An *In-Vivo* Analysis of the Protective Effect of the Methanol Extract of *Ocimumbasilicum* (L.)(MAEOB) on Ethanol-Induced Oxidative Stress in Albino Rats.

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ABSTRACT

This study was conducted to evaluate the protective effects of graded doses of methanol and aqueousextract of Ocimumbasilicum(L.)(MAEOB) onthe oxidative stress induced by ethanol toxicity. Forty male albino rats of Wister strain were divided into eight groups of five rats each andfour groups were named as positive control(PC) and another four as experimental groups(EX). The experimental groups(EX) were treated with graded doses of extract together with alcoholand a pretreated group , the treatment was carried out for 30 days and the blood and liver tissue were collected for histopathological and biochemical estimations.In the four experimental groups,the biochemical estimations indicated that the extract could control and prevent the toxic effects produced during alcohol metabolism in a dose dependent manner. The results indicated that there were no significant difference between the EX group and normal control in all the parameters checked. The control groups were used to compare the results with all the parameters checked.The results were compared with the pretreated groups in both PC and EX groups showed that there was a slight improvement on the antioxidant status in the pretreated groups. This result was also supported by the biochemical estimations and the histopathological studies of the liver tissue.The use of antioxidants is an important preventive method to minimize the pathological and toxic effects of oxidative stress induced by alcohol. Ocimumbasilicumis a naturally occurring medicinal herb with antioxidants, which can be effectively used in preventing the harmful effects of ethanol toxicity without many side effects.

KEYWORDS: *hepatotoxicity, lipid peroxidation, methanoland aqueous extract of Ocimumbasilicum (MAEOB), oxidative stress,phytochemicals.*

INTRODUCTION

Oxidative stress plays an important role in the adverse effects of ethanol-associated liver injury. Ethanol administration induces an increase in lipid peroxidation either by the production of oxygen reactive species or by decreasing the level of endogenous antioxidants and thereby creating oxidative stress [1].Alcohol abuse is a major problem worldwide. Being a small molecule and soluble in both water and lipids, ethanol penetrates to all tissues of the body and affects most vital functions of virtually all organs, including the liver, kidney, brain, heart and pancreas [2]. Most ingested toxic substances, such as ethanol, must first be broken down by the liver, which possesses special enzymes not present in other tissues. Enzymes in the liver first oxidize ethanol (CH_3CH_2OH) to acetaldehyde (CH_3CHO), removing two hydrogen atoms and reducing a molecule of $NADP^+$. Hepatocytes contain three pathways for ethanol metabolism: the alcohol dehydrogenase (ADH) pathway in the cytosol: the microsomal ethanol oxidizing system (MEOS) located in the smooth endoplasmic reticulum and the catalase pathway located in peroxisomes.About 90% of alcohol in the blood is metabolized in the liver and the other 10% is eliminated through the lungs and in the urine. If the alcohol is consumed at a faster rate than the liver can break it down, the blood alcohol concentration will rise. This may result in some of the immediate effects seen in people who have consumed excess alcohol[3].

In spite of decades of research the after- effects of liver injury caused by the abuse of alcoholic beverages are still 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 [4].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 diseaseand age related degenerative conditions[5].Neurodegenerative diseases such as Alzheimer's disease and cancer are also linked to damage from ROS as a result of an imbalance between the rate of generation of free radicals and the scavenging of these radicals. The cellular radical –scavenging systems include enzymes such as superoxide dismutase (SOD), which scavenges the superoxide ion by speeding up its dismutase, catalase (CAT), a haeme enzyme, which removes hydrogen peroxide and glutathione peroxidase (GPX), a selenium-containing enzyme, which scavenges other peroxides as well as hydrogen peroxide [6].Other molecules that can counteract ROS include glutathione, flavonoids, ubiquinol-10, glucose and albumin. External sources of antioxidative protection include antioxidant vitamins C, E, β-carotene and carotenoids as well as minerals such as selenium and zinc [7]. Changing one's life style and moving towards the natural diet can control many of the physiological problems [8]. Again the alcohol-induced hepatic injury might be caused by minor genetic abnormalities due to single nucleotide polymorphism(SNP) that predisposes people but this is still under investigation.

