*

Table 2. Levels of antioxidants

No	Groups	Parameters		
		GSH (mg/dl) Plasma	CAT (U/mg Hb) Haemolysate	SOD (U/mg Hb) Haemolysate
1	NC	35.07± 1.09	48.75 ±1.32	2.65 ± 1.17
2	EC	17.88 ±0.34*	28.48 ±1.19*	1.28 ±0.48*
3	ET	38.93 ±0.67	49.84 ±0.19	2.86 ±0.09
4	EX-1	33.56 ± 0.09	44.83 ±0.53	2.28 ±0.84
5	EX-2	35.88 ± 0.01	46.88 ±0.89	2.59 ±0.65
6	EX-3	36.21 ± 0.09	49.09 ± 0.85	2.76 ±1.09

n= 6in each group, shows significant difference and the results were given as mean ±SEM. P ≤0.05. NC-Normal Control rats; EC- Ethanol Control rats; ET-Extract treated rats; EX-1 Experimental rats ; EX-2 Experimental rats; EX-3 Experimental rats.*

3.2 In Liver Homogenate

Table 3. Levels of antioxidants and TBARS

No	Groups	Parameters checked			
		TBARS (nmol/1gm of wet tissue)	GSH (mg/gm wet tissue)	CAT (U/mg-^{1} protein	SOD (U/mg-^{1} protein
1	NC	2.28 ±0.08	73.17 ± 0.04	71.75 ±0.43	3.33 ±1.17
2	EC	6.97 ± 0.06*	43.75 ±0.09*	38.48 ±0.08*	1.89 ±1.23*
3	ET	2.22 ± 0.14	75.83 ± 0.04	72.43 ±1.12	3.35 ±.09
4	EX-1	2.29 ±0.12	70.89 ±0.06	69.09 ±0.02	3.12 ± 0.34
5	EX-2	2.26 ±1.02	72.57 ± 0.17	70.98 ±0.92	3.32 ±0.05
6	EX-3	2.24 ±0.05	74.32 ±0.43	72.08 ±0.45	3.34 ±0.3

n= 6in each group, shows significant difference and the results were given as mean ±SEM. P ≤ 0.05. NC-Normal Control rats; EC- Ethanol Control rats; ET-Extract treated rats; EX-1 Experimental rats ; EX-2 Experimental rats; EX-3 Experimental rats.*

3.3 Hisopathological Studies

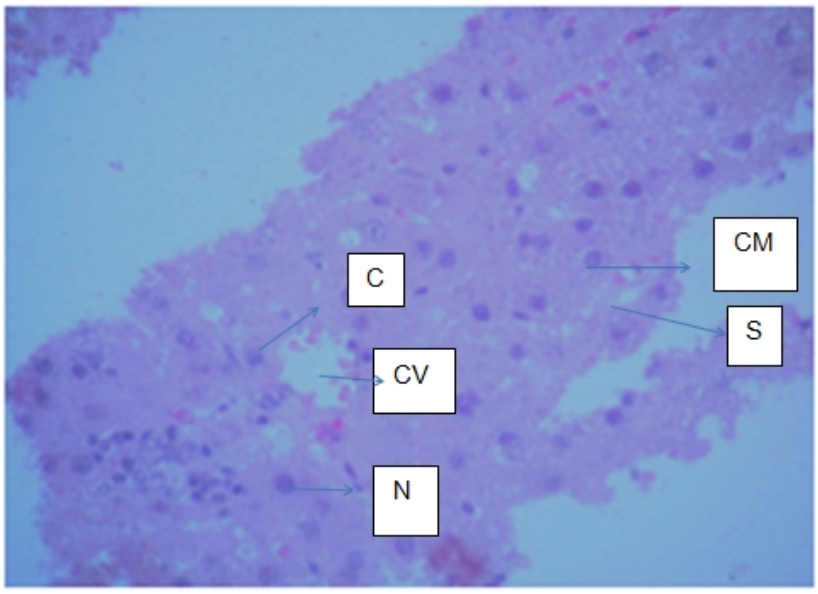

Fig. 1. Normal Control (NC)

(HE stain, 400Xmagnification). Normal hepatic cells with well-preserved cytoplasm; well brought out central vein; prominent nucleus and nucleolus. The hepatocytes were intact with proper cell membrane and sinusoids.
N-Nucleus, CM-Cell membrane, S- sinosoids, CV-Central vein, C-Cytoplasm.

