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GINGER ETHANOLIC EXTRACT, GINGER OIL OR RICE BRAN OIL INDUCED HEPATOPROTECTIVE EFFECT AGAINST FATTY LIVER IN RATS

[85]

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ABSTRACT

Hepatoprotective effect of ethanol extract of ginger, ginger oil or rice bran oil against fatty liver disease which induced by ethanol stress was investigated in the present study. Thirty six (36) male albino rats were classified into 6 groups as follows: 1- Normal control (NC), 2- Positive control (induced fatty liver by ethanolic stress) (PC+), 3- rats group administered ethanol and ginger extract (GE group), 4- rats group administered ethanol and ginger oil (GO group), 5- rats group administered ethanol and rice bran oil (RBO group) and 6- rats group administered ethanol and DMSO (DMSO control group, because GE, GO and RBO were dissolved in DMSO as a vehicle). Results revealed that hepatic triglycerides was significantly ($p \leq 0.05$) raised to 80.7 mg/g liver, in positive control (PC+), compared to 15.98 mg/g liver in normal control (NC). Also significant increase ($p \leq 0.05$) in levels of ALT (69.41 U/L), AST (62.98 U/L) and ALP (121.65 U/L) in PC+, compared to their levels in NC (23.35 U/L), (27.95 U/L) and (73.45 U/L) respectively. In addition, high significant level was observed in serum triglycerides (214.37 mg/dl), total cholesterol (TC) (99.81 mg/dl) and LDL cholesterol (47.75 mg/dl) in PC+, compared with its values in NC group: (74.22 mg/dl), (31.45 mg/dl), (4.21 mg/dl) respectively. However, significant ($p \leq 0.05$) decrease was noticed in HDL cholesterol level (9.18 mg/dl) in PC+, compared to NC (12.39 mg/dl). On the other hand, treatment by ethanolic ginger extract (200 mg/kg body weight) showed a hepatoprotective effect which confirmed by remediation the values of hepatic TG, ALT, AST, ALP, TP, Alb, besides serum TG, TC, HDL-C and LDL-C in GE group as compared with their values in NC and PC+. Moreover, treatment by ginger oil (200 mg/kg body weight) and rice bran oil (200 mg/kg body weight) displayed a protective effect in GO or RBO groups, but lower than GE. In addition, ethanol extract of ginger disclosed very high antioxidant activity (IC_{50} = 18.25 µg/ml) compared to both ginger oil (IC_{50} = 6714.38 µg/ml) or rice bran oil (IC_{50} = 1409.57 µg/ml). Finally the present study indicates that ethanol extract of ginger showed hepatoprotective effect more than either ginger oil or rice bran oil.

INTRODUCTION

Fatty liver (steatosis) is a common histological finding in human liver biopsies which is not caused simply by eating fatty foods but it is correlated with health problems, almost attributed to the effects of alcohol excess, obesity, diabetes, or drugs. Fatty liver disease (FLD) is caused by the excessive accumulation of fat (mainly triglycerides) in the hepatic cells which displays a morphological conditions consisting of hepatic steatosis (fatty liver) and steatohepatitis (cells inflammation) that can progress to fibrosis, cirrhosis and hepatocellular carcinoma. Fatty liver disease (FLD) is classified into alcoholic fatty liver disease (AFLD) and nonalcoholic fatty liver disease (NAFLD)**, (Day and yeaman 1994; Cao et al 2016).** Etiologies of steatosis such as oxidative stress in both AFLD and NAFLD are associated with the development of many advanced diseases like steatohepatities (inflammation) which can lead to fibrosis and cirrhosis according to **Bacon et al (1994).**

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Alcoholic fatty liver disease (AFLD) is a major reason of chronic liver disease, morbidity and mortality worldwide and it can develop to fibrosis and cirrhosis, it accounted for 3.8% of all deaths in 2004 **(Rehm et al 2009; Bin and Ramon, 2011; ceni et al 2014)**. Moreover, evidence has emerged that oxidative stress/lipid peroxidation and endotoxin-mediated cytokine release play an underlying important role in the pathogenesis of AFLD and NAFLD. These observations provide an explanation for the striking histological similarity between NAFLD and AFLD, in addition steatosis implicate as a direct contributor to inflammation, fibrosis and cirrhosis. **(Diehl et al 1988; Bin and Ramon, 2011)**

Although AFLD remains a major cause of morbidity and mortality in many countries, there is no therapy approved from Food and Drug Administration (FDA) for either alcoholic cirrhosis or alcoholic hepatitis. However, several drugs have been used "off label." **(Marsano et al 2003)** and it have many side effects. Many drugs used for fatty liver therapy have adverse side effects such as Statins drugs (HMG-CoA reductase): Atorvastatin, Lovastatin, Pravastatin, Fluvastatin, Rosuvastain and Simvastatin, besides Fibrate drugs: Gemfibrozil, Clofibrate, and Fenofibrate, which side effects are muscle problems, an increased risk of diabetes mellitus, and increased liver enzymes in the blood due to liver damage, other possible adverse effects of these drugs include: neuropathy, pancreatic and hepatic dysfunction, sexual dysfunction and formation of hepatic tumor in some conditions. **(Braun et al 1999; Golomb & Evans, 2008; Bellosta and Corsini, 2012 and Naci et al 2013).**

Ginger (Zingiber officinale Roscoe) is used worldwide in foods as a spice and herbal medicines (**Wang and Wang, 2005; Tapsell et al 2006).** Rhizomes of ginger contain protein 12%, crude fiber 7%, starch 46%, water extract 20%, alcohol extract 6%, ash 7%, minerals, vitamins (niacin and vit. A), and volatile oil 2%. The major components in the essential oil are zingiberene (40%) and curcumene (20%). The warm pungent taste is due to nonvolatiles such as gingerols, shogaols, paradols, and zingerone which account for many of its health beneficial effects **(Kundu et al 2009).** Ginger has beneficial uses in both traditional and modern medicine for the treatment of nausea, motion sickness, diarrhea, vomiting, and digestive and respiratory disorders. Moreover, ginger also provide numerous significant pharmacological properties such as anti-inflammatory, antimicrobial, anticarcinogenic, analgesic and antioxidant activities **(Ali et al 2008; Butt and Sultan, 2011).** The U.S. Food and Drug Administration (FDA) marked ginger as generally recognized as safe (GRAS) as a food additive **(Kubra and Rao, 2011).**