For a long time, plants have been a valuable source of natural antioxidant products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies [9].Plant extracts and phytochemicals derived from medicinal plants are used extensively in pharmaceutical products. A diet rich in antioxidants is recommended for health maintenance.Although most relevant studies have suggested that the supplementation of antioxidants could suppress ethanol-induced liver injury; there is no antioxidant that has been advised for clinical treatment.In the absence of a reliable liver -protective drug in the modern medicine,there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders: therefore such plants should be investigated to better understand their properties, safety and efficiency.Indian medicinal plants belonging to about 40 families were investigated for liver protective drugs[10].One of the families referred to above is Labiateae, which includes the basil family. The objective of this study was therefore to evaluate the efficacy of *Ocimumbasillicum* for its hepatoprotective properties by preventing the free radical formation and the damage caused by the toxicity of ethanol metabolism. Also checking the

pretreatment with these extract can protect the damage caused by ethanol toxicity or boost up the intrinsic antioxidant system.

METHODOLOGY

Plant Material

Tender parts of *O. basilicum*were collected locally from Botswana and the identification was done by Dr. M. P. Setshogo at the University of Botswana Herbarium (UCBA). The voucher specimen was submitted to the herbarium and voucher no. Was given as (2006/G, A02) for *O. basilicum.*

Preparation of Extract

The plant was cut into small pieces and dried in the shade, then coarsely powered and soaked in 70% +30%methanol and distilled water (MAEOB) for three days at room temperature. The extract was filtered and made it solvent free byBuchi type rotary evaporator at 65 ^{o} C and the yield was 7.8% (W/W).Extract was administered to the rats orally after dissolving it in the drug carrier (distilled water + tween-80 in the ratio of 9:1) with rubber tubing attached to a syringe.

Animals

Male albino rats of Wistar strain (*Rattusnorvegicus*) of body weight ranging 200-250 grams were housed in colony cages at ambient temperatures of 25 ^{o} C ± 2 ^{o} C 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.

Chemicals

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

Experimental Design

This experiment was designed to check the protective effects of MAEOB on antioxidant status and theliver tissue in ethanol-induced toxicity. This experiment was conducted in a completely randomized design with 40 male albino rats of Wistar strain into 8 groups of 5 rats each and the groups were as follows:

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

Group 2: PC-2- AC-Alcohol Control (rats received ethanol 5g/Kg body weight).

Group 3: PC-3–EC- Extract control (rats administered with the extract only.100mg/Kg body weight.)

Group 4: PC-4PEC – Pretreated extract control (rats administered with the extract only100mg/Kg body weight for 30 days and continued the treatment)

Group 5: EX-1 Experimental rats Pretreated with the extract (100mg/Kg body weight) for 30 days and administered ethanol (5g/Kg body weight) plus extract (100mg/Kg body weight).

Group 6: EX-2 Experimental rats administered ethanol (5g/Kg body weight) plus extract (100mg/Kg body weight).

Group 7: EX-3 Experimental rats administered ethanol (5g/Kg body weight) plus extract (120mg/Kg body weight).

Group 8: EX-4 Experimental rats administered ethanol (5g/Kg body weight) plus extract (140mg/Kg body weight).

The pre-treated groups were given the extract for 30 days before the experiments. Treatment was carried out for 30 days and at the end of the experimental period the rats were sacrificed to collect the blood and the liver tissue for histopathological observations and biochemical estimations.

BIOCHEMICAL ANALYSIS

Preparation of the liver homogenate

Rats were dissected and the liver was perfused with phosphate buffer saline (PBS) through the hepatic portal vein. The liver lobes were collected and pressed gently between filter paper to remove the blood. The liver lobes

were later cut into smaller pieces and then transferred to the tubes of the homogenizer and cold PBS was added. The mixture was centrifuged at 2000rpm for 10 minutes and the supernatant was collected and finally suspended in 2.5 ml of PBS to be used later for biochemical estimations.

Acute toxicity study

An acute toxicity study was conducted on MEOB according to OECD guidelines[11].

Estimation of haemoglobin

The haemoglobin content of the blood was measuredwith Sahlihaemometer and method described in the instructions given with the instrument was followed and calculation was done as described in the method.

TBARS was estimated by the method described by Niehaus and Samuelsson (1986)[12] and the reduced glutathione was estimated by the method of Ellman (1959)[13]' Superoxide dismutase was assayed by the method of Kakkar*et al., (*1984)[14].A single unit of enzyme was expressed as 50 % inhibition of NBT (nitroblue tetrazolium) reduction /min/mg protein. Catalase was estimated by the method of Hans Bisswagner, (2004)[15] and∝- tocopherol(Vit –E) was estimated by the method of Martinek. (1964)[16].Ascorbic acid(Vit –C) was estimated by the method of Roe (1961) [17] with slight modification. Protein was determined by the method of Lowry *et al.*, (1951)[18] using bovine serum as standard, at 660 nm. Alanine aminotransferase (ALT), alanine aspartatetransaminase (AST) total proteinand bilirubin were estimated by the kits bought from Sigma and the manufacture's guidelines were followed[19,20].