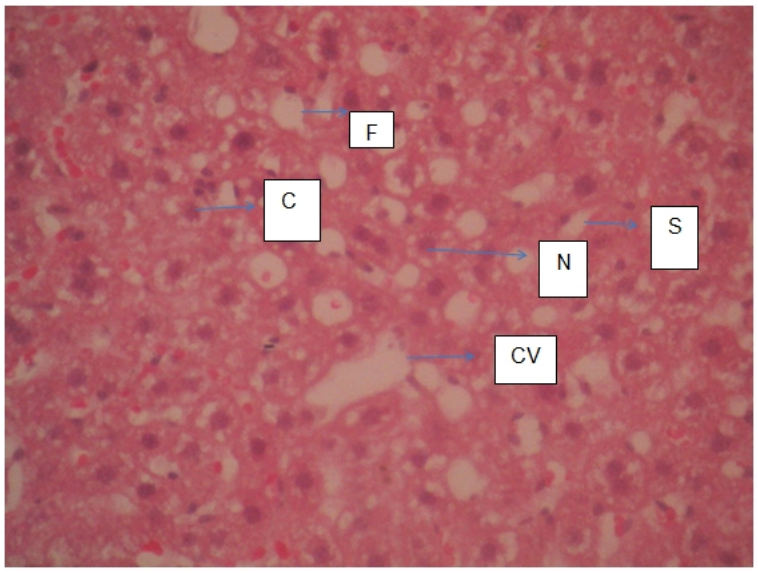

Fig. 2. Ethanol Control (EC)

(HE stain, 400X magnification). Massive fatty changes necrosis, ballooning degenerative and the loss of cellular boundaries. The hepatocytes had undergone lipid peroxidation as a result the cells lost their limiting membrane structures. Central vein distorted
F-Faty ballooning, N-Nucleus, C-Cytoplasm, CV-Central vein, CM-Cell membrane, S- sinosoids

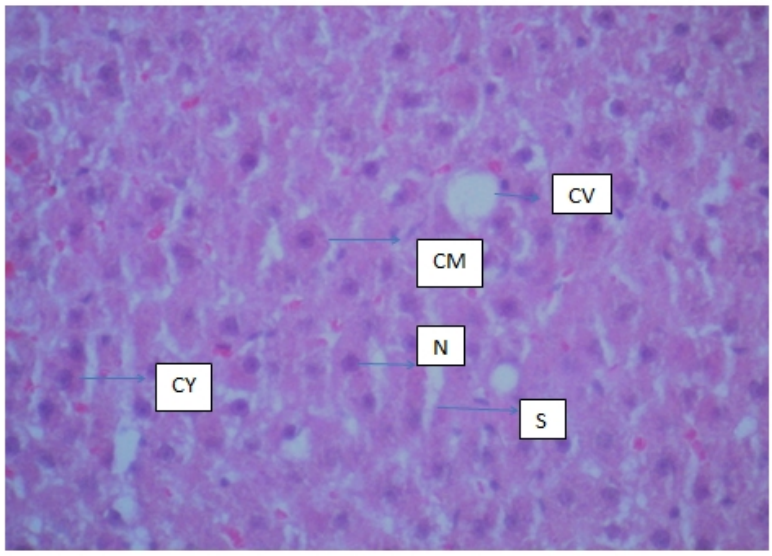

Fig. 3. Extract Treated (ET*)*

(HE stain, 400X magnification). Hepatic cells with well-preserved cytoplasm ,prominent nucleus and nucleolus. The normal architecture of the liver tissue without any alterations.
N-Nucleus, C-Cytoplasm, CV-Central vein, CM-Cell membrane, S- sinosoids

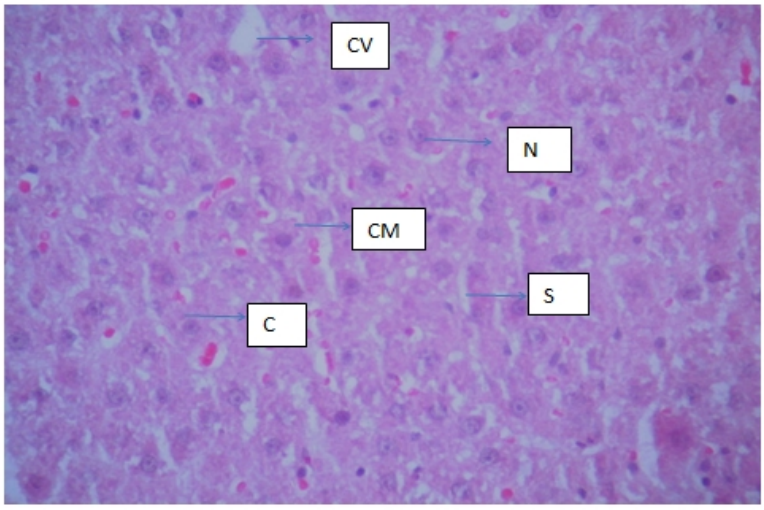

Fig. 4. Extract Treated (80 mg/kg body wt+ ethanol) (EX1)