Rice bran oil (RBO) contains a rich unsaponifiable fraction (up to 5%) mainly composed by sterols (43%), triterpene alcohols (28%) 4-methylsterols (10%) and less polar components (19%) **(Sayre and Saunders, 1990).** Phytosterols include β-sitosterol (900 mg%), campesterol (500 mg%), stigmasterol (250 mg%), squalene (320 mg%) and gamma-oryzanol (1.6%). Gamma-oryzanol, often identified as the active molecule of rice bran oil, is a mixture of ferulic acid esters of triterpene alcohols such as cycloartenol (106 mg %) and 24-methylene cycloartanyl (494 mg %) **(Metwally et al 1974 and Norton, 1995).**

Rice bran oil reduce by about 40% TC and LDL-C plasma levels and at the same time increasing HDL-C. Also, contribute in lowering the risk of colon cancer (anti-tumor activity) and benign prostatic hypertrophy and cardiovascular health benefits. However, it has strong antioxidant property. Previous studies provide that RBO and its components are non-toxic and non-carcinogenic **(Kim et al 1995; Deckere and Korver 1996; Sugano and Tsuji, 1997; Cicero and Derosa, 2004 and Nagendra et al 2011).** The aim of this study is to investigates the protective effect of natural plant extracts (ethanolic ginger extract or ginger oil or rice bran oil), against fatty liver disease induced by ethanol, to avoid the side effects of chemical therapy.

MATERIALS AND METHODS

Materials

Ginger and rice bran: ginger powder and ginger oil were obtained from Harazz Stores of Medicinal Plants, Cairo, Egypt. While rice bran oil was obtained from Carrefour hyper market, Cairo, Egypt.

Chemicals

All chemicals used in this study were analytical grade and purchased from El Gomhoureya Company for Drugs Trade & Medical Supplies, medical supply store in Egypt, Cairo.

Biochemical parameters kits

All kits used in this study were purchased from Egyptian Company for Biotechnology, Obour city Industrial area, block 20008 pieces 19A. Cairo, Egypt.

Methods of Analysis

Preparation of Ginger Extract

Ethanolic ginger extract was prepared according to the method of **Santosh et al (1996).** In brief, as follows: five hundred grams (500g) of ginger powder (Zingiber officinalis Roscoe) were saturated with 95% ethanol and was shaken well for 30 min, then soaked overnight. The supernatant of upper layer was collected and filtered, the residual ginger powder was re-extracted with 95% ethanol for three times. All the ethanolic filtrates were collected and re-filtered. Finally the filtrate was air dried. The obtained ethanolic ginger extract was oily and viscous and it does not soluble in water, So, ginger extract was dissolved in dimethyl sulphoxide (DMSO) at different concentrations.

Antioxidant Activity of GE, GO and RBO

Scavenging activity on DPPH radical

The hydrogen atom or electron donation ability of the corresponding extract was measured from the bleaching of a methanolic purple colored solution of DPPH according to (**Gulluce et al 2004).**

This spectrophotometric assay uses the stable radical 2, 2′- biphenyl picryl hydrazyl (DPPH) as a reagent, as follows: Different amounts of each fraction dissolved in methanol were added to 2 ml of 0.004% (2mM) methanolic solution of DPPH, then incubated for 30 min at room temperature. After that the absorbance was read against the blank at

$$\% \text{ Scavenging activity} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

517 nm. The inhibition of free radical DPPH (1%) was calculated according to the following equation:

Liver and blood Biochemical analysis

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total protein (TP), Albumin (Alb), total Cholesterol (TC), High density lipoprotein cholesterol (HDL-C), hepatic triglycerides (h-TG) and serum Triglycerides (s-TG) were determined according to the methods as described in pamphlets. (**Young 1990; Belfield and Goldberg 1971; Tietz 1994 and Richmond 1973)**

Determination of hepatic triglycerides

At the end of the experiment, rats were sacrificed then liver was removed and washed with saline solution, triglycerides was determined in liver according to the method described by **Haug and Hostmark (2010).** One gram (1g) of liver was homogenated in 10 ml isopropanol for 2 min. after that, the homogenate was kept at 4 ºC for two days, and then centrifuged for 10 min at 4000 rpm. Triglycerides determined in the supernatant enzymatically by triglyceride kit reagent.

Biological experiment

Experimental design

Thirty six (36) male albino rats of Wistar strain, weighing about 100g ±2.36 were obtained from Animal Health Institute Dokki, Giza, Egypt. The animals were kept under normal laboratory condition and fed on normal diet for one week as an adaptation period before starting of the experiment. Rats were divided into 6 groups (6 rats/group) as follow: **Group 1 (NC):** Normal Control group, only fed on normal diet. **Group 2 (PC+):** positive control group, fed on normal diet and received orally ethanol treatment. **Group 3 (GE):** Ginger Extract Group, fed on normal diet + ethanol treatment + Ginger extract [200mg/Kg body weight]. **Group 4 (GO):** Ginger Oil Group, fed on normal diet + ethanol treatment + Ginger oil (GO) [200mg/Kg body weight]. **Group 5 (RBO):** Rice Bran Oil group, fed on normal diet + ethanol treatment + Rice Bran oil (RBO) [200mg/Kg body weight]. **Group 6 (DMSO):** fed on normal diet + ethanol treatment + dimethyl sulfoxide (DMSO) and served as the DMSO control group.

Ginger and rice bran treatments

Ethanolic Ginger Extract, Ginger Oil, Rice Bran Oil and DMSO were gavaged daily from first day to the end of the Experiment (30 days). DMSO used as a vehicle for GE, GO and RBO so we made a group for DMSO control.

Induction of fatty liver by ethanol treatment

All rats in group 2, 3, 4, 5 and 6 orally received 10 % w/v ethanol in drinking water starting from the day 22 of experiment period for 7 days. Then at the day 29 all these groups orally received a single

acute dose of ethanol 30 % calculated as (5g/kg body weight) by gavage syringe, **(Gonçalvesa et al 2016).** After 10 hours of gavaging the single acute dose of Ethanol Rats were sacrificed.