Histological examination

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. The lobes were cut in to smaller pieces with a heavy duty blade and transferred them to 10% formalin as the fixative.The liver tissue was, dehydrated 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 microscopic observations including cell necrosis, fatty deposit change, cell membrane degeneration, ballooning, and cytoplasmic degeneration[21].

Statistical analysis

All data were expressed as the mean ±S.E. mean of n=5. Analysis of variance was performed by one way ANOVA and the significant difference between the means were determined by the Holm-Sidak method. The p value ≤ 0.05 was regarded as significant. In all these cases, Statistical Software Sigmastat, 3.1 was used to analyze the data.

RESULTS

The overall results indicated that there was significant protective effects of MAEOB were shown in all the parameters checked when the EX groups were compared with the PC groups. There was significant difference between the levels of TBARS in the NC group and the AC group ($P \leq 0.05$) as well as the levels ALT and AST. In rats treated with MAEOB (EX group) only, the levels of TBARS, ALT and AST were significantly lower than the levels in the EC group ($P \leq 0.05$) and were not significantly different from those of NC group. The levels of enzymatic antioxidants such as SOD and CAT in blood also showed significant differences between the NC and the EC groups ($P \leq 0.05$) which indicate the decline of the enzymatic activity in the AC group when the animals were in oxidative stress due to ethanol toxicity. However the experimental groups did not show significant difference with NC group. The effects of MAEOB non enzymatic antioxidants demonstrate that in the AC group the non- enzymatic antioxidants such as Vit E, Vit C and GSH levels were diminished by 42-66% but the EX groups did not show any significant difference when compared with the NC groups. There were significant protective effect in the pretreated groups and has shown some increase in the antioxidant status in all the parameters checked.

Acute toxicity studies

MAEOB was found to be practically non-toxic when tested and the lethal was higher than 2500mg/kg bw.

BIOCHEMICAL ESTIMATIONS

TABLE 1: Assessment of liver function indices in blood

GROUPS	TBARS (nmol/L) Plasma	ALT (U/L) Plasma	AST (U/L) Plasma	ALP (U/L) Plasma	TB (mg/dl) Plasma	TP (mg/dl) Plasma
NC	1.28±0.08	40.16±1.18	39.78±2.52	60.38±0.58	11.98±2.03	61.17±0.13
AC	6.87±0.18*	78.01±1.26*	82.08±.056*	85.73±0.22*	18.28±0.08*	45.05±0.08*
EC	1.18± 0.56	39.15±0.78	35.78±.54	55.18±.054	11.28±0.21	62.43±0.61
PEC	0.98±0.34	36.56±0,98	34.53±0.87	53.12±0.76	11.09±0.56	62.75±0.43
EX-1	0.93±065	37.86±0.77	35.88±0.64	54.84±0.82	11.22±0.34	62.66±0.09
EX-2	1.29±.12	44.65±0.08	43.18±.56	65.48±.94	13.58±0.12	59.09±0.65
EX-3	1.27±.02	42.15±0.38	41.73±1.52	59.72±.34	12.22±0.36	60.04±0.21
EX-4	1.26±0.45	40.05±0.58	38.28±0.59	58.43±.84	11.28±0.23	61.98±0.63

TBARS: Thiobarbituric acid reactive substances, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase,ALP: Alkaline phosphatase, TB Total bilirubin, TP: Total protein.* Significantly different,n= 5 in each group and the results were given as mean ±SEM.

The results reflected in (Table 1) shows the liver function indices of alanine aminotransferases (ALT), aspartate amino transferases (AST), alkaline phosphatases (ALP), total bilirubin(TP) and total protein (TP) concentrations in the serum after 30 days of oral administration of the extract MAEOB. There was a significant (P<0.05) increase in the activities of all these liver marker enzymes (ALT, AST and ALP) in the ethanol -induced control group (AC) when compared with the NC and EC groups. The EX groups did not show any significant difference in the liver marker enzymes when compared with the NC and the EC groups.