(HE stain, 400X magnification). Some of the cells are showing the degenerative effect, the sinosoids are widened and the central veins are distorted. The effect of the ethanol is shown here, but the extract is excreting the preventive action. The present dose was not enough to control the ethanolic effect. N-Nucleus, C-Cytoplasm, CV-Central vein, CM-Cell membrane, S- sinosoids

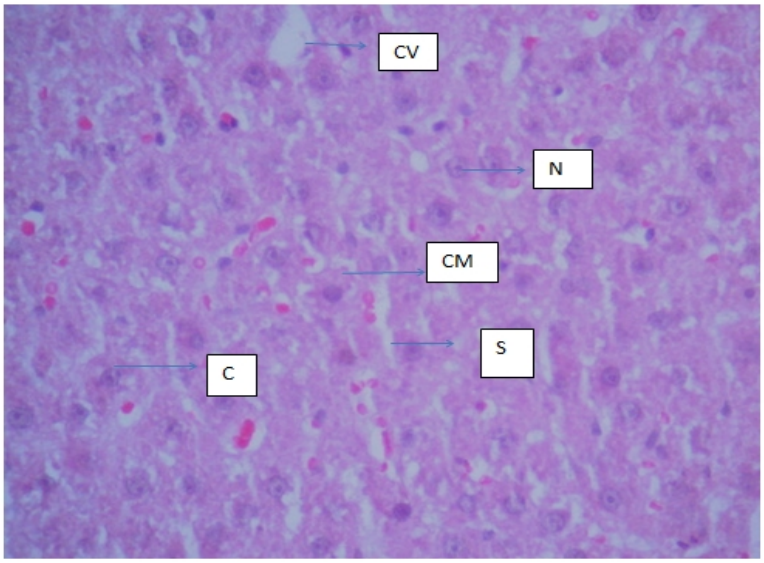

Fig 5.Extract Treated (120 mg/kg body wt + ethanol)(EX2)

(HE stain, 400X magnification). Normal hepatic condition: Hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus. Sinosoids still shows widening, with well-maintained central vein. N-Nucleus, C-Cytoplasm, CV-Central vein, CM-Cell membrane, S- sinosoids

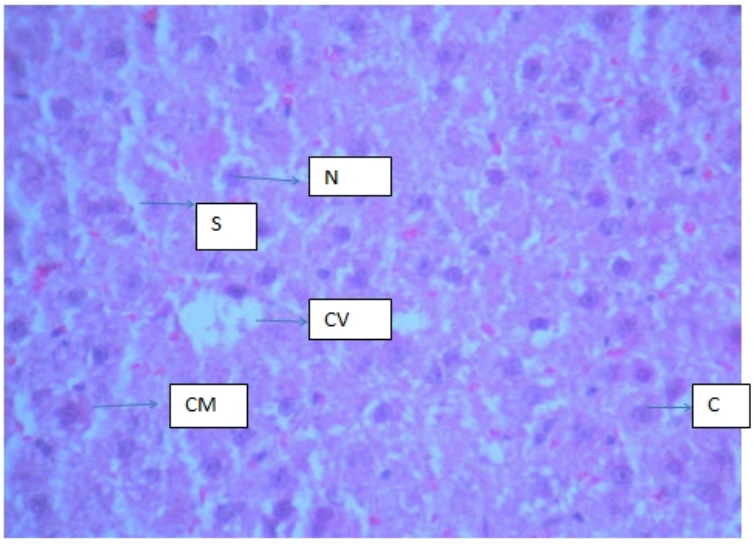

Fig. 6. Extract Treated (160 mg/kg body weight + ethanol) (EX 3)
(HE stain, 400 x magnification). Normal hepatic condition: Hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus. Normal sinosoids with well-maintained central vein. N-Nucleus, C-Cytoplasm, CV-Central vein, CM-Cell membrane, S- sinosoids