Blood sampling

Blood samples were taken at the end of the experiment after 10h of the acute single dose of ethanol as previously mentioned. The blood samples obtained from the sacrificing of rats were centrifuged at 4000 rpm for 10 min to obtain the serum which separated and kept in a deep freezer until biochemical analyses.

Statistical analysis

All results calculated were presented as means ± SD from six replicates and subjected to one way analysis of ANOVA test. The means of different treatments were compared using Duncan's multiple range test (L.S.D) at $p \leq 0.05$. Statistical analyses were performed using SPSS statistical software (IBM SPSS Statistics, version 20) **(Snedecor and Chochran, 1980)**.

RESULTS AND DISSCUSIONS

The present work was conducted to study the effects of ethanolic ginger extract (GE), ginger oil (GO) or rice bran oil (RBO) on fatty liver disease (steatosis) induced by ethanolic treatment in experimental rats. Relevant blood biochemical parameters and liver triglycerides were determined to investigate these effects.

Blood biochemical analysis

Liver functions

Activity of ALT, AST and ALP

Serum ALT activity of NC, PC+ (the rats induced fatty liver disease by ethanol), GE, GO, RBO and DMSO groups are presented in **Table (1) and Figure 1(a).** Results in **Table (1)** revealed that ALT activity showed different significant values in all groups under study. PC+ group displayed high Significant ($p \leq 0.05$) increase in ALT activity compared to NC group. Meanwhile, no significant difference was observed between NC group and rats treated by GE and RBO. Also, a significant increase was found in GO group compared to NC group. However, ALT level in GO group was less than PC+. Moreover, No significant difference was found among DMSO group and PC+. These results indicated that liver induced injury by alcohol treatment in PC+, meanwhile, liver was remediated by both GE, GO and RBO as shown in **Table (1)**.

Serum AST activity of NC, PC+, GE, GO, RBO and DMSO groups are presented in **Table (1) and Figure 1(b).** Data in **Table (1)** revealed that AST activity showed different significant values in all groups of the experiment. PC+ group displayed high significant ($p \leq 0.05$) increase in AST activity compared to NC group. However, no significant difference was observed between NC group and GE group. Also, a significant increase was observed in GO and RBO group compared to NC group. However, level of AST in both GO and RBO groups were less than PC+ or DMSO. Moreover, No significant difference was found among DMSO and PC+ groups. These results also, indicated that liver induced injury in PC+ and remained as normal in GE group.

Serum ALP activity of NC, PC+, GE, GO, RBO and DMSO groups are presented in **Table (1) and Figure 1(d)**. Significant ($p \leq 0.05$) differences were found among the activities of ALP for the groups of the experiment. A significant increase ($p \leq 0.05$) was noticed in the activity of ALP for the rats in PC+ compared to the control group. Also, a significant decrease ($p \leq 0.05$) was noticed between normal control group NC and GE group. In addition, there was a significant increase observed among both of GO group and RBO group as compared to NC group. These results reflected liver induction injury in PC+ and it is still in normal level in GE as shown in **Table (1) and Figure 1(d).**

Regarding, damage or injury induced to liver by ethanolic stress that determined by elevation in levels of aminotransferases activities: ALT and AST **(Black, 2002),** also increasing of ALP level. It is well known that more fats in liver is an indicator for damage or injury of liver cells, when it happened liver enzymes (ALT, AST and ALP) are released in the plasma. Elevation of activities of ALT, AST and ALP in plasma which, is a relevant marker of the extent and type of hepatocellular damage or injury **(Atta et al 2010)**, and elucidates altered plasma membrane permeability **(Boyd, 1962).** Results obtained showed that treated rats with ethanol caused fatty liver, these histological changes were correlated with the high significant increase in activities of serum ALT, AST and ALP as shown in **Table (1)**. This results are in accordance with **(Asha et al 2007),** who interpret that this significant increase may be due to increase in free

Table 1. Effect of Ginger extract (GE), Ginger oil (GO) or Rice bran oil (RBO) on the levels of ALT, AST and ALP in rats treated with ethanol

Enzyme	NC	PC+	GE	GO	RBO	DMSO
ALT (U/L)	23.35^{cd} ±1.11	69.41^{a} ±1.91	19.75^{d} ±1.03	30.51^{b} ±1.29	27.51^{bc} ±0.77	70.3^{a} ±2.38
AST (U/L)	27.95^{bc} ±0.76	62.98^{a} ± 2.22	23.96^{c} ±0.94	32.21^{b} ±1.17	32.13^{b} ±1.43	66.83^{a} ±3.00
ALP (U/L)	73.45^{c} ±1.32	121.65^{a} ±1.17	69.03^{d} ±2.22	82.03^{b} ±2.22	85.75^{b} ±1.67	123.55^{a} ±1.89

Data represent the means ± SE from six replicates. Different letters refer to significant differences at (P ≤ 0.05).

