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Effects of the methanol extract of *Ocimum gratissimum* on cumen hydroperoxide–induced oxidative stress on rat liver tissue

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ABSTRACT

The present study was conducted to evaluate the *in vitro* effects of the methanol extract of *O.gratissimum* (MEOG) on DPPH and liver homogenate. All the assays with DPPH (2,2-diphenyl-1-picrylhydrazyl) including the radical scavenging assay, indicated that MEOG has a notice able effect on scavenging the free radicals. The phenolic content of the sample was determined using Folin-Ciocalteu reagent and it was found to be 55.28 ± 5.24 mg gallic acid equivalents (GA)/g dry weight. There was a statistically significant correlation between the amount of phenolic compounds and antioxidant activity ($r^2 = 0.836$, $P < 0.05$). A group was treated with the extract only to check whether this plant has any toxic effect of its own or produced any side effect when added to the liver homogenate. The results established the fact that this extract is safe to be used without any toxic or side effects. In the liver homogenate, the levels of reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were significantly reduced ($P < 0.05$) in cumen hydroperoxide (CHP)-treated control group (CC), but the extract-treated groups (EX) did not show any significant difference with the normal control (NC), which clearly indicated that the extract could prevent the toxic effects of CHP. These results suggest that MEOG could protect the liver tissue from lipid peroxidation induced by CHP by its antioxidative effect, and hence prevent the deleterious effects of CHP- induced hepatotoxicity.
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Keywords: *Ocimum gratissimum,* phytochemicals, oxidative stress, reactive oxygen species, lipid peroxidation

INRODUCTION

The liver, an important organ actively involved in metabolic functions, is a frequent target of a number of toxicants which causes liver injury by the formation of free radicals. Oxidative stress plays a key role in liver injury which can increase in lipid peroxidation either by enhancing the production of oxygen reactive species (ROS) or by decreasing the level of endogenous antioxidants (Finkel and Holbrook, 2000). Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species

(ROS). These radicals are formed by a one electron reduction process of molecular oxygen (O_2). ROS can easily initiate the lipid peroxidation of the membrane lipids, causing damage of phospholipids, lipoprotein (of the cell membrane) by propagating a chain reaction cycle (Astiz et al., 2009). Almost all organisms are well protected against free radical induced oxidative damage by antioxidant enzymes such as superoxide dismutase and catalase or antioxidant compounds such as ascorbic acid, tocopherols, and glutathione (Chaturvedi et al., 2007). However, numerous investigations have proved that the phyto-constituents are responsible for scavenging the free radical and there by exhibiting the different beneficial pharmacological properties (Geetha and Vasudavan, 2004; Gulcin et al., 2007)such as hypoglycemic effect, cardiovascular protective, anti-carcinogenesis, stress depressant and many more to mention (George et al., 2004; Karthikayan et al., 1999).

Basil is one of the most popular herbs grown in gardens all over the world and is an important constituent of many Ayurvedic preparations and called the "wonder herb". The genus *Ocimum* (Lamiaceae formerly Labiatae), collectively called basil has long been recognized as a diverse and rich source of essential oils (Prabhu et al., 2009). *Ocimum* contains between 50 to 150 species of herbs and shrubs from the tropical regions of Asia, Africa, and Central and South America.*O. gratissimum*is one of the species which is widely distributed throughout Africa especially in Botswana and is commonly known as the wild basil. It is widely used for treatment of nasal congestion and cough, abdominal pain, well known for its anti-inflammatory antimicrobial and hypoglycemic activity (George et al., 2004; Chaturvedi et al., 2007). Recent studies on *Ocimum gratissimum* proved it to be a useful medication for people living with Human Immuno-deficiency Virus (HIV) and Acquired Immuno Deficiency Syndrome virus

AIDs and this was reported by Elujoba in 2000.

The aim of this study was to estimate the amount of total phenolic content in methanol extract of *O. gratissimum* (MEOG) and to estimate the radical scavenging activity. Also to find out whether there is any correlation between the total phenolic content and the radical scavenging activity of MEOG and to evaluate the *in vitro* effect of methanol extract of *O. gratissimum* on liver homogenate in a dose dependent manner.

