

Therapeutic effect of aqueous extracts of three dietary spices and their mixture on lipid metabolism and oxidative stress in a rat model of chronic alcohol consumption

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Abstract: The protective effect of aqueous extracts of three dietary spices, garlic, (*Allium sativum*), ginger (*Zingiber officinale*) and pepper (*Capsicum frutescens*) singly and combined was investigated using a rat model of chronic alcohol intake. Rats were given 30% ethanol, with or without aqueous extracts of garlic, ginger, pepper or mixture of the three administered at 200mg/kg body weight by oral gavage for 28 days. Lipid profile, lipid peroxidation, oxidative and antioxidative profiles of serum, faecal, liver, kidney, heart and brain tissues of the rats were analyzed. Alcohol treatment significantly elevated liver enzymes, lipid peroxidation, depleted antioxidant system and induced histopathological changes in the liver. These alterations were markedly ameliorated by treatment with aqueous extracts of the three spices singly or mixed at 200mg/kg body weight. These results suggest that aqueous extracts of garlic, ginger, pepper or a blend of the three protects against alcohol- induced hypercholesterolemia, lipid peroxidation, oxidative stress and liver damage.

Keywords: chronic alcoholism; dietary spices; lipid peroxidation; liver injury; oxidative stress.

INTRODUCTION

Chronic alcohol use in human results in functional changes and disease in multiple organ systems as almost all organs in the body are affected by ethanol intake due to its ability to cross the membrane barrier of most tissues particularly liver, heart and pancreas (Jones 2005). Increased production of reactive oxygen species (ROS), enhanced oxidative stress in many tissues especially the liver and reduced cellular antioxidant levels has been reported in acute and chronic alcohol consumption (Lieber 2000; Albano 2006; Arulmozhi *et al.*, 2012).

Herbs and spices have been used since ancient times not only to enhance the flavor of foods but also to prevent and treat chronic health diseases. More recently, herbs and spices have attracted attention as physiologically functional foods, which are excellent sources of polyphenols, flavonoids, and saponins that could boost the antioxidant defense system. The use of dietary spices singly or in combination to treat or manage chronic diseases is increasingly becoming more popular and successful. Several herbal mixtures and spices such as grape seeds, gardenia, *Aframomum meleguata*, livomap, bael leaf, garlic, ginger, pepper, *Ocimum gratissimum*, *Garcinia kola*, curcumin, *Piper guineense* (Nwozo *et al.*, 2012) and cinnamon have been reported to have protective effects against chronic diseases (Kim *et al.*, 2006; Singanan *et al.*, 2007; Adaramoye *et al.*, 2009; Ighodaro and Omole 2012; Kanchana and Jayapriya 2013).

Allium sativum (garlic), *Zingiber officinale* (ginger) and *Capsicum frutescens* (pepper) are three common culinary spices used by most populations of the world as both spices and indigenous medicines. These three spices have been reported to have hypolipidemic, anti-inflammatory, anti-cancer, anti-nausea properties, as well as circulatory, pain relieving and warming effects amongst others (Srinivasan 2005; Rahman and Lowe 2006; Beltran *et al.*, 2007).

Previous studies on the chemical composition of each of these spices have shown that garlic, ginger and pepper possess nutrients and minerals in varying proportions, as well as phytochemicals such as alkaloids, tannins, carotenoids and polyphenols (Otunola *et al.*, 2010; Otunola and Afolayan 2013). These reports suggest that the three spices and a mixture of the three may possess beneficial activity as antioxidant and hepatoprotective agents. However, the protective effects of the spices singly or blended together as a mixture against alcohol toxicity are yet to be investigated. The present study therefore evaluates the protective action of extracts of the three spices singly or in combination in a rat model of ethanol toxicity.

MATERIALS AND METHODS

Materials: Ethanol and assay kits for cholesterol, HDL-C, LDL-C, triglycerides, total protein, albumin, glucose, gamma glutamyl transferase, alanine and aspartate amino transferase, superoxide dismutase, glutathione reductase, and alkaline phosphate assay kits were purchased from Randox Laboratories Ltd (Ardmore, Co, Antrim, UK). The spices garlic, ginger and pepper were purchased at