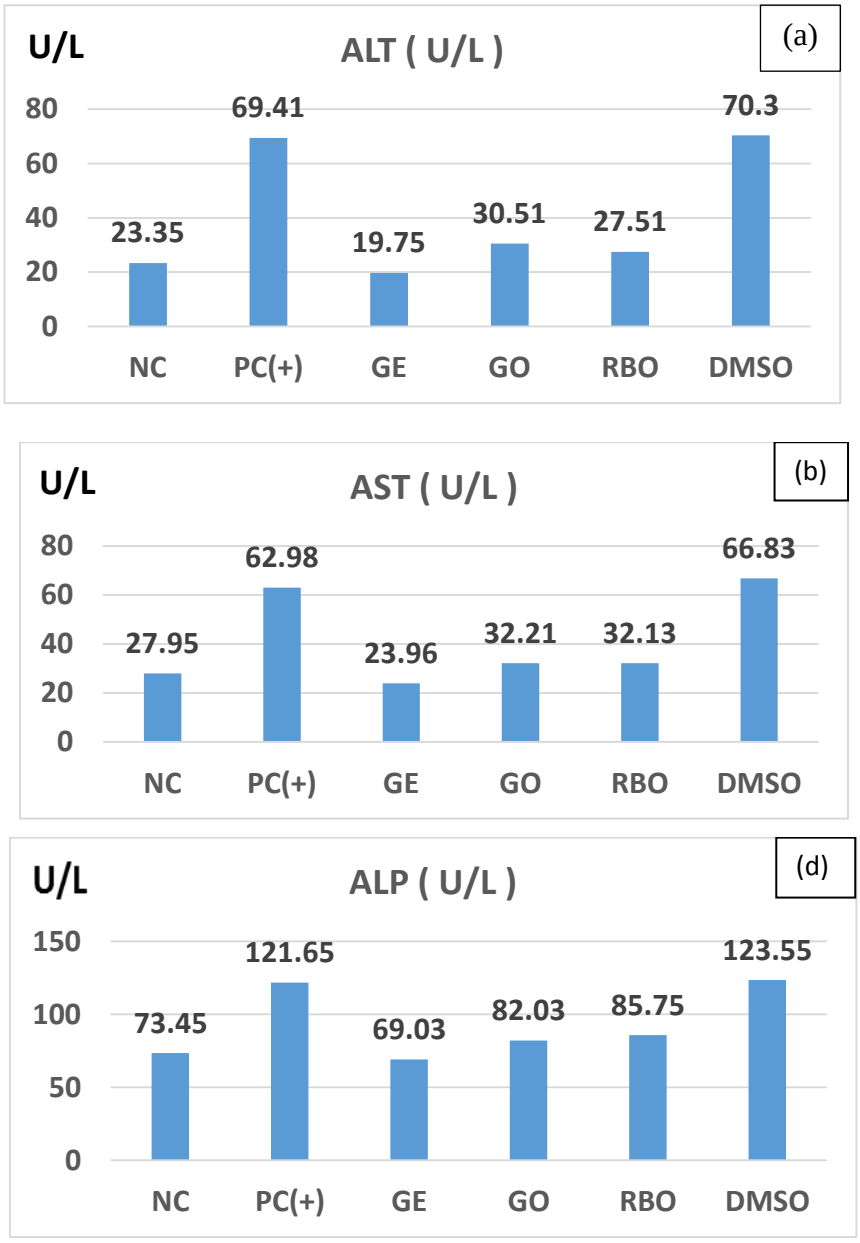


Fig. 1. (a, b, d): Effect of GE, GO, RBO or DMSO on the levels of ALT (a), AST (b) & ALP (d) in rats treated with ethanol

radicals, associated with decrease in the antioxidant enzyme levels. Generally the normal levels of serum transaminases AST, ALT and ALP activities in ethanolic treated rats with GE get indicates the hepatoprotective effect of ethanolic ginger extract, as coincided by **Yaccout et al (2007).** The liver enzymes in the present study of rats induced fatty liver which treated with GE are probably due to a membrane stabilizing effect of ginger. Meanwhile**,** many studies reported the hepatoprotective induction of ginger against liver toxicity and damage induced by ethanol, bromobenzene, acetaminophen and carbon tetrachloride by significant decrease in the levels of ALT, AST and ALP activities **(Mallikarjuna et al 2008; El-Sharaky et al 2009; Yemitan & Izegbu 2006; and Ajith et al 2007)**

The present results are in accordance with those studies which confirm that GE, GO and RBO decreased the levels of serum ALT, AST and ALP. And so, GE is the best treatment for induction hepatoprotective effect as shown in obtained results.

Serum Total protein, albumin and globulin

Serum total protein of PC+, GE, GO, RBO and DMSO groups are presented in **Table (2) and Figure 2(a)**. It is show High significant (p≤0.05) increase in TP was found both in rats induced fatty liver PC+ and DMSO group compared with NC rats. Meanwhile, rats of Positive control group kept on GO and RBO showed significant increase of TP compared to NC but lesser than PC+. However, no significant difference was noticed between NC and positive treated with GE which mean that GE has remediate effect on protein. These results revealed that GE treatment maintain the level of total protein to be close to normal level as displayed in obtained results.

Table 2. Effect of Ginger extract (GE), Ginger oil (GO), Rice bran oil (RBO) and DMSO on the levels of total protein, Albumin, Globulin and A/G Ratio in rats treated with ethanol

	NC	PC(+)	GE	GO	RBO	DM
Total protein (g/dl)	$6.41^{c} \pm 0.05$	$7.46^{a} \pm 0.07$	$6.25^{c} \pm 0.04$	$6.91^{b} \pm 0.06$	$6.78^{b} \pm 0.18$	$7.52^{a} \pm 0.05$
Albumin (g/dl)	$4.53^{c} \pm 0.04$	$4.88^{a} \pm 0.04$	$4.51^{c} \pm 0.04$	$4.74^{b} \pm 0.03$	$4.72^{b} \pm 0.06$	$4.95^{a} \pm 0.05$
Globulin (g/dl)	$1.83^{cd} \pm 0.06$	$2.55^{a} \pm 0.08$	$1.72^{d} \pm 0.02$	$2.15^{b} \pm 0.06$	$2.03^{bc} \pm 0.21$	$2.54^{a} \pm 0.04$
A/G Ratio	$2.49^{a} \pm 0.09$	$1.92^{b} \pm 0.07$	$2.62^{a} \pm 0.05$	$2.21^{ab} \pm 0.08$	$2.51^{a} \pm 0.38$	$1.94^{b} \pm 0.04$

Data represent the means ± SE from six replicates. Different letters refer to significant differences at (P ≤ 0.05)

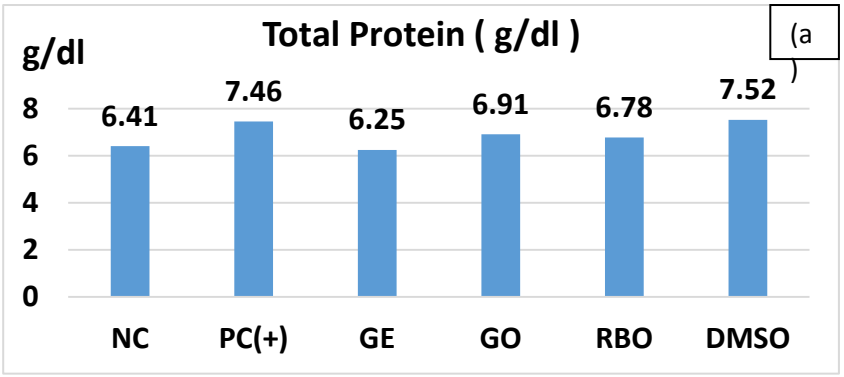
Serum albumin of NC, PC+, GE, GO, RBO and DMSO groups are presented in **Table (2) and Figure 2(b)**. Results showed that no significant (p≤0.05) differences were found among the NC group and GE group. While, high significant (p≤0.05) elevation was observed in both PC+ and DMSO compared to NC. Moreover, GO, RBO treatment induced significant (p≤0.05) increase in serum albumin compared to NC. These finding revealed that GE was the best treatment for remaining the level of serum albumin to kept on value close to normal control.