MATERIALS AND METHODS
Plant material
Tender parts of *O. gratissimum* were collected locally from Botswana and plant was identified 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/G, A01) for *O. gratissimum*.

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 65 ^{o}C and the yield was 7.8% (W/W).

Animals
Male albino rats of Wistar strain of body weight ranging 200-250 grams were housed in colony cages at ambient temperatures of 25± 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.

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 smaller pieces and transferred to a glass Teflon homogenizing tube to prepare the homogenate (1 g, w/v) in phosphate buffer saline (pH 7.4) in 4 °C. Then the liver tissue was centrifuged at 2000 rpm for ten minutes and the supernatant was taken for further use.

Chemicals

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

Experimental design
Effect of plant extract on liver homogenate

This was conducted to establish that the extract had no toxic effect on liver homogenate and was safe to be used. Five replicates of liver homogenate were mixed with different doses of plant extracts ranging from 50 μL to 250 μL, which was incubated for 30 minutes. The biochemical estimations were done on TBARS, GSH, SOD and CAT.

Effect of MEOG on CHP- induced lipid peroxidation

In this experiment, the antioxidant property of plant extract was investigated during CHP induced lipid peroxidation. Five replicates of the following groups were used:

− 3 mL homogenate + 1.3 mL of distilled water- normal control- NC

− 3 mL homogenate + 1 mL CHP + 300 μL of distilled water- CC

− 3 mL homogenate + 1 mL CHP +100 μL of the extract + 200 μL of distilled water- EX1

− 3 mL homogenate +1 mL CHP +200 μL of the extract + 100 μL of distilled water- EX2

− 3 mL homogenate +1 mL CHP +300 μL of the extract-EX 3

200 μL of the above mixture was pipetted from each group at 0, 20, 40 and 60 minutes

and estimated the quantity of TBARS, GSH, SOD and CAT in each of them.

Biochemical analysis
TLC - semi quantitative DPPH assay

A 0.2% DPPH solution in methanol was prepared and kept in the fridge for further use. The grid space was marked with 1.0 cm^2 space on an aluminum based TLC sheet (Merck silica gel 60F$_{254}$) and a stock solution of all the extracts together with the standard were prepared in methanol. A series of dilutions of the stock together with the standard were prepared ranging from 400 μL to 0.0 μL for the last dilution. The grid on the TLC sheet was labeled with extract on the horizontal axis and amount of extract on the vertical axis. The extracts of different concentrations and the standards were plotted on the TLC sheets and allowed the spots to dry for at least 2 hours. Care was taken to keep the volume of the extracts spotted were the same for all the spots so that all the spots were of the same size for a fair comparison. The sheet was sprayed with 0.2% DPPH solution and the appearance of yellow spots against the white background showed the antioxidant activity. Photographs of the TLC were taken after 2 hours and this could be used for further references because the DPPH gets faded with time. This procedure was adapted and revised from the methods which were previously used by Juma and Majinda (2004).

TLC- Autographic assay

The solvent systems used to develop the chromatograms were given in Table 1 and spots of the extracts in methanol were in duplicate on an aluminum based TLC sheet (Merck silica gel 60F$_{254}$). One of the sheets was sprayed with vanillin-sulphuric acid reagent and heated then on a hot plate until the colours were developed. (1 g of vanillin dissolved in 5 mL of conc. H$_2$SO$_4$ and added about 95 mL of methanol). The other with one was sprayed with 0.2% DPPH which were shown as yellow spots. Photographs of the sheets were taken against a white background

after 30 min while the colors were prominent. The sheets were placed side by side and the components which showed the antioxidant activity were identified (Chacha et al., 2005).

