

Antihepatotoxic Effect of Garlic and Onion Oils on Ethanol-induced Liver Injury in Rats

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Abstract: The objective of this study is to investigate the effect of antihepatotoxic and antioxidant effect of both garlic and onion oils in ethanol induced liver injury. Rats were divided into four groups; group I received saline, and group II, III, IV received ethanol for 30 days, then group II received corn oil, group III received garlic oil and group IV received onion oil (100 mg oil /kg b.w/day) orally for 60 days. Serum lipid profile, liver functions and antioxidant parameters were estimated. Garlic and onion oils improved the reduction of antioxidant parameters (SOD and reduced GSH) and the increasing of thiobarbituric acid which caused by ethanol administration, serum hepatic markers (ALT, AST, ALP and γ GT), serum cholesterol and triglycerides significantly increased by ethanol abuse and returned to the normal value after garlic and onion oils treatment. Histopathological examination showed a damaging effect, necrosis and fibrosis of liver cells after ethanol abuse and a marked improvement was seen after garlic and onion oils treatment. These results prove the potent antioxidant activity of both garlic and onion oils.

Keywords: Garlic oil, Onion oil, antioxidant, antihepatotoxic, liver injury

INTRODUCTION

Alcoholic liver disease remains one of the most common causes of chronic liver disease in the world^[1]. It is well known that, ethanol administration can elicit disturbances in the delicate balance between the pro- and antioxidant system of the organism leading to oxidative stress^[2]. The resulting oxidative stress is a reason of enhanced lipid peroxidation and change in structure and functions of other important cellular components, such as protein and DNA^[3]. Moreover, ethanol-induced hypoxia especially in pericentral zone of the liver acinus such as oxygen is consumed in order to detoxify ethanol^[4].

Thus, there is an urgent need to develop potent antihepatotoxic and antioxidants especially among natural product agents against alcohol-induced hepatic disorders.

Garlic (*Allium sativum* L.) is widely consumed herb in foodstuffs and medicines. Numerous studies have shown that garlic exhibits diverse biological activity, including antitumorogenic, antiatherosclerosis and detoxification^[5]. However, researchers have only been focused on the use of garlic as an antimicrobial ingredient while its pharmacological effects might have long been neglected^[6]. Similarly, onion (*Allium cepa*) is used as a traditional remedy in the treatment of a variety of disorders. The pharmacological evidence for the use of onion as an anti-asthmatic, anti-hypertensive, anti-hyperglycemic, anti-hyperlipidemic and anti-tumor agent was reported^[7,8].

Thus, the aim of this study is to investigate the antihepatotoxic and antioxidant effect of both onion and garlic oils on ethanol induced liver injury in rats.

MATERIALS AND METHODS

The steam-distilled garlic and onion oils were purchased from El-captain Comp. (CAP PHARM-Egypt).

Ethanol of E. Merck, Darmstadt, Germany. Was used.

Experimental Animals: 48 Male rats weighing 180-200 g were used. Rats were fed standard chow and water ad libitum. They were divided into four groups, containing 12 rats each. Group I received saline orally (control group), group II, III and IV received 25% ethanol (5ml/ kg body weight/ day) orally for 30 days^[9], after that, group II received corn oil, group III received garlic oil, group IV received onion oil (100 mg oil /kg body weight/ day) orally^[10]. After 60 days of treatment, rats were fasted over night, anesthetized with diethyl ether, blood was withdrawn from the optical vein according to Madway^[11] and left to clot at 37°C then centrifuged at 3000 r.p.m. for 10 minutes, serum was separated and stored at -20°C till used for biochemical estimations.