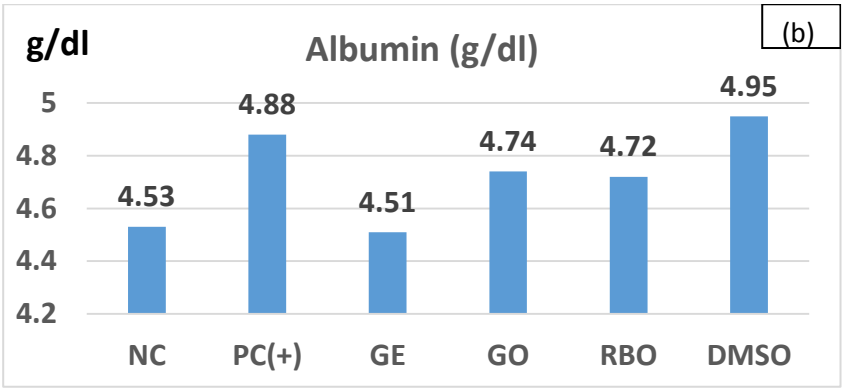
Effect of different treatments with PC+, GE, GO, RBO and DMSO on the levels of serum globulins in rats displays in **Table (2)** and illustrated in **Figure 2(d)**. As shown in Table (2) significant differences (p≤0.05) was observed among the levels of serum globulins for the rats received different treatments.

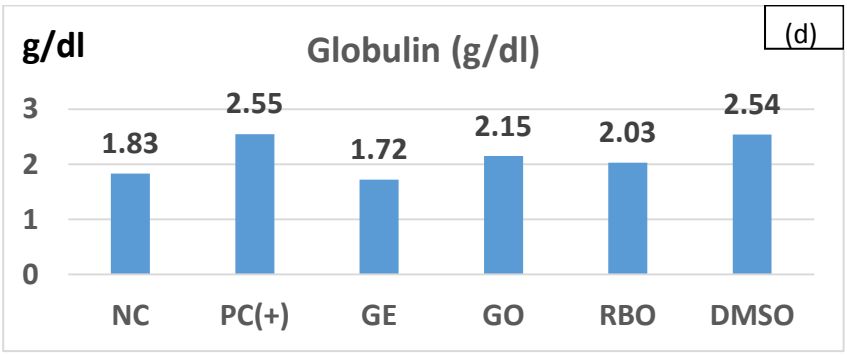
The effect of PC+, positive treated by GE, GO, RBO and DMSO on the levels of A/G ratio in rats revealed that, there were a significant (p<0.05) differences among the levels of A/G ratio for the rats received the different treatments as reported in **Table (2) and Figure 2(f).**

From liver functions results, it could be observed that ethanol treatment in PC+ induced liver dysfunction or damage, while GE treatment induced hepatoprotective effect more than either GO or RBO treatments.

In the present study, elevated levels of total protein, albumin and globulin in PC+, could be attributed to liver dysfunction or increasing of protein synthesis. Also, may be related to damage of biological tissues and processes or altering in permeability of liver and kidney cells membrane that lead to leakage of proteins **(Roushdy et al 1989)**. However, treatment with GE remained these levels as normal, which could be due to the hepatoprotective and beneficial effect of ginger.







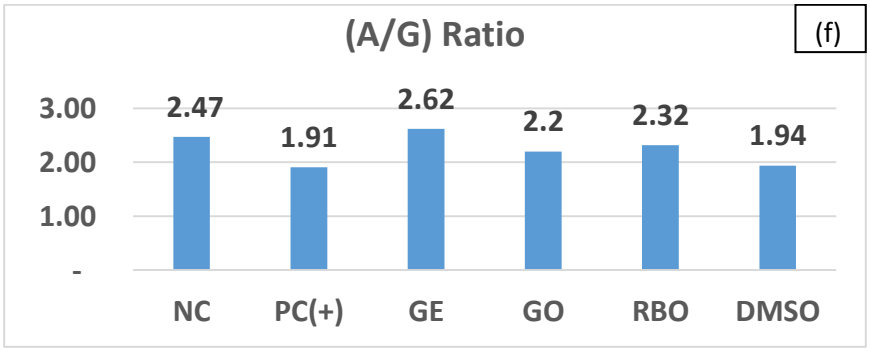


Fig. 2. (a, b, d, f). Effect of GE, GO, RBO or DMSO on the levels of TP (a), Alb (b), globulin (d) and A/G ratio (f) in rats treated with ethanol

Liver Triglycerides

Liver triglycerides of NC, PC+, GE, GO, RBO and DMSO groups are presented in **Table (3) and Figure 3(a)**. High significant increase ($p<0.05$) was noticed in liver triglycerides level in PC+ group and DMSO group as compared with NC group. Despite, insignificant difference was found in liver triglycerides between either GE group or RBO group and NC group. However, a significant elevation ($p<0.05$) in liver triglycerides was observed in GO group compared to NC group.