Spectrophotometric method

The free radical scavenging activity was measured using DPPH method modified by Yeboah and Majinda (2008). Solutions of 500 µM DPPH (i.e. 0.02 % or 0.2 mg/mL) in methanol (AR) was prepared. Also different concentrations of each of the plant extracts and standards were prepared (ascorbic acid and gallic acid) ranging from 0.001-0.05 mg/mL in methanol. Each extract or standard solution (2 mL) was added to an equal volume of the DPPH solution, making a total reaction volume of 4 mL. A control reaction mixture was prepared consisting of 2 mL methanol without extract and an equal volume of DPPH solution. The test tubes were tightly closed, vigorously shaken and placed in a dark cupboard for 30 minutes. The absorbance of each solution was measured at 517 nm, and methanol was used as the blank for baseline correction, after 2 h and finally after 24 h. The percentage inhibition of DPPH, I% was calculated using the following formula-

$$I\% = \frac{(\text{Absorbance control- Absorbance sample}) \times 100}{\text{Absorbance control}}$$

From the inhibition curves (I % versus sample concentration in µg/mL) the concentration of extract or standard required to inhibit DPPH radical activity by 50% (IC_{50}) was determined from non-linear regression equations that best fitted the curves. The experiment was carried out in triplicate and the IC_{50} values reported as the average of three trails in µg/mL + the standard deviation.

Total phenolic content

Total soluble phenolic compounds in the extracts were determined using the Folin-Ciocalteu reagent method modified byYeboah and Majinda (2008). Five different concentrations of the standard, gallic acid, in methanol were prepared ranging from 0.01 to 0.0 5 mg/mL. A 1mg/mL solution of each of the above extracts was prepared in methanol. To 0.5 mL of the test sample wre added is 5 mL of 90% aqueous methanol and 0.5 mL Folin-Ciocalteu reagent followed by 1 mL 2% Na_2CO_3 after 3 min. Subsequently, the mixture was shaken for 2 h at room temperature and absorbance was measured at 725 nm. 90% aqueous methanol was used for baseline correction (solvent blank). All the tests were performed in triplicate. The concentration of total phenolic compounds was determined as gallic acid equivalents using the following equation obtained from a standard curve using linear regression.

Estimation of lipid peroxidation

TBARS in liver homogenate can be estimated by the method described by Niehaus (1986) with few modifications. Lipid peroxidation was measured by the formation of thiobarbituric acid reactive substances (TBARS) such as malonyl dialdehyde (MDA). MDA formed from breakdown of fatty acids, served as a convenient index for determining the extent of peroxidation reaction. MDA has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give red species absorbing at 535 nm. 0.1 mL of liver homogenate was treated with 2 mL of TCA-TBA-HCL (1:1:1) mixture and incubated in boiling water bath for ten minutes ,then the mixture was cooled, to that were added 2 mL of freshly prepared 1N NaOH and the absorbance was measured at 535 nm.

Estimation of reduced glutathione (GSH)

Reduced glutathione can be estimated by the method of Ellman (1959). 0.25 mL of liver homogenate was mixed with 0.5 mL of precipitating buffer (5% TCA in 1m M EDTA), centrifuged and supernatant was collected. This was mixed with 2.5 mL of 0.1 M phosphate buffer (pH 8.0). The colour was developed by adding 100 µl DTNB (0.01%) and the absorbance was measured at 412 nm.

Table 1: % inhibition of DPPH versus concentration of extracts and standards.

Extracts and standards	concentrations (Mg/Ml)					
	0	0.001	0.01	0.1	0.25	0.5
GAE	0	82.03±0.05	90.8±0.12	92.3±0.43	97.8±0.07	97.9±0.11
AA	0	52.8±0.65	62.2±0.55	92.1±0.21	97.7±0.62	97.8±0.13
MEOG	0	38.87±0.61	18.38±0.65	43.56±0.09	60.8±0.77	98.45±0.08

GAE: Gallic acid, AA: Ascorbic acid, MEOG: Methanol extract of *O. gratissimum.*

Estimation of Superoxide Dismutase (SOD)

Superoxide Dismutase was assayed by the method of Kakkar et al. (1984). The role of SOD is to accelerate the dis-mutation of the superoxide radical $(Oz^{.})$ produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. The assay of SOD activity is 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 (Nitroblue tetrazolium) reduction /min/mg protein.

The reaction mixture contained 150 µl EDTA, 600 µl L-methionine, 300 µl NBT and the volume was made up to 2.8 mL by the addition of SOD buffer. To the reaction mixture were added 200 µl of the liver homogenate except in the control. Finally to this were added 200 µl of riboflavin to start the reaction. The test tube was kept under a fluorescent lamp and the reaction kinetics measured for four minutes. The absorbance was taken at 560 nm for the four minutes.