Biochemical Estimations: The activity of both serum

Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) were assayed according to Rietman and Frankel^[12], serum alkaline phosphatase was determined by the method of Henry^[13], and gamma glutamyltransferase (γ -GT) was estimated by the method of Whitfield *et al.*,^[14]. Serum cholesterol was estimated according to Allein *et al.*,^[15] and triglyceride determined by the method of Fredrickson *et al.*,^[16]. Serum protein and albumin were estimated by Biuret method^[17]. Lipid peroxidation was determined by measuring thiobarbituric acid reactive substance (TBARS) according to the method of^[18]. Reduced glutathione was assayed according to Ellman^[19]. Superoxide dismutase was determined by the method of Misra and Fridovich^[20] as modified by Sykes *et al.*,^[21].

Histopathological Examination of the Liver: Samples of liver tissue were dissected from the animals immediately after decapitation. They were fixed in buffered 10% formal saline solution at room temperature for 72 hours. Tissue samples were then dehydrated in ascending grades of ethyl alcohol, cleared and then embedded in soft paraffin. Tissue sections of about 6 μ m were obtained, stained by Haematoxylin and Eosin^[22] and examined.

Statistical Analysis: Data were analyzed by one-way analysis of variance (ANOVA) followed by tukey test. Results were presented as mean \pm S.D. p-values <0.05 were regarded as statistically significant.

RESULTS AND DISCUSSIONS

Results: There was no mortality in any of the studied groups. Table (1) shows that alcohol administration increased serum transaminase ALT & AST, ALP and γ -GT significantly which indicated severe liver damage. Garlic and onion oils co-administration showed significant decrease in AST and ALP but there was a reversal in the values of ALT and γ GT towards normal.

The mean values of total protein and albumin were significantly decreased by ethanol intake when compared with normal group while it returned to normal by garlic and onion oils administration (table. 2).

The mean value of total cholesterol and triglycerides were significantly increased in ethanol intake rats compared to normal while it improved significantly after administration of garlic and onion oils (table. 3).

The mean value of reduced glutathione and superoxide dismutase activities were decreased significantly by ethanol administration while it returned to increase significantly again to reach the normal values by garlic

and onion oils administration, in addition TBARS level was increased by ethanol administration while garlic and onion oils decreased it significantly after treatment (table. 4).

Histopathological Results: Histopathological examination of liver tissue reveals that ethanol abuse for 1 month had a damaging effect on the liver tissue. Dilatation with or without congestion of blood vessels, necrosis of cells and the presence of fibrosis were used as parameters for damage.

Sections taken control rats showed the normal structure of liver tissue composing of hepatocytes arranged in cords radiating from a central vein in an astomosing manner to form a sponge work or labyrinth. These cords are separated from each other by blood sinusoids, which are nearly equal in size (Fig.1)

The hepatocytes appear polyhedral in shape. Their acidophilic cytoplasm takes a lace-like or granular appearance with clumps of basophilic material. The nuclei are vesicular, large and rounded or ovoid in shape with well-defined one or two nucleoli. (Fig. 2). Ethanol treatment for 1 month had an obvious damaging effect on liver tissue in the form of dilatation with congestion of the portal vein, with thickening of its wall and marked fibrosis in the portal area extending from it in between the hepatocytes in many directions. The fibrous tissue in the portal area is formed of irregular collagenous fibers with fibrocytes. (Fig. 3, 4).

Using garlic oil showed marked improvement in liver tissue structure in spite of the presence of slight thickening and fibrosis in blood vessels' walls especially central veins. Hypertrophy of Kupffer cells in blood sinusoids can be explained by increased phagocytic activity to remove debris of dead cells in the stage of regeneration (Fig.5, 6) Using onion oil showed slightly little improvement as fibrosis remained surrounding the components of the tract and extending between the hepatocytes towards neighboring areas. Slight dilatation of blood sinusoids and hypertrophy of Kupffer cells denote the presence of edema, while the hepatocytes retain their normal structure (Fig.7,8).