Table 3. Effect of Ginger extract (GE), Ginger oil (GO), Rice bran oil (RBO) or DMSO on the levels of liver triglycerides and serum triglycerides in rats treated with ethanol

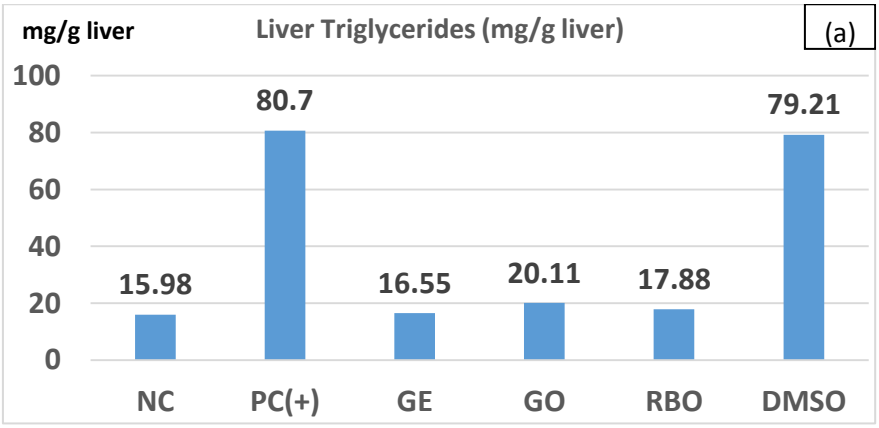
	NC	PC+	GE	GO	RBO	DMSO
Liver triglycerides (mg/g)	$15.98^{c} \pm 0.24$	$80.7^{a} \pm 0.41$	$16.55^{c} \pm 0.35$	$20.11^{b} \pm 0.32$	$17.88^{c} \pm 0.18$	$79.21^{a} \pm 1.62$
Serum triglycerides (mg/dl)	$74.22^{c} \pm 1.45$	$214.37^{a} \pm 2.69$	$72.76^{c} \pm 1.53$	$87.46^{b} \pm 3.67$	$78.39^{c} \pm 1.84$	$213.15^{a} \pm 4.03$

Data represent the means ± SE from six replicates. Different letters refer to significant differences at ($P \leq 0.05$)

Serum Triglycerides

Serum triglycerides Levels of NC, PC+, GE, GO and RBO or DMSO groups are presented in **Table (3) and Figure 3(b)**. Results display that PC+ and DMSO groups significantly elevated ($p<0.05$) the level of serum triglycerides compared to NC group. However, insignificant ($p<0.05$) difference was recorded in GE group as compared to NC group. Meanwhile, a significant increase ($p<0.05$) in serum triglycerides level was noticed in GO compared to NC group. Moreover, data showed that no significant ($p<0.05$) differences in serum triglycerides observed between PC+ and DMSO group.

Triglycerides measurements are used in the diagnosis and treatment of patients with fatty liver, diabetes mellitus, liver obstruction, nephrosis and other diseases involving lipid metabolism. In the present study, hepatic triglycerides were highly elevated in PC+ group compared to NC group which indicate that triglycerides accumulated in the liver (fatty liver induced). However, hepatic triglycerides level in GE group was still not affect to be close to level in NC group, this mean that GE induced hepatoprotective effect, this effect may be attributed to the antioxidant action of ginger that reduce the oxidative stress on liver, consequently safe the plasma membrane permeability. **(Ajith et al 2007; Stoilova et al 2007; Mallikarjuna et al 2008).**



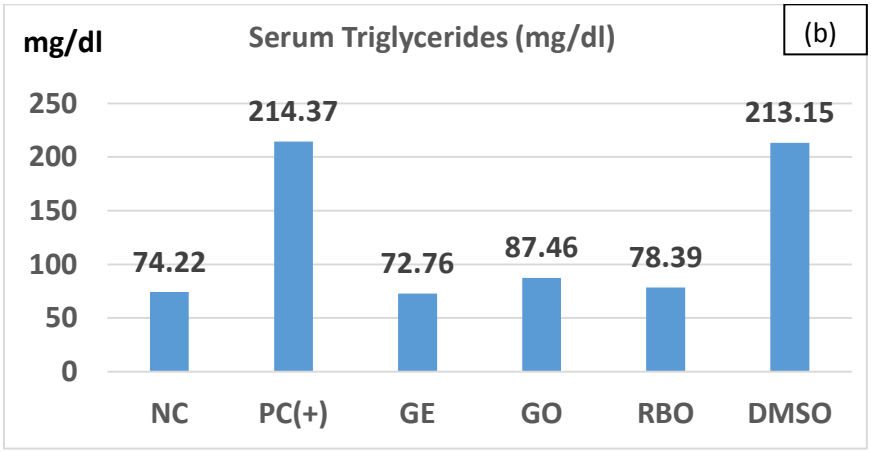


Fig. 3. Effect of GE, GO, RBO and DMSO on the levels of Liver triglycerides (a) and serum triglycerides (b) in rats treated with ethanol

Total Cholesterol (TC)

TC levels of NC, PC+, GE, GO, RBO and DMSO groups are presented in **Table (4) and Figure 4(a)**. A significant increase ($p<0.05$) in TC level was found in PC+ and DMSO group as compared to NC group. Also data show that, there was a significant increase ($p<0.05$) in TC level in GO and RBO compared to NC group. However, insignificant differences were observed in TC level between NC group and GE group.

High density lipoprotein-cholesterol (HDL-C)

HDL-C levels of NC, PC+, GE, GO, RBO and DMSO groups are displayed in **Table (4) and Figure 4(b)**. Data recorded significant increase ($p<0.05$) in HDL level in GE group compared to NC group. wheras, PC+ induced significantly decrease in HDL-C level compared with NC.

Low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein (VLDL-C)

LDL-C and VLDL-C levels of NC, PC+, GE, GO, RBO and DMSO groups are presented in **Table (4)**. High significant increase ($p<0.05$) was noticed in both LDL-C and VLDL-C in PC+ group and DMSO group as compared to NC. However, GE group showed insignificant difference for both VLDL-C and LDL-C levels compared with NC. Meanwhile, a significant increase ($p<0.05$) was observed in LDL-C level in both GO group and RBO group as compared to NC group. In addition, in VLDL-C level, GO group recorded significant ($p<0.05$) elevation in comparison with NC, while, RBO group showed insignificant difference as compared to NC.