Estimation of Catalase (CAT)

Catalase was estimated by the method of Hans Bisswagner (2004). To 0.98 mL of H_2O_2 _ solution (10 mM), 0.2 mL of liver homogenate was added. The absorbance was taken at 240 nm and the catalase activity was calculated using the extinction coefficient of H_2O_2 (0.071). The activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.

Estimation of protein

Protein was determined by the method of Lowry et al. (1951), using Bovine Serum albumin as standard, at 660 nm. One ml of sample was mixed with 3 ml of Lowry's reagent (prepared by mixing 98 ml of 2% sodium bicarbonate solution, 1 ml of 1% copper sulfate solution, and 1 ml of sodium potassium tartrate) and incubated for 10 minutes at room temperature. After incubation, 0.3 ml of Folin-Ciocalteau reagent (diluted with equal volume of water) was added. The color formed was read against blank (1 ml of distilled water) at 670 nm after 30 minutes.

Statistical analysis

All data are expressed as the mean ±S.E. mean of n=6. Analysis of variance was performed by one-way ANOVA and the significant differences between the means were determined by Holm-Sidak method at *p*-value of ≤ 0.05. Statistical software Sigmastat 3.1 was used to analyze the data.

RESULTS
Radical scavenging activity- TLC - semi quantitative DPPH assay

Three different methods were used for DPPH radical scavenging activity namely (a) a quantitative TLC autographic assay (b) a semi quantitative TLC assay (c) quantitative spectrophotometric assay which was modified by Yeboah and Majinda. In all the above three tests conducted, the presence of antioxidants was clearly indicated (Figures 1, 2 and 3).

Total phenolic content

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. Many natural antioxidants are phenolic compounds, so the determination of total phenolic content could give useful information that could be correlated with antioxidant capacity of the

sample. In the extract used, TPC was calculated from the linear regression equation of the standard curve $y = 36.84x + 0.1069$. From this equation, the equivalent concentration of gallic acid (x mg/mL) was determined for each extract and converted to mg of gallic acid equivalents/g of dry extract (mg GAE/g). The total phenolic content of MEOG was 254.21 ± 0.43 (mg GAE/g).

Effect of MEOG on liver homogenate

This was done to check the i*n vitro* effect of the extract on liver homogenate and the results show (Table 2) that the MEOG did not change the antioxidant status in EX groups when compared with the NC groups. Also it was evident from the results obtained that this extract could improve the antioxidant status in a dose dependent manner. This clearly indicated that the extracts did not have any toxic effect and could be used without any side effects.

Effect of MEOG on CHP- induced lipid peroxidation

Tables 3-6 show the level of antioxidants in liver homogenate, when it was used with CHP. The results obtained in 60 min show a significant difference between the NC and the CHP treated groups in all the parameters checked but there was no significant difference between the NC and EX groups. There was a threefold increase of TBARS in CC groups when compared with NC groups but EX groups could control the lipid peroxidation initially. Later the lower two doses could not withstand the toxicity of CHP so it started giving the indication of lipid peroxidation after 40 min in both the doses, EX1 and EX2. The GSH levels in both the extracts showed almost the same type of reactions, the levels were decreased significantly in CC groups by almost 50%. The levels of GSH were increasing significantly in EX groups up to 34% in EX 3 groups showed that the extracts had components which could boost up the GSH levels and this may be through its antioxidant capacity. Both CAT and SOD showed the same type of reaction as GSH.The results of the above parameters checked, TBARS, GSH, CAT and SOD with MEOG showed significant potential of prevention of lipid peroxidation in 60 min time. The EX-groups were significantly different from the CC-group in 20 min and 40 min.

Table 2: Effect of MEOG on liver homogenate after 60 min of incubation.