Ethanol toxicity is closely related to its metabolism in the liver. Ethanol is metabolized into acetaldehyde by alcohol dehydrogenase (ADH)in the liver generates NADH and increased production of ROS in different organelles [23]. Later this acetaldehyde is oxidizedintoacetate by aldehyde oxidase or xanthine oxidase giving rise to ROS via Cytochrome P 450 2EI, excess ROS production plays an important role in the development of lipid per oxidation [24]. The elevated NADH/NAD ratio results in alterations of the intermediary metabolism of lipids, carbohydrates, proteins, purines, hormones and porphyrins [25]. The ethanol-associated redox changes are pronounced in the perivenular zone, since this is the area of low oxygen tension and of high ADH activity [26].

The microsomal enzyme oxidizing system (MEOS) is an alternate pathway for alcohol metabolism in the liver. Microsomal enzymes belong to a family of proteins called cytochromes. Some cytochromes, located in a cellular substructure called the endoplasmic reticulum detoxify harmful substances that enter the body. The MEOS oxidizes alcohol to acetaldehyde by means of a cytochromeP450 2E1, or CYP2E1, which is found in the endoplasmic reticulum of liver cells. Normally functioning at a low level, CYP2E1 is induced in higher level by the presence of alcohol [28]. Thus, the MEOS becomes increasingly important as alcohol consumption becomes heavier and chronic. After chronic ethanol consumption, the activity of the microsomal ethanol-oxidizing system (MEOS) increases, with an associated rise in cytochromes P-450, especially CYP2E1. This induction is associated with proliferation of the endoplasmic reticulum, both in experimental animals and in humans. Ethanol metabolism generates oxygen radicals, which inhibits GSH synthesis and depletes GSH levels in tissues, increase MDA levels and generally impair the anti oxidative defense system in humans and experimental animals [28]. Lipid peroxidation, as reflected in TBARS values were higher in all ethanol treated groups when compared to normal controls clearly indicate the oxidative stress in hepatic and extra-hepatic tissues [28] induced by ethanol and its oxidation.

A significant increase in the levels of liver markers (ALT and AST) were observed in ethanol treated groups, this may be due to the consequence of change in membrane phospholipids composition caused by the peroxidation process which in turn increase the degree of membrane permeability (P≤0.0001).Treatment with MEOB helped to prevent the above condition in EX –groups in a dose dependent manner. The administration of ethanol together with the extract could reduce the effect of ethanol and the lipidperoxidation caused by the metabolism of ethanol [30].

GSH is a critical factor that determine the susceptibility of tissues to oxidative damage and marked depletion of GSH occur in a variety of tissues after acute and chronic ethanol intoxication. Liver cells contain an abundance of glutathione, especially in mitochondria, where the energy is synthesized. The key enzymes in mitochondria are certain cytochromes that are integral components of the inner mitochondrial membrane. Like CYP2E1, these cytochromes can produce free radicals hence the need for antioxidant protection. Glutathione is not synthesized in mitochondria; they are supplied from the cytoplasm by active transport through the mitochondrial membrane. Alcohol interferes with the transport of glutathione through membranes, leading to its depletion from mitochondria [31]. Ethanol is believed to be generating free radicals which inhibit GSH synthesis and deplete GSH levels in tissues, partly a result of binding of cysteine in GSH by acetaldehyde. CAT acts as a preventive antioxidant which plays an important role in protection against the deleterious effects of lipid peroxidation. The inhibition of CAT activity suggest the enhanced synthesis of superoxide ions during ethanol ingestion since superoxide can be a powerful inhibitor of CAT. Normally in haemolysate both catalase and glutathione peroxidase work hand in hand to remove H_2O_2 from the physiological systems. CAT needs to be regarded as the major H_2O_2 decomposing enzyme in normal erythrocytes. SOD scavenges the superoxide ions produced as cellular by-products during ethanol metabolism [38]. The reduced activity of the SOD in ethanol treated groups results in the accumulation of superoxide radical in tissues which produces the oxidative stress. SOD catalyzes the dismutation reaction of the toxic superoxide radical to molecular oxygen and hydrogen and thus forms a crucial part of the cellular antioxidant defense system [32]. Superoxide anions indirectly initiate lipid peroxidation as a result of superoxide and hydrogen peroxide, serving as precursors of singlet oxygen and hydroxyl radicals. In the present study in Table 2 a significant decrease in plasma GSH, CAT and SOD were observed in ethanol treated groups when compared to normal control groups and in all the experimental groups the levels of GSH were not significantly different from the NC group. In all the three experiments the alcohol treated groups shows significant difference when compared with the normal control (P≤0.0001) and there was no significant difference with the experimental groups (EX 1-3) [33].