TABLE 2: Antioxidant status in blood

GROUPS	GSH(mg/dl) Plasma	SOD (U/mg Hb) Haemolysate	CAT (U/mg Hb) Haemolysate	VITC (mg/dl.) Plasma	VIT E (mg/dl.) Plasma
NC	35.07±0.09	2.68±1.17	48.75±1.32	2.37±0.18	2.28±0.14
AC	17.95±0.97*	1.28±1.21*	28.48±1.19*	1.38±0.18*	0.94±1.23*
EC	36.09±0.57	2.98±0.91	49.18±1.09	2.56±0.15	2.31±0.08
PEC	38.98±0.87	3.09±0.65	49.88±1.02	2.86±0.21	2.78±0.56
EX-1	37.94±0.54	3.01±0.78	48.65±0.88	2.76±1.09	2.77±0.31
EX-2	33.01±0.64	2.28±0.48	44.66±1.13	2.12±0.1.38	2.21±0.1.32
EX-3	34.08±1.78	2.42±0.41	46.88±0.06	2.35±0.19	2.23±0.27
EX-4	35.04±0.88	2.62±1.01	47.33±0.26	2.36±0.07	2.27±0.78

GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase,VIT E:Vitamin E, VIT C: Vitamin C, * significantly different. n= 5in each group and the results were given as mean ±SEM.

The antioxidant status in the blood included the non-enzymatic antioxidants such as GSH, Vit E and Vit C as well as the enzymatic antioxidants such as CAT and SOD (Table 2). The results indicate that all the parameters checked showed a significant decrease in EC groups when compared to NC groups ($P \leq 0.05$). The EX groups did not show any significant difference between the NC and the EC groups but they showed slight a variation in a dose dependent-manner.

TABLE 3: Protective effect of MAEOB on liver tissue

GROUPS	TBARS (nmol/ 1gm of wet tissue)	GSH mg/gm wet tissue)	CAT (U/mg-^{1} protein	SOD (U/mg-^{1} protein	VIT E mg/gm wet tissue)	VIT-C mg/gm wet tissue)
NC	2.28±0.28	75.07±0.09	71.75±1.12	3.38±1.17	5.81±0.14	1.23±0.44
AC	6.87±0.18*	32.88±0.16*	38.48±1.09*	1.08±1.18*	2.18±0.11*	0.93±1.06*
EC	2.18±0.12	79.08±1.18	72.28±0.76	3.45±0.78	5.83±1.03	1.25±0.56
PEC	2.11±0.78	80.88±0.54	76.98±0.09	3.88±0.43	5.98±0.08	1.87±0.03
EX-1	2.03±0.98	79.21±1.09	73.76±0.21	3.56±0.94	5.65±0.09	1.34±0.77
EX-2	2.35±0.15	73.03±1.12	70.25±0.85	3.25±0.16	4.19±0.45	1.02±0.1.12
EX-3	2.25±0.58	75.91±1.09	71.29±0.23	3.32±0.08	5.63±1.09	1.19±0.45
EX-4	2.21±0.89	76.06±1.03	72.20±0.12	3.35±1.04	5.78±1.34	1.21±0.78

TBARS: Thiobarbituric acid reactive substances, GSH: Reduced Glutathione, SOD: Superoxide dismutase, CAT: Catalase,

VIT E: Vitamin E, VIT C: Vitamin C,

*** Significantly different. n= 5 in each group and the results were given as mean ±SEM.**

The results of the protective effect of MEOB indicate that the extract could control the production of free radicals induced by ethanol or it could effectively remove them from the site before it initiated the lipid peroxidation, therefore the EX groups in all the parameters checked did not show any significant difference when compared with the NC-group. But after 30 days' the results could show a significant difference when compared with the EC-group. The extract did not show any toxic effects but it slightly improved the antioxidant status in a dose dependent manner.

Histopathological examinations

Table 4: Observations of histopathological studies:

GROUPS	BODY WEIGHT	LIVERWEIGHT % Change in Liver Weight	HISTOPATOLOGICAL OBSERVATIONS	COMMENTS
NC	256.31±1.34	4.31±0.34	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.
AC	211±0.36*	4.91±0.84	Massive fatty changes necrosis, ballooning degenerative and the loss of cellular boundaries.	The hepatocytes underwent the lipid peroxidation as a result the cells lost their limiting membrane structures.
EC	258.01±1.09	4.29±0.64	Hepatic cells with well preserved cytoplasm ,prominent nucleus and nucleolus-	The normal architecture of the liver tissue without any alterations.
PEC	256.78±1.23	4.67±0.82	Well preserved hepatocytes with normal sinusoids and central vein	The normal architecture of the liver tissue without any alterations.
EX-1	259.65±0.67	4.56±0.78	Hepatocytes well preserved	No effect of ethanol
EX-2	246.81±1.56	4.35±1.35	Some of the cells are show the degenerative effect, the sinusoids are widened and the central veins are distorted.	The effect of the ethanol is shown here, but the extract is exerting the preventive action.
EX-3	254.06±0.84	4.34±0.84	Hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus. Normal sinusoids with well-maintained central vein.	Normal hepatic condition
EX-4	257.41±1.04	4.30±1.09	Hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus. Normal sinusoids with well-maintained central vein.	Normal hepatic condition