S NO	GROUPS	TBARS (n mol/g wet tissue)	GSH (mg /g /wet tissue)	CAT (Umg/-^{1} protein)	SOD (Umg/-^{1} protein)
1	NC	$2.56 \pm .06$	$31.83 \pm .05$	$71.73 \pm .06$	$3.23 \pm .03$
2	EX-1	$2.54 \pm .77$	$33.68 \pm .34$	$73.65 \pm .08$	$3.26 \pm .06$
3	EX-2	$2.54 \pm .66$	$36..96 \pm .07$	$75..46 \pm .27$	$3.41 \pm .15$
4	EX-3	$2.53 \pm .04$	$38.26 \pm .04$	$76.06 \pm .03$	$3.44 \pm .07$
5	EX-4	$2.53 \pm .06$	$39.03 \pm .14$	$78.43 \pm .84$	$3.49 \pm .13$
6	EX-5	$2.53 \pm .02$	$40.76 \pm .64$	$80.46 \pm .06$	$3.61 \pm .04$

n=4 replications in each group, p<0.001.
NC- Normal control– 3mL of Liver homogenate +250µ L of Distilled Water
EX 1 Extract treated– 3mL Liver homogenate+ 50µ L of the extract +200µ L of Distilled Water
EX 2 Extract treated – 3mL Liver homogenate+ 100µ L of the extract +150µ L of Distilled Water
EX 3 Extract treated – 3mL Liver homogenate+150µ L of the extract+1000µ L of Distilled Water
EX 4 Extract treated – 3mL Liver homogenate+ 200mL of the extract+50µ L of Distilled Water
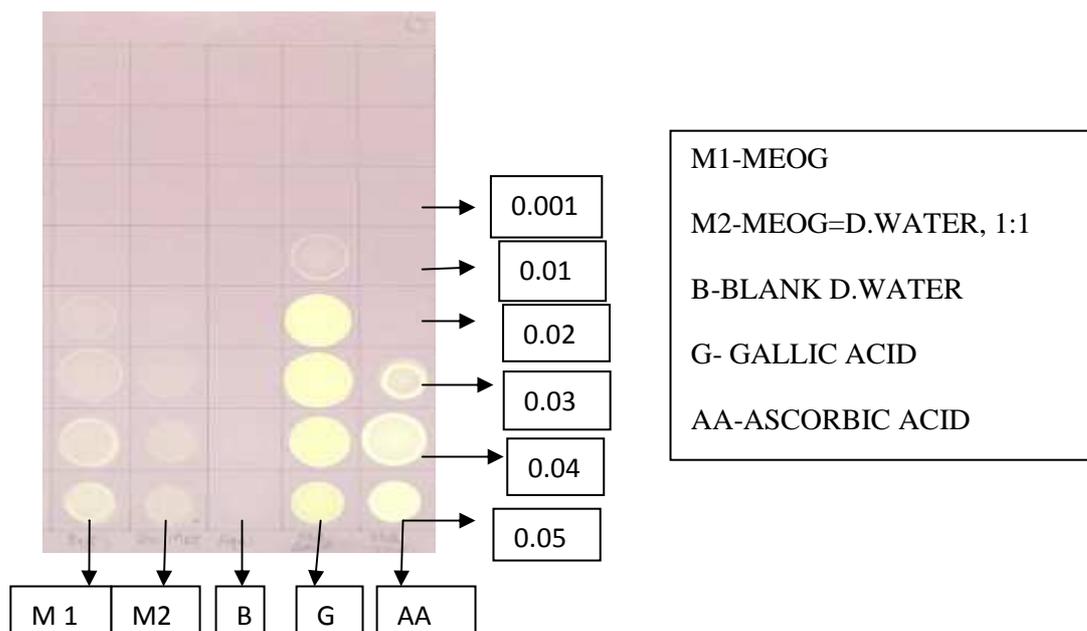EX 5 Extract treated – 3mL Liver homogenate+ 250µ L of the extract
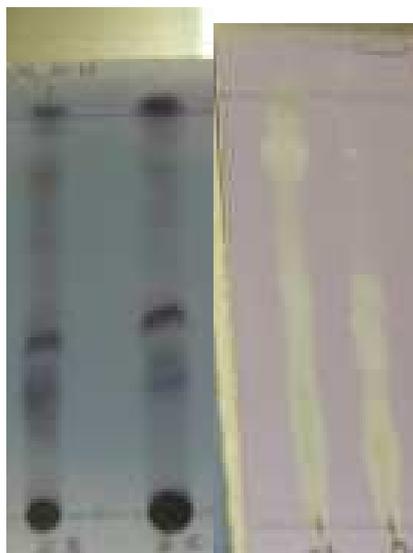
Figure 1: TLC of *O.gratissimum* after 2 hours.