The histopathological studies also supported the biochemical estimations; The structural architecture of the hepatic tissue was similar in normal control (NC) extract treated (ET) and EX3 groups. The EC group showed the fatty deposits due to excessive alcohol metabolism. The primary cause of fatty liver syndrome induced by ethanol are the altered NADH/NAD^+ levels that in turn inhibits gluconeogenesis, inhibits fatty acid oxidation and also inhibits the TCA cycle. Each of these inhibited pathways will lead to excess production of acetyl coA which finally end up in fatty acid synthesis. Again the large amount of acetate produced as intermediate also lead to the production of acetyl CoA which will be leading into the biosynthesis of fatty acids and cholesterol. As a result of all these the ethanol treated group showed the hepatic steatosis and alteration of the normal architecture. About the EX1 and EX2 the treatment was given with extract and the ethanol for thirty days and the results

showed some degree of prevention of the adverse effects of ethanol metabolism but the treatment either needed more time or higher doses [37,38].

All the parameters checked in liver tissue also showed the same type of antioxidant status, so the results obtained in TBARS levels were significantly increased ($P \leq 0.0001$) in EC groups when compared to all other groups. Again there was no significant difference between the experimental groups in all the parameters checked. So also the levels of GSH, SOD and CAT were significantly reduced between EC groups and all other groups [37]. The histopathological observations also showed the changes in the membrane structure in par with the observations of the blood and liver tissue biochemical estimations. Some degree of fat deposition in the liver occurs in almost all heavy drinkers. It also may occur transiently in non-alcoholics after a single drinking session

4. CONCLUSION

In conclusion the use of antioxidants is an important preventive method to prevent the patho physiological effects of oxidative stress. Thus from the data generated from this work, MEOB is a potent antioxidant agent in controlling the toxicity induced by ethanol metabolism on liver tissue and exerts its protective effect by decreasing the lipid peroxidation and improving the antioxidant status.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENT

Author is thankful to the Office of Research and Development Committee of the University of Botswana for providing fund to carry out this study.

The author hereby declares that "Principles of laboratory animal care" (published in 2005) were followed. All experiments have been examined and approved by the appropriate ethics committee."

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408:831-49.
2. Lieber CS. Ethanol metabolism, cirrhosis and alcoholism. Clin Chim Acta. 1997;257:59–84.

3. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet. 1994;344:721–724.
4. Chaturvedi P, George S, John A. Preventive and protective effects of wild Basil in ethanol–induced liver toxicity in rats. Br. J. of Biomed. Sci. 2007;64(1):10-12.
5. Montoliu C, Valle`s S, Renau-Piqueras J, Guerri C. Ethanol induced oxygen radical formation and lipid peroxidation in rat brain. Effect of chronic alcohol consumption. J Neurochem. 1994;63:1855–1862.
6. Saravanan N, Nalini N. Antioxidant Effect of *Hemidesmus indicus* on Ethanol-Induced Hepatotoxicity in Rats. J. Med Food. 2007;10(4):675-82.
7. Lieber CS. Hepatic, metabolic and nutritional disorders of alcoholism: from pathogenic to therapy. Crit Rev Clin Lab Sci. 2000;37(6):551-84.
8. Nordmann R, Ribière, C, Rouach H. Implication of free radical mechanisms in ethanol-induced cellular injury. Free Radical Biology and Medicine.1992;12:32:219–240.
9. Witztum JL. The role of oxidized low density lipoproteins in atherigenesis. British Heart Journal. 1993;69:12-14.
10. Husain K and SM Somani. Interaction of exercise and ethanol on hepatic and plasma antioxidant system in rat. Pathophysiol. 1997;4:69-74.
11. Richardson SJ. Free radicals in the genesis of alzheimer's disease. Annals of New York Academy of Science. 1993;695:73-76.
12. Borek C. Free radical processes in multistage carcinogenesis. Free Radical Research Communications. 1990;12:745–50.
13. George S and Chaturvedi P. Protective role of *Ocimum canum* plant extract in alcohol-induced oxidative stress in albino rats. 2008;65(2):1-6.
14. Chatterjee TK. Medicinal plants with hepatoprotective properties In: Herbal Options, 3rd ed. Books & Allied (P) Ltd., Calcutta. 2000;135–137.
15. Manikandan P, Murugan RS, Abbas H, Abraham SK, Nagini S. *Ocimum sanctum* Linn. (holy basil) ethanolic leaf extract protects against 7,12-dimethylbenz[a]anthracene-induced genotoxicity, oxidative stress, and imbalance in xenobiotic-metabolizing enzymes. J Med Food. 2007;10:495–502.
16. Beric T, Nicolic B, Stanojevic J, Vucovic-Gacic B, Knezevic-Vukcevic J: Protective effect of basil (*Ocimum basilicum L.*) against oxidative DNA damage and mutagenesis. Food Chem Toxicol. 2007;46:724–732.
17. Akinmoladun AC, Ibukun EO, Emmanuel A, Obuotor EM, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum.* Sci Res Essay. 2007;2:163-6.
18. Niehaus WG, Samuelson B. Formation of MDA from phospholipid arachidonate during microsomal lipid peroxidation. Eur. J. Biochem. 1986;6:126–130.
19. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70–77.
20. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21:130–132.
21. Bisswanger H. Practical Enzymology. Wiley-VCH Verlag GmbH & Co., Weinheim. 2004;79.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;93.
23. Kono H, Rusyn I, Yin M, Yamashina SG. NADPH oxidase-derived free radicals are key oxidants in alcohol-induced liver disease. J. Clinic. Invest. 2000;106:867–872.
24. Reinke LA, Moore DR, Hague CM, McCay PB. Metabolism of ethanol to 1-hydroxyethyl radicals in rat liver microsomes: comparative studies with three spin trapping agents. Free. Radic. Res. 1994;24(4):213-22.
25. Lieber CS. Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev. 1999;77:517–544.

26. George S, Chaturvedi P. A comparative study of the antioxidant properties of two different species of *Ocimum* of Southern Africa on alcohol induced oxidative stress. J. Med. Food. 2009;12(5):1154-58.
27. Gopi S, Setty OH. Beneficial effect of the administration of *Hemidesmus indicus* against bromobenzene induced oxidative stress in rat liver mitochondria. J Ethnopharmacol. 2010;127(1):200-03
28. Geetha RK, Vasudavan DM. Inhibition lipid peroxidation by botanical extracts of *Ocimum sanctum in vivo* and *in vitro* studies: Life Sci. 2004;19:76(1):21-28.
29. George S, Chaturvedi P, Moseki B. A comparative study of the restorative effects of *Ocimum gratissimum* on alcohol induced hepatotoxicity in albino rats.. FANAD. 2011;11(7)56:14-28.
30. Gülçin I, Elmastas M, Aboul-Enein HY. Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum*) assayed by different methodologies. Phytother. Res. 2007;21(4):354-361.
31. George S, Caturvedi P. Protective role of *Ocimum canum* (OCM) plant extract on alcohol induced oxidative stress in albino rats. Br. J. of Biomed. Sci. 2008;65(2):80-85.
32. Anita Murali, Purnima Ashok, Varadharajan Madhavan. Antioxidant activity of leaf of *Hemidesmus indicus* (L.) R. Br. var. *pubescens* (W.& A.) Hk.f. (Periplocaceae)-an *in vivo* analysis. Spatula DD. 2011;(2):91-100.
33. Astiz M, MJ de Alaniz, Marra CA. Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. Environ. Toxicol. Pharmacol. 2009;28**:**465-473.
34. Shivasankara AR, Azmidash A, Haniadha R, Rai MP, Arora R, Baliga MS. Dietary agents in the prevention of alcohol-induced hepatotoxicty: preclinical observations. Food Funct. 2012;3(2):01-9.
35. Galilia AY, Nihal ME, Eman F El A. Hepatoprotective effect of basil (*Ocimum basilicum* L.) on CCl_4- induced liver fibrosis in rats. African Journal of Biotechnology. 2012;11(90):15702-11.
36. Meera R, Devi P, Kameswari B, Madhumitha B, Merlin NJ. Antioxidant and hepatoprotective activities of *Ocimum basilicum* Linn. and *Trigonella foenum-graecum* Linn. against H_2O_2 and CCL_4 induced hepatotoxicity in goat liver. Indian Journal of Experimental Biology. 2009;47:584-590.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=362&id=13&aid=2642