*** Significantly different. n= 5 in each group and the results were given as mean ±SEM.**

When comparing the average body weight of all the groups only the AC group was showing a significant decrease (P<0.05) but this did not affect the weight of the liver. The histopathological observations indicated the changes in each group with its cellular architecture which supports the previous biochemical estimations.

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

Group 2: AC-Alcohol Control (rats received ethanol 5g/Kg body weight).

Group 3: EC –Extract control (rats administered with the extract only.100mg/Kg body weight.)

Group 4: PC-4 PEC – Pretreated extract control (rats administered with the extract only 100mg/Kg body weight for 30 days and continued the treatment)

Group 5: EX-1 Experimental rats Pretreated with the extract (100mg/Kg body weight) for 30 days and administered ethanol (5g/Kg body weight) plus extract (100mg/Kg body weight).

Group 6: EX-2 Experimental rats administered ethanol (5g/Kg body weight) plus extract (80mg/Kg body weight).

Group 7: EX-3 Experimental rats administered ethanol (5g/Kg body weight) plus extract (100mg/Kg body weight).

Group 8: EX-4 Experimental rats administered ethanol (5g/Kg body weight) plus extract (120mg/Kg body weight).

DISCUSSION

Ethanol-induced tissue damage occurs in a variety of organs whereliver is the main organ involved in the metabolism of biological toxins which is always associated with the generation of reactive oxygen species (ROS)[22].ROS are

generated under basal conditions as by-products of cellular metabolism, primarily in the mitochondria.ROS, produced as a result of ethanol metabolism cause lipid peroxidation of cellular membranes, proteins and DNA oxidation, which results in hepatocytic injury[23]. Hepatic lipid peroxidation associated with acute ethanol administration is an indicator of the oxidative stress and theantioxidant defense system protects the aerobic organism from the adverse effects of reactive oxygen metabolites[24].Free radical induced lipid peroxidation is one of the major causes of cell membrane damage leading to a number of pathological situations. Free radicals are chemically active atoms or molecular fragments that have a charge due to an excess or deficient number of electrons.The animal body is equipped with an antioxidant defense system that deactivates these highly reactive free radicals, through the activities of antioxidant enzymes and other antioxidantsIt has been reported that SOD, CAT and GSH constitute a mutually supportive team of defense against ROS[25].

Ethanol metabolized into acetaldehyde by alcohol dehydrogenase (ADH) in the liver generates NADH and increased production of ROS by NADH in different organelles[26].Later this acetaldehyde is oxidized to acetate by aldehyde oxidase or xanthine oxidase giving rise to ROS via P 450 2EI. Excess ROS production plays an important role in the development of lipid peroxidation.All of the three pathways result in ROS generation[27]. However, the microsomal ethanol-oxidizingsystem, especially the cytochrome P450 2E1 (CYP2E1), has beenshown to play a critical role in ethanol-induced oxidative stress and long-term ethanol exposure significantly increases the CYP2E1pathway. A recent study using CYP2E1 transgenic mice hasdemonstrated that over-expression of CYP2E1 enhances liver damageby chronic ethanol exposure.The physiological role of this system comprises gluconeogenesis from ketones, fatty acid metabolism, and detoxification of xenobiotics, including ethanol. After chronic ethanol consumption, the activity of the microsomal ethanol-oxidizing system (MEOS) increases with an associated rise in cytochrome P-450, especially CYP2E1[28]. This induction is associated with proliferation of the endoplasmic reticulum, both in experimental animals and in humans. In addition, metabolism by CYP2E1 could result in a significant free radical release and acetaldehyde production, which, in turn, diminishes the GSH and otherdefence systems against oxidative stress. Acetaldehyde also forms adducts with proteins, thereby altering the functions of mitochondria and of repair enzymes[29]. Increases of CYP2E1 and its mRNA prevail in the perivenular zone, the area of maximal liver damage. One of the secondary effects of ROS formation is mitochondrial dysfunction which results in ATP depletion and oxidative stress. ALT and AST are transaminases localized in the hepatic cells are released in the circulation after hepatic cell damage [23]. High levels of ALT and AST in EC groups indicates hepatic cell damage by ethanol byproducts that caused lipid peroxidation [30].