Discussion: Chronic alcohol abuse provokes successive hepatic changes, consisting of alcoholic steatosis (Fatty liver), alcoholic fibrosis, alcoholic hepatitis and cirrhosis^[23]. In the present study, ethanol intake significantly increased liver enzymes (ALT, AST and ALP). These results were in agreement with Pari and Karthikesan^[24] who indicated that chronic alcohol intake leads to many cellular and tissue abnormalities such as alteration in liver enzymes (ALT, AST and ALP), which indicated the increased permeability, damage and /or necrosis of hepatocytes^[25]. In the current study, these enzymes decreased significantly by onion

Table 1: Effect of garlic and onion oils on serum hepatic markers in different studied groups.

	AST(IU/l)	ALT(IU/l)	ALP(IU/l)	γGT(U/l)
Normal control	90.5±3.0	40.1±4.3	70.4±3.10	3.1±1.2
Ethanol	211.9±8.1 ^a	82.4±7.3 ^a	212.6±12.70 ^a	26.3±2.7 ^a
Ethanol+onion oil	145.6±8.8 ^b	43.3±5.4 ^b	95.6±9.50 ^b	3.1±1.7 ^b
Ethanol+garlic oil	125.0±8.4 ^b	40.6±3.9 ^b	96.0±12.80 ^b	4.1±2.3 ^b

Values are expressed as means± SD. For twelve rats in each group. ^a p< 0.05 in comparison between normal control group and ethanol group
^b p< 0.05 in comparison between ethanol and treated group.

Table 2: Effect of garlic and onion oils on the serum cholesterol and triglyceride levels in different studied groups..

	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)
Normal control	65.1±0.4	35.0±0.4	30.10±0.1
Ethanol	55.1±0.6 ^a	27.0±0.2 ^a	28.1±0.36 ^a
Ethanol+onion oil	77.3±0.2 ^b	39.1±0.1 ^b	38.2±0.35 ^b
Ethanol+garlic oil	66.5±0.6 ^b	36.1±0.3 ^b	30.4 ±0.29 ^b

Values are expressed as means± SD. For twelve rats in each group. ^a p< 0.05 in comparison between normal control group and ethanol group
^b p< 0.05 in comparison between ethanol and treated group.

Table 3: Effect of garlic and onion oils on lipid profile in different studied groups..

	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal control	84.5±6.24	91.02±5.30
Ethanol	117.75±6.07 ^a	140.50±7.21 ^a
Ethanol+onion oil	83.50±8.31 ^b	88.43±8.65 ^b
Ethanol+garlic oil	70.75±8.7 ^b	84.54±4.6 ^b

Values are expressed as means± SD. For twelve rats in each group ^a p< 0.05 in comparison between normal control group and ethanol group
^b p< 0.05 in comparison between ethanol and treated group.

Table 4: Effect of garlic and onion oils on antioxidant and lipid peroxidative markers in different studied groups.

	TBARS (μmol/l)	reduced GSH (μmol/l)	SOD(U/ml)
Normal control	0.50±0.07	16.25±0.27	0.62±0.02
Ethanol	0.71±0.01 ^a	13.83±0.32 ^a	0.51 ±0.04 ^a
Ethanol+onion oil	0.54±0.07 ^b	15.35±0.65 ^b	0.59 ±0.01 ^b
Ethanol+garlic oil	0.53±0.02 ^b	15.48±0.61 ^b	0.61 ±0.03 ^b

Values are expressed as means± SD. For twelve rats in each group. ^a p< 0.05 in comparison between normal control group and ethanol group
^b p< 0.05 in comparison between ethanol and treated group.

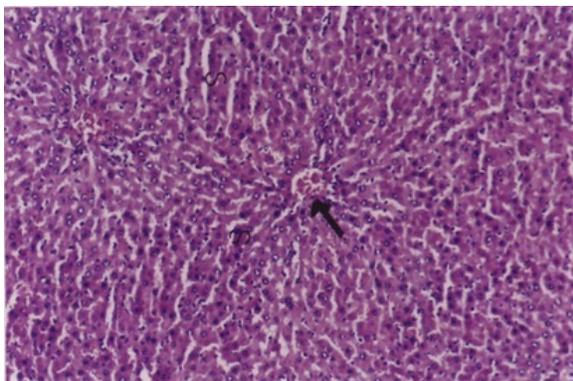


Fig. 1: A photomicrograph of a control liver showing the central vein (arrow) and the hepatocytes from it in plates.