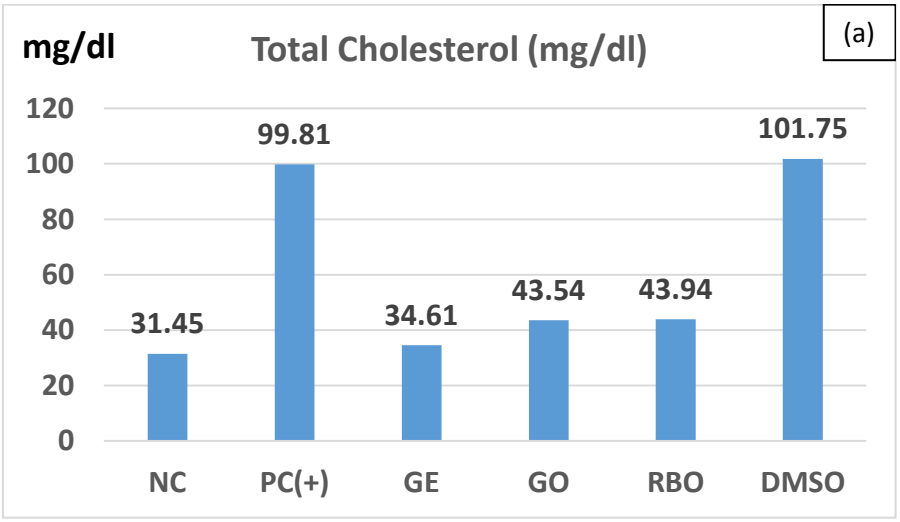
It is well known that of serum total cholesterol, HDL-C and LDL-C levels is important as an indicator of liver function and diagnosis of some diseases.

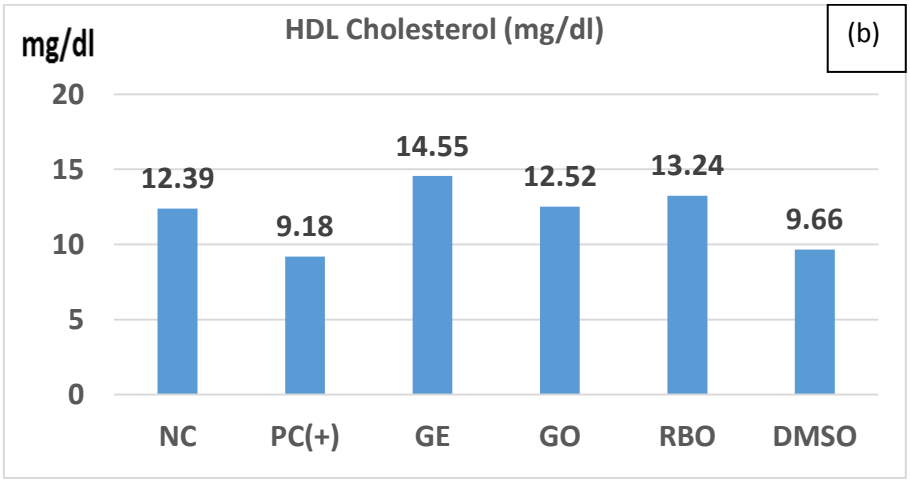
In the present study, total cholesterol, LDL-C and VLDL-C levels were raised while HDL-C dose not increase in PC+ group as compared to NC group, this observation was an indicator of liver dysfunction. However, TC, LDL-C and VLDL-c levels in GE group were decreased to be close to the levels in NC group, which revealed that GE exhibit hepatoprotective effect more than GO and RBO as shown in obtained results. The protection effect of GE extract could be attributed to decreasing of lipid biosynthesis or enhancing elimination of cholesterol from body by converting it to bile acids as reported by **Tanabe et al (1993); Verma et al (2004) and Nammi et al (2008).**

Table 4. Effect of Ginger extract (GE), Ginger oil (GO) and Rice bran oil (RBO) on the levels of total cholesterol (TC) and cholesterol fractions in rats treated with ethanol

	NC	PC+	GE	GO	RBO	DMSO
Total cholesterol (mg/dl)	$31.45^{c} \pm 1.23$	$99.81^{a} \pm 1.77$	$34.61^{c} \pm 1.11$	$43.54^{b} \pm 1.43$	$43.94^{b} \pm 1.92$	$101.75^{a} \pm 2.01$
HDL.C (mg/dl)	$12.39^{b} \pm 0.30$	$9.18^{c} \pm 0.25$	$14.55^{a} \pm 0.66$	$12.52^{b} \pm 0.33$	$13.24^{ab} \pm 0.69$	$9.66^{c} \pm 0.32$
LDL.C (mg/dl)	$4.21^{c} \pm 1.11$	$47.75^{a} \pm 1.70$	$5.50^{c} \pm 0.79$	$13.52^{b} \pm 0.89$	$15.01^{b} \pm 1.62$	$49.46^{a} \pm 2.64$
VLDL.C (mg/dl)	$14.85^{c} \pm 0.29$	$42.88^{a} \pm 0.53$	$14.56^{c} \pm 0.30$	$17.50^{b} \pm 0.73$	$15.68^{c} \pm 0.36$	$42.63^{a} \pm 0.80$

The data are presented as means ± SE from six replicates. Different letters refer to significant differences at ($P \leq 0.05$)