Figure 2: TLC of *Ocimum gratissimum* (MEOG) extracts with vanilin–H_2SO_4 and DPPH spray.

Figure 3: TLC of *O. gratissimum* extracts with vanilin –H$_2$SO$_4$ spray showing the active bands.

Table 3: Effect of MEOG on TBARS in CHP-induced lipid peroxidation in liver homogenate.

S NO	GROUPS	0 MIN	20 MIN	40 MIN	60 MIN
		(nmol/gwet tissue)	(nmol/gwet tissue)	(nmol/gwet tissue)	(nmol/gwet tissue)
1	NC	2.64 ± .09	2.65 ± .03	2.66 ± .04	2.69 ± .18
2	CC	2.95 ± .28	6.41 ± .15*	7.59 ± .07*	7.6 ± .03*
3	EX -1	2.65 ± .02	2.29 ± .08	2.93 ± .39	4.83 ± .42
4	EX -2	2.48 ± .34	2.14 ± .13	2.75 ± .04	4.48 ± .39
5	EX - 3	2.55 ± .43	1.61 ± .06	1.92 ±.05	2.8 ± .13

Table 4: Effect of MEOG on GSH in CHP-induced lipid peroxidation in liver homogenate.

S NO	GROUPS	0-MIN	20 MIN	40 MIN	60 MIN
		(mg/g wet tissue)	(mg/g wet tissue)	(mg/g wet tissue)	(mg/g wet tissue)
1	NC	31.96 ± .06	31.66 ± .04	40.46 ± .04	39.99 ± .04
2	CC	30.98 ± .14	16.41 ± .16*	21.59 ± .08*	33.86 ± .07
3	EX -1	31.68 ± .21	39 ± .07	39.03 ± .39	39.33 ± .42
4	EX -2	32.69 ± .64	42.14± .13	41.75 ± .04	43.48 ± .39
5	EX- 3	31.96 ± .05	42.61 ± .06	43.02 ±.05	44.98 ± .13

*Considered as significantly different n=4 replications in each group, p<0.001.
NC- 3 mL homogenate-normal control +1.3 mL of Distilled Water
CC - 3 mL homogenate +1 mL CHP –CHP control +300 μL of Distilled Water
EX 1- 3 mL homogenate +1 mL CHP +100 μL of the extract+200 μ L of Distilled Water
EX 2 – 3 mL homogenate +1 mL CHP +200 μL of the extract +100 μL of Distilled Water
EX 3 – 3 mL homogenate +1 mL CHP +300 μL of the extract
The results of the above parameters checked, TBARS, GSH, CAT and SOD with MEOG showed significant potential of prevention of lipid peroxidation in 60min time. The EX-groups were significantly different from the CC-group in 20 min and 40 min.

Table 5: Effect of MEOG on CAT in CHP-induced lipid peroxidation in liver homogenate.

S NO	GROUPS	0-MIN	20 MIN	40 MIN	60 MIN
		$(Umg/-^1$ protein	$(Umg/-^1$ protein	$(Umg/-^1$ Protein)	$(Umg/-^1$ Protein)
1	NC	$71.56 \pm .12$	$71.66 \pm .08$	$71.46 \pm .04$	$71.39 \pm .03$
2	CC	$70.61 \pm .02$	$36.41 \pm .15*$	$41.59 \pm .07*$	$63.86 \pm .03*$
3	EX -1	$71.88 \pm .13$	$71.29 \pm .07$	$72.06 \pm .69$	$72.08 \pm .42$
4	EX -2	$71.61 \pm .45$	$72.14 \pm .13$	$72.75 \pm .04$	$80.28 \pm .89$
5	EX - 3	$72.12 \pm .58$	$72.61 \pm .04$	$73.05 \pm .09$	$80.68 \pm .17$

Table 6: Effect of MEOG on SOD in CHP-induced lipid peroxidation in liver homogenate.