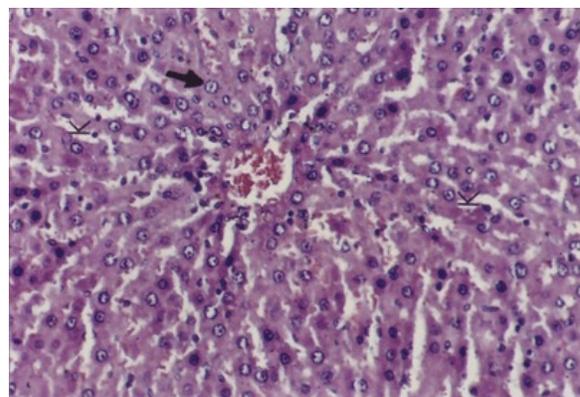


Fig. 2: Notice the hepatocytes having rounded vesicular nuclei (arrow) and the Kupffer cells (K) in the lumen of blood sinusoids.

and garlic oils treatment. The observed decrease in these enzymes shows that onion and garlic oils preserve the structural integrity of the liver from the toxic effect of ethanol.

In our study, ethanol administration increased the mean value of gGT significantly. Concomitantly^[26], reported that serum gGT concentration may be elevated in response to many drugs and toxins, thus, alcohol is

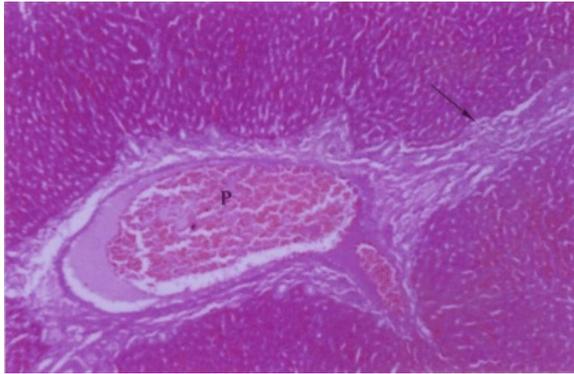


Fig. 3: Shows the effect of ethanol on liver tissue in the form fibrosis (arrow) and dilatation with congestion of portal vein (P).

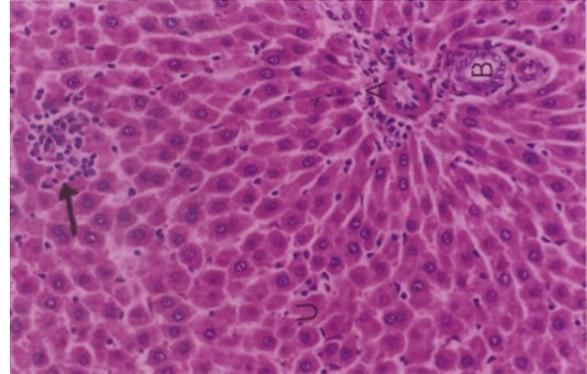


Fig. 6: The higher magnification shows focal lymphocytic infiltration in between the hepatocytes.

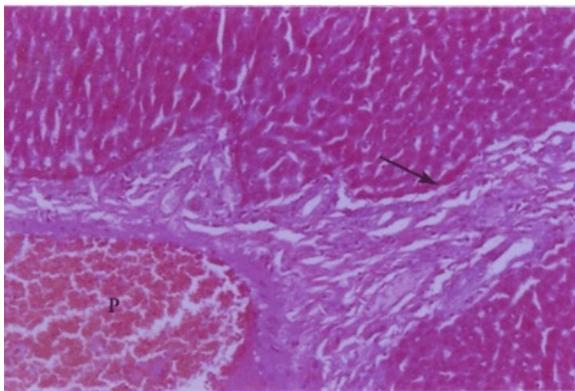


Fig. 4: The fibrous tissue appears composing of collagenous fibers (arrows).