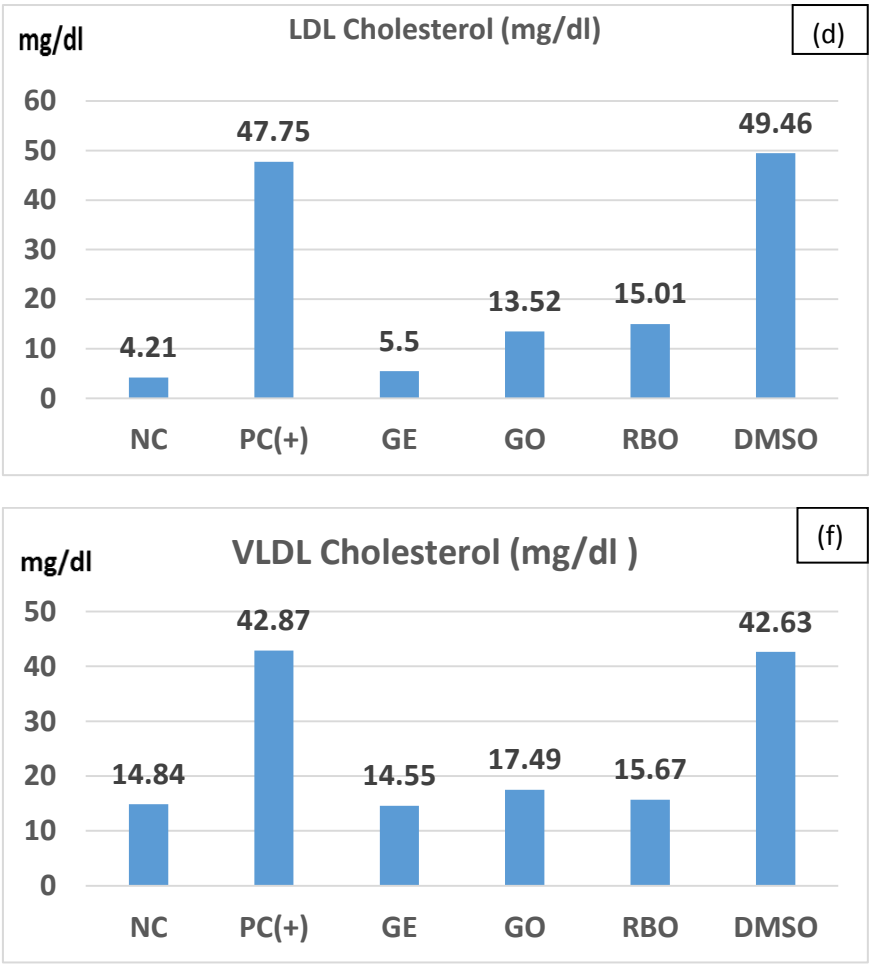


Fig. 4. (a, b, d, f). Effect of GE, GO, RBO and DMSO on the levels of Total Cholesterol (TC) (a), HDL-C (b), LDL-C (d) and VLDL-C (f) in rats treated with ethanol

Antioxidant activity of GE, GO and RBO

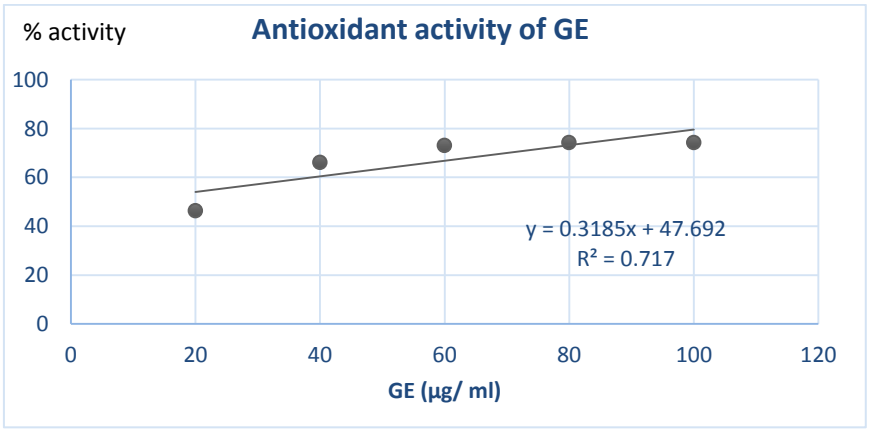


Fig. 5. Antioxidant activity of ginger extract (GE)

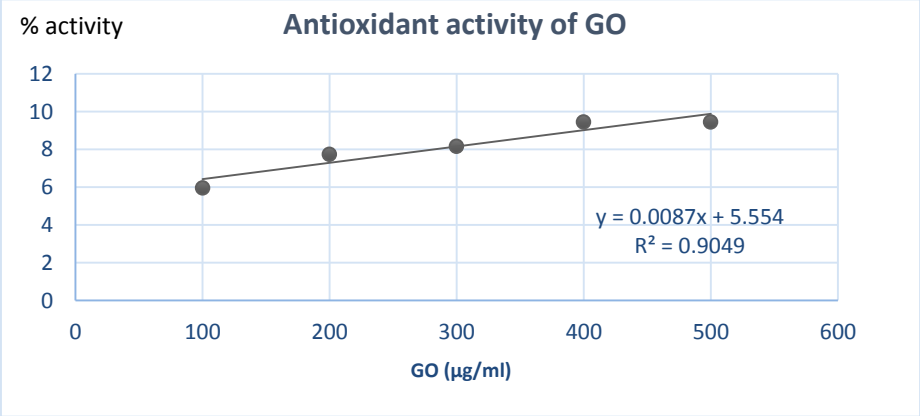


Fig. 6. Antioxidant activity of ginger oil (GO)

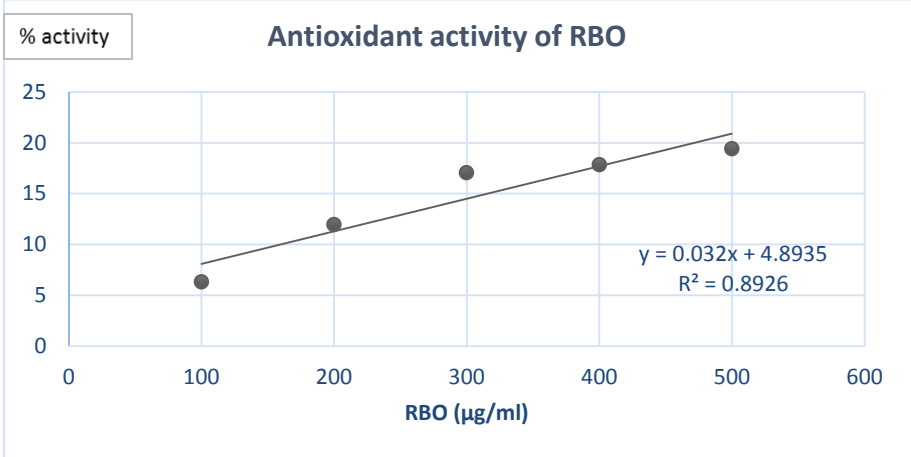


Fig. 7. Antioxidant activity of rice bran oil (RBO)

Table 5. The half maximal inhibitory concentration (IC50) of GE. GO and RBO

Extract	IC50 (µg/ml)
ginger extract (GE)	18.25
Ginger oil (GO)	6714.38
Rice bran oil (RBO)	1409.57

As shown in **Table (5)**, antioxidant activity of GE recorded very high antioxidant effect compared to GO and RBO. So, high antioxidant activity of GE could be interpret that it has hepatoprotective effect more than GO and RBO. **(El-Sharaky et al 2009; Masuda et al 2004).** In addition to**,** antioxidant activity of GE explain many of its hepatoprotective mechanisms.

Conclusion

Finally, ethanolic ginger extract, ginger oil and rice bran oil have a hepatoprotective effect. However, Ethanolic ginger extract was the best in liver protection as found from results obtained in the present study.

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