S NO	GROUPS	0 MIN	20 MIN	40 MIN	60 MIN
		$(Umg/-^1$ Protein)	$(Umg/-^1$ Protein)	$(Umg/-^1$ Protein)	$(Umg/-^1$ Protein)
1	NC	$3.65 \pm .18$	$3.66 \pm .06$	$3.46 \pm .12$	$3.99 \pm .17$
2	CC	$3.63 \pm .36$	$1.41 \pm .15*$	$1.59 \pm .05*$	$2.86 \pm .07*$
3	EX -1	$3.68 \pm .32$	$2.29 \pm .07$	$3.03 \pm .35$	$3.93 \pm .44$
4	EX -2	$3.67 \pm .09$	$2.14 \pm .13$	$3.78 \pm .06$	$3.88 \pm .30$
5	EX-3	$3.64 \pm .03$	$2.61 \pm .03$	$3.02 \pm .06$	$3.98 \pm .17$

*Considered as significantly different n=4 replications in each group, p<0.001

NC- 3 mL homogenate-normal control +1.3 mL of Distilled Water

CC - 3 mL homogenate +1 mL CHP –CHP control +300 µ L of Distilled Water

EX 1- 3 mL homogenate +1 mL CHP +100 µ L of the extract+200 µ L of Distilled Water

EX 2 – 3 mL homogenate +1 mL CHP +200 µ L of the extract +100 µ L of Distilled Water

EX 3 – 3 mL homogenate +1 mL CHP +300 µ L of the extract

The results of the above parameters checked, TBARS, GSH, CAT and SOD with MEOG showed significant potential of prevention of lipid peroxidation in 60min time. The EX-groups were significantly different from the CC-group in 20 min and 40 min.

DISCUSSION

Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species (ROS) (Gulcin et al., 2002).Reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals (OH^-) and non-free-radical species such as H_2O_2 and singlet oxygen ($^1O_2$), are various forms of activated oxygen (George and Chaturvedi, 2009). Free radical

induced lipid peroxidation is one of the major causes of cell membrane damage leading to a number of pathological situations (Halliwell and Gutteridge, 2000). Free radicals are chemically active atoms or molecular fragments that have a charge due to an excess or deficient number of electrons. Examples of free radicals are the super oxide anion, hydroxyl radical, transition metals such as iron and copper, nitric acid and ozone. Free radicals are highly unstable because they have one or more unpaired electron. They scavenge in the body to grab or donate electrons, thereby damaging cells, proteins and DNA (George and Chaturvedi, 2011). Animal body is equipped with antioxidant defense system that deactivates these highly reactive free radicals, through the activities of antioxidant enzymes and other antioxidants (Gopi and Setty, 2010).

DPPH Radical scavenging activity

The results of TLC gave much information on the active components of MEOG and the semi-quantitative TLC of *O. gratissimum* showed almost the same type of kinetics. Yellow spots start to appear immediately after the spraying but got intensified after 30 minutes (Figure 1). This extract thus has the strong radical scavenging antioxidants with fast kinetics. So this procedure provided a rapid visual identification of fractions with antioxidant potential and could give a clear-cut idea for an activity guided isolation of active compounds (Gulcin et al., 2010).

This reaction has been widely used to test the ability of compounds to act as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activityof plant extracts. The DPPH radical is considered to be a model of lipophilic radical which is a stable free radical in an aqueous or methanol solution. Due of the odd electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy (purple colour). The antioxidant activity is measured by the capacity of an odd electron of the radical become spaired off in the presence of extract (Hydrogendonor). When it become spaired off, the absorption strength is decreased, and the resulting decolourization is stochiometric with respect to the number of electronscaptured (Afolabi et al., 2007). A chain reaction in lipophilic radicals was initiated by lipid autoxidation. The radical scavenging activity of *O. gratissimum* was determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radical. The positive DPPH test suggests that the samples possesses free radical scavenging activities. The scavenging effects of *O. gratissimum* on the DPPH radical are illustrated and different concentrations of extract had significant scavenging effects on the DPPH radical which increased with increasing concentration in the 0.001-0.5 mg/mL range.