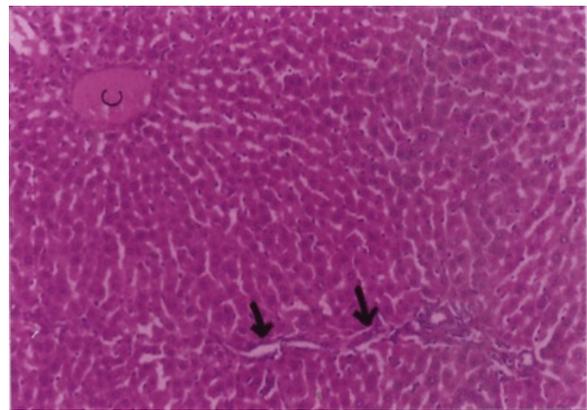


Fig. 7: Shows the effect of onion oil on the ethanol-damaged liver tissue, in which marked decrease of fibrosis occurs, however bundles of collagenous fibers are observed extending from the portal area outward (arrows). The higher magnification in.

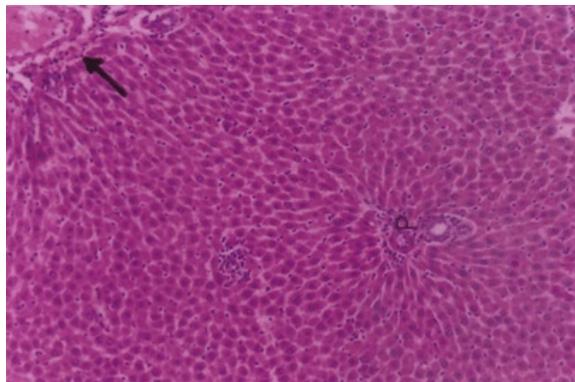


Fig. 5: Shows marked decrease in fibrous tissue appear (P) although remnants of fibrosis are still observed around the central vein (arrow) in liver of rats treated with garlic oil.

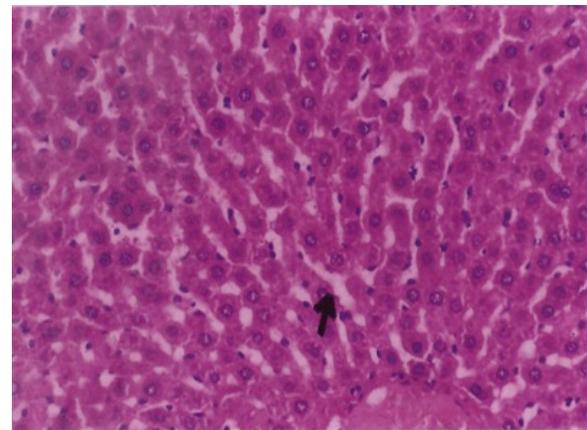


Fig. 8: Reveals slight dilatation of blood sinusoids denoting a mild degree of edema.

a potent inducer of gGT and the enzyme is commonly elevated in regular drinkers. After treatment with onion and garlic oils, these enzymes decreased significantly indicated antihepatotoxic effect of these oils.

Since albumin and globulin are two key components of serum proteins, because albumin is synthesized in the liver, one element is used to monitor the liver function^[27]. In the present study, there was a concomitant decrease in serum albumin and protein levels. These results were in agreement with Ahmed *et al.*,^[28] who found significant decrease in serum protein and albumin in ethanol-administered rats. It demonstrates the decrease functional ability of ethanol-administered rat liver.

In the current study, significant increase in serum total protein and albumin was observed in garlic and onion oils co-administered rats, that indicates the ability of these oils to stimulate the regeneration of hepatic tissue which increase protein synthesis in damaged liver and improvement of the functional status of the liver cells.

Several studies demonstrated that alcohol (or ethanol) intake is associated with changes in plasma lipid concentrations and whole-body lipid balance^[29]. In the present study ethanol intake increased the mean values of serum cholesterol and triglycerides significantly while after treatment by garlic and onion oils, these values significantly decreased. These results were in agreement with Mukherjee *et al.*^[30].

The mechanism of action was suggested by Sodimu *et al.*^[31] who indicated that garlic oil prevented an increase of cholesterol, triglyceride and total lipids by inactivation of thiol group enzymes as HMG-CoA reductase and CoASH, the rate limiting enzyme for cholesterol biosynthesis and the multi-enzyme complex for fatty acid biosynthesis.

In this work, the mean value of reduced glutathione and superoxide dismutase decreased significantly by ethanol intake while excess lipid peroxidation as measured by TBARS was found. In the same line,^[25] observed a lower level of plasma reduced GSH in alcoholic rats. He suggested that reactive oxygen intermediates, generated during the metabolism of ethanol, leads to glutathione oxidation and lipid peroxidation that are responsible for the toxic effects of ethanol. In addition,^[32] found a decrease in superoxide dismutase activity after alcohol consumption.

In the current study, onion and garlic oils increased superoxide dismutase and reduced glutathione significantly while TBARS decreased significantly. These results were in agreement with Helen *et al.*^[10] who indicated that garlic and onion oils are effective antioxidant against the oxidative damage.

Also, Prakash *et al.*^[33] reported that onion is a rich source of polyphenols with promising antioxidant and free radical scavenging activities and has the ability to provide protection against DNA damage caused by reactive oxygen species.

Moreover, Tang and Cronin^[34] indicated that Quercetin, is the main onion juice antioxidant, which considered as free radical scavenging, chelating of transition metal ions, and inhibition of oxidases such as lipoxigenase^[35].

Furthermore, De vries *et al.*,^[36] indicates that absorption and bioavailability of flavonoids in onions have been shown to be more effective than other sources.

Garlic oil is as effective as onion oil. Garlic has anti-oxidant effects, which means it can reduce toxicity associated free-radical damage and it contains the trace elements germanium and selenium, which have been thought to play a role in improving host immunity and 'normalizing' the oxygen utilization in cells. In addition, garlic compounds have been found to inhibit lipid peroxidation, which is considered one of the main features of aging in liver cells^[37,38].

MacDonald *et al.*,^[39] reported that there are varieties of antioxidants in garlic, which protect against disease-causing oxidative damage. This effect may be due to the fact that, garlic and related organosulfur compounds have antioxidant, detoxifying and other properties. These detoxifying effects are related to their ability to inhibit phase I enzymes and induce phase II enzymes or bind to exogenous toxins through sulfhydryl groups^[40]. Moreover, He *et al.*,^[6] indicated that, the enzyme activity of SOD in 100 g of garlic ranges from 20 000 to 30 000 units much more than that of another SOD in abundant plant, *Rosa Roxburghii*.

In addition, Liver histology of ethanol administered animal showed pathomorphologic alterations in the form of obvious dilatation and congestion of blood vessels accompanied with marked fibrosis extending from the portal area in-between the hepatocytes. Such findings are supported and explained by those of Charles S. Leiber *et al.*,^[41] who stated that alcohol increases hepatic collagen type I with a significant rise in mRNA for [alpha] 1 procollagen. This leads to cirrhosis, septal and perivenular fibrosis, this result was in agreement with MacSween and Burt^[42] who observed a spectrum of histological abnormalities in the liver by alcohol administration. Treatment with garlic and onion oils reduced morphological changes produced by ethanol and greatly reverted the microanatomy of the liver to normal which can be explained by containing of garlic as well as onion of certain compounds such as germanium and selenium, that play an important role in 'normalizing' the oxygen utilization in cells.

In conclusion, ethanol significantly impairs the antioxidant defense system and inconsequence may lead to significant increase in oxidative stress mainly in the

liver. Garlic and onion oils can influence on normalization of cellular metabolism and functions by their powerful antioxidant effect against oxidative damage caused by ethanol.

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