Thiobarbituricacid reactive substances are one of the diagnostic indices of lipid peroxidation due to oxidative stress. An increase in TBARS in alcohol treated groups in all the experiments indicated the adverse effect of ethanol metabolism generating the lipid peroxidation. Lipid peroxidation is measured by the formation of TBARS such as malondialdehyde (MDA) which was formed from the breakdown of fatty acids. The amount of lipid peroxidation is directly proportional to the amount of MDA produced during lipid metabolism.[31] Lipid peroxidation, as reflected in TBARS values were higher in all ethanol treated-groups when compared to normal controls clearly indicating the oxidative stress in hepatic and extra hepatic tissues induced by ethanol and its oxidation. This increase was significantly inhibited in the experimental groups, which were treated with the extract together with ethanol, clearly showing the preventive effects of the extract within 30 days of treatment

Reduced glutathione plays a major role in coordinating the cellular defenses against oxidative damage. Liver injury mediated by free radicals is characterized by the depletion of endogenous antioxidants such as GSH. In the present study the ethanol administration markedly decreased hepatic levels of GSH in alcohol–treated control animals and this was associated with enhanced lipid peroxidation. The GSH ratio can serve as a good indicator of the cellular redox state[32]. This ratio in GSH may be determined by the rates

of hydrogen peroxide reduction by glutathione peroxidase and GSSG reduction by glutathione reductase[33]. The levels of reduced glutathione were significantly restored by treatment with MEOB.SOD is an important intracellular antioxidant enzyme present in all aerobic cells which scavenges superoxide anion. Superoxide dismutase (EC 1.15.1.1) destroys the free radical superoxide by converting it to peroxide that can in turn be destroyed by catalase or GPX reactions[34] and the low level of superoxide is constantly generated by aerobic respiration. The electron-transport chain of mitochondria, which is meant to escort four electrons to molecular oxygen to form water, occasionally leaks a single electron. Superoxide reduces Fe^{+++} to Fe^{++}, releasing the iron from storage sites so that it can react with hydrogen peroxide and produce hydroxyl radicals. SOD converts superoxide to hydrogen peroxide and molecular oxygen[35]. Catalase is an enzymatic haemoprotein localized in the peroxisomes and micro-peroxisomes, which decomposes hydrogen peroxide to water and oxygen, protecting tissues against highly reactive hydroxyl radicals [36]. The reduction in the activity of these enzymes may result in accumulation of superoxide radicals and hydrogen peroxide. The body has an effective mechanism of preventing and neutralizing free radical induced damage, accomplished by the endogenous antioxidant enzymes such as SOD, catalase, peroxidase. When the antioxidant defence are lost, oxidative stress develops, which through a series of events leads to the development of various diseases[37] thus capturing H_2O_2 before it can escape the cell and converting it into O_2. In this way, catalase can maintain the concentration of O_2 either for repeated rounds of chemical reduction or for direct interaction with the toxin[38].Any compound natural or synthetic with antioxidant properties alleviates this damage partially or totally in controlling the production of free radicals so prevent the oxidative stress.Vit-E is a reduced form of coenzyme Q, a major lipophilic radical scavenging antioxidant and plays an important role in the total defense system against oxidative stress [39].Ascorbic acid appears to be the most important non-protein antioxidant and *in-vitro* studies have indicated that ascorbic acid may function synergistically with ∞-tocopherol by regenerating the ∞-tocopherol from tocopheroxyl radicals[40].Antioxidants such as ascorbic acid, ∞-tocopherol and glutathione remove free radicals by reacting directly with them. In all these experiments the alcohol-treated groups show significant differences between the normal control groups (P=0.0001) and there was no significant difference with the experimental groups [41].The cellular integrity of the hepatocytes was also examined in this study. The following was fond in the liver tissue, severe fatty change, sinusoidal dilation and congestion, mild periportal inflammation, fibrosis, severe feathery degeneration and necrosis in AC groups when compared with NC groups[42]. All other groups showed almost the same cellular architecture as the NCgroups. Histopathological studies conducted in the laboratory were also in agreement with the biochemical estimations[43]. The hepatoprotective effect of the extract can be correlated directly to its ability to reduce the activity of ROS by destroying the activity of the free radicals which are formed during metabolic reactions and enhancing the system with antioxidant defense status [44].

CONCLUSION

The observations discussed above showedthat MAEOB is a potent antioxidant agent in controlling the toxicity induced by ethanol and that it exerts its protective effect by decreasing the lipid peroxidation and the oxidative stress.

RECOMMENDATIONS

Further research is needed to establish the efficacy of the plant and its antioxidant properties for other ailments such as diabetes, cardiovascular diseases.

It is suggested that research also be done on the isolation and characterization of the active components from the crude extract so that these can be used for pharmaceutical and therapeutic purposes.

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REFERENCES

[1] Halliwell B, Gutteridge JMC. FreeRadicals in Biology and Medicine.Oxford University Press: Oxford. 2000; 148–149.

[2] Lieber CS. Ethanol metabolism, cirrhosis and alcoholism. ClinChim Acta.1997; 257:59–84.

[3] Lieber CS: Hepatic, metabolic and nutritional disorders of alcoholism: from pathogenic to therapy.Crit Rev Clin Lab Sci2000; 37: 6:551-84.

[4] George S, Caturvedi P. 2008. Protective role of Ocimumcanum(OCM) plant extract on alcohol induced oxidative stress in albino rats. Br. J. Biomed. Sci., 65(2): 80-85.

[5] Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing.Nature.2000; 408:831-49.

[6] McClain, C.J., Song, Z., Barve, S.S., Hill, D.B., Deaciuc, I. Recent advances in alcoholic liver disease. IV. Dysregulated cytokine metabolism in alcoholic liver disease American Journal of Physiology.2004; 287:G497-G502

[7] 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.

[8] Kumar S. Free radicals and antioxidants: human and food system. AdvApplSci Res. 2011; 2(1):129–35.

[9] Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 2nd Edition Spectrum Books Limited, Ibadan, Nigeria.1993; 1-153.

[10] Chauhan CK, Nanivadekar SA and Billimoria FR.Effect of an herbal hepato-protective product on drug metabolism in patients of cirrhosis and hepatic enzyme function in experimental liver damage. Indian Journal of Pharmacology. 1992;24 107–110.

[11] OECD. OECD Guidelines for Testing of Chemicals [internet].France: OECD Publishing; 2006. Section 4, Health effects: Test No. 425: Acute oral toxicity: Up and down procedure (Adopted 2006. March 23, cited 2008 September 27).

[12] Niehaus WG, Samuelson B: Formation of MDA from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem1986, 6: 126- 130.

[13] Ellman, GL: Tissue sulhydryl groups. Archive of Biochemistry and Biophysics.1959, 82:70-77.

[14] Kakkar P, Das B, Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase..Indian J BiochemBiophys1984; 21:130-132.

[15] Bisswanger, H. Practical Enzymology. Wiley-VCH.2004; 79.

[16] Martinek RG: Method for the determination of Vitamin E (total tocopherols) in serum. Clinical Chem.1964; 10:1078-86.

[17] Roe JH: In Methods of Biochemical analysis. Interscience Publishers Inc, New York. 1961, 115-139.

[18] Lowry OH, Rosebrough MJ, Farr L, Randall R J: Protein measurement with the folin phenol reagent. J. BiolChem 1951; 93:265-275.

[19] Jendrassik, L. and Grof. In-vitro determination of total and direct bilirubin in serum.Journal of Biochemistry.1938; 299:81-88

[20] Aebi, H.E. and Bergmeyer, H.U. (1983). Methods in enzymatic analysis.New York Academicpress Inc. 1983; 3: 276-286.

[21] Chavan, Sandeep, D., Patil, Sandeep, B. and Naikwade, N.S. (2012). Biochemical and Histopathological Studies of Butea Monosperma(Lam) Taub Leaves on Paracetamol-Induced Hepatotoxicity in Albino Rats.Journal of Pharmacy Research.2012; 5 (8): 4006-4008.

[22] George, S., Chaturvedi, P. &Kamau, J. Hepatoprotective potential of Ocimumgratissimum on ethanol induced hepatotoxicity in albino rats. AJPBR.2012; 2 (1), 27-32.

[23] Gopi S, Setty OH. Beneficial effect of the administration of Hemidesmusindicusagainst bromobenzene induced oxidative stress in rat liver mitochondria. J Ethnopharmacol. 2010; 127(1): 200-03.

[24] George S, Chaturvedi P. A comparative study of the antioxidant properties of two different species of Ocimumof Southern Africa on alcohol induced oxidative stress. J. Medicinal Food.2009;12 (5): 1154- 1158.

[25] Awah FM, Uzoegwu PN, OyugiJO,Rutherford J, Ifeonu P, Yao X, Fowke KR, Eze MO. Free radical scavenging activity and immunomodulatory effect of Stachytarphetaangustifolialeaf extract.Food Chem.2010; 119: 1409–1416.

[26] ReinkeLA,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.

[27] Lieber CS: Cytochrome P-4502E1- its physiological and pathological role. Physiol Rev1997; 77:517–544.

[28] Kono H, Rusyn I, Yin M, Gabele, E, Yamashina S: NADPH oxidase-derived free radicals are key oxidants in alcohol-induce liver disease. J Clin Invest. 2000, 106:867-872.

[29] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39:44–84.

[30] Gülçin I, Elmastas M, Aboul-EneinH.Y.Determination of antioxidant and radical scavenging activity of basil (Ocimumbasilicum) assayed by different methodologies. Phytother. Res.2007; 21(4): 54-361.

[31] Ugwu, G.C. and Eze, E.I. In vivo evaluation of the effects of ethanolic leaf extract of Gongronemalatifoliumon AST and bilirubin secretions in albino rats. Journal ofMedical and Applied Biosciences.2010;2:1-2.

[32] Gülçin I, KirecciE,Akkemik E, Topal F, Hisar,O. Antioxidant and antimicrobial activities of an aquatic plant: Duckweed (LemnaminorL.). Turk.J. Biol.2010; 34(2): 175-188.

[33] Fernandez-Checa, J.C and Kaplowitz N: Hepatic mitochondrial glutathione: transport and role in disease and toxicity. ToxicolAppl Pharm;2005.204:263–273.

[34] Speisky H, MacDonald A, Griles G, Orrego H, Israel L: .Increased loss and decreased synthesis of hepatic glutathione after acute ethanol administration. Biochem J. 1993, 255: 565- 572.

[35] Tamilarasi, R., Sivanesan, D. and Kanimozhi, P. Hepatoprotective and antioxidant efficacy of Anethumgraveolenslinn in carbon tetrachloride-induced hepatotoxicity in albino rats. Journal of Chemical and Pharmaceutical Research.2012; 4(4): 1885-1888.

[36] Shaker, E., Mahmoud H. and Mnaa S. Silymarin, the antioxidant component and Silybummarianumextracts prevent liver damaged. Elsevier journal of food and chemical toxicology.2010; 48: 803-806.

[37] Sebastiann M, Hans DR, Wolfgang S. Direct evidence for catalase as the predominant H_2 O_2 removing enzyme in human erythrocytes. Blood .1997; 12: 4973-4978.

[38] Meera R, Devi P, Kameswari B, Madhumitha B, Merlin NJ. Antioxidant and hepatoprotective activities of OcimumbasilicumLinn.andTrigonellafoenum-graecumLinn. against H_2O_2 and CCL_4 induced hepatotoxicity in goat liver. Indian Journal of Experimental Biology.2009; 47:584-590.

[39] Sevan A, Guzel S, Seymen O CivelekS ,Bolayili M, Uncu M and Burcak G. Effects of Vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats :investigation of liver and plasma. YonseiMedicl Journal .2004;45: 4: 703-10

[40] Suresh MV, John JL, Kumar CVS, Indra M. Interaction of ethanol and ascorbic acid on lipid metabolism in guinea pigs. Ind J. ExptBiol1997; 35:1065-69.

[41] 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.

[42] Ephraim,K.D, Jacks ,T.W, Sopido,O.A. Histopathological Studies on the toxicity of Ocimum gratissimum leave extract on some organsof rabbit.2003 Afr.J. Biomed. Res .6:21-25.

[43] Galilia AY, Nihal ME, Eman F El A. Hepatoprotective effect of basil (OcimumbasilicumL.) on CCl4- induced liver fibrosis in rats. African Journal of Biotechnology. 2012; 11(90):15702-11.

[44] MamtaSachdeva, Maninder Karan, Tejvir Singh, Sameer Dhingra. Oxidants and Antioxidants in Complementary and Alternative Medicine: A Review: Spatula DD: 2014; 4(1):1-16.

My area of specialization is animal physiology, and my research work is mostly concentrated to antioxidant activities of medicinal plants. Right now we have on-going projects on isolation and characterisation of phytochemicals and checking its antioxidant activities. One of the major studies going on in our lab is on histopathological studies of hepatic tissues on alcohol induced toxicity and the protective effects of some of the indigenous medicinal plants.