Effect of total phenolic content on antioxidant activity

Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation. More than 4000 phenolic compounds (flavonoids, monophenols and polyphenols) are found in vascular plants (Chauhan et al., 1992). Phenols found in plants have been considered anti nutrient due to their potential to "tie up" nutrients such as copper and iron and especially proteins, and prevent them from being absorbed (McDonald et al., 2001). Their function in the plant is to prevent free radical damage to proteins,

carbohydrates, lipids and DNA caused by UV light from the sun. Phenols, especially those with multiple phenolicgroups, are better antioxidants than the well-known antioxidant vitamins. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Rice-Evans et al., 1995). A number of plants have been shown to possess hepatoprotective effect by improving antioxidant status. Thus the efficacy of the extract would be preventative and passive for defending against damages. The result indicates a strong association between antioxidative activities and phenolic compounds (r^2 = 0.836), suggesting that phenolic compounds are probably responsible for the antioxidative activities of *Ocimum gratissimum*. Phenolic compounds are also effective hydrogen donors, which make them good antioxidants (Shahidi and Naczk, 1995). Similarly, Yeboah and Majinda (2008) also reported that naturally occurring phenolic compounds exhibit antioxidative activity in many other plants. Many *Ocimum* species possess phenolic compounds such as euginol, methylchavicol, linalool in their chemical composition. Thus the therapeutic properties of different species of *Ocimum*may be possibly attributed to the phenolic compounds present (Afolabi et al., 2007).

Effect of MEOG on liver homogenate

The effect of MEOG on liver homogenate was checked to find the toxic effect of the extract on liver tissue and it was established that the extract was safe to be used without any toxic effects. All the parameters checked after 60 minutes did show any significant difference from the normal control used. This was in agreement with results obtained in phytochemical analysis and antioxidant activity of *O. gratissimum* extracts from leaves by Acinomoldan in 2007.

Effect of MEOG on CHP- induced lipid peroxidation

The trend of the *in vitro* effect of MEOG on four parameters (TBARS, GSH, CAT and SOD) showed that the CHP treated groups showed the lipid peroxidation within 10 minutes time, up to 40 minutes. In all the parameters checked, there were significant difference between the NC groups and CC groups but not significantly different from the EX groups, which showed that the extract could prevent the lipid peroxidation and the depletion of its indigenous antioxidants by scavenging the free radicals (George and Chaturvedi, 2008). The present study was conducted to evaluate the protective effect of MEOG on liver tissue and the toxicity was induced by cumen hydroperoxide because other toxicants such as ethanol and others needed higher doses and more time to induce the toxic effects. This was in agreement with the findings of Geenley and Davis (1992) to use azo initiators such as cumen hydroperoxide in which the alkoxyl radicals can undergo β–scission to give methyl radicals. The results suggest that the extract possesses protective action against hepatic dysfunctions induced by CHP in dose and time dependent manner. Maximum protective activity of the extract was obtained with administration of 300 µL of the extract at 20 minutes in all the four parameters checked. It has been reported that SOD, CAT and GSH constitute a mutually supportive team of defense against ROS (Awah et al., 2010). The decreased levels of these antioxidants may be because of the enhanced lipid peroxidation in CHP treated groups. The levels of TBARS were increased in CC groups which clearly indicated the amount of malondialdehyde (MDA). MDA was identified as the product of lipid peroxidation by Di Luzio in 1963. Evidence suggests that various enzymatic and non-enzymatic defense mechanisms have

been developed by liver cells to cope with the production of ROS and free radicals. The decrease in GSH has been related to an enhanced oxidation of GSH to oxidized glutathione (GSSG) as a consequence of increased generation of reactive oxygen species (Anitha et al., 2011). Prevention of GSH depletion in EX groups has been shown to inhibit the CHP-induced liver tissue injury. Superoxide dismutase catalyzes the dis-mutation of superoxide into oxygen and hydrogen peroxide (Saravanan and Nalini, 2007). As such, it is an important antioxidant defense in nearly all cells exposed to oxygen. Hydrogen peroxide is then converted to oxygen and water by glutathione peroxidase and catalase and they constitute a mutually supportive team of defense against ROS (Gulcin et al., 2007).

In conclusion, the results indicate a strong association between antioxidative activities and phenolic compounds suggesting that phenolic compounds are probably responsible for the radical scavenging activity of *Ocimum gratissimum*. The above observations show that MEOG is a potent antioxidant agent in controlling the toxicity induced by cumen hydroperxide on liver homogenate and exerts its protective effect by decreasing the lipid peroxidation and improving the antioxidant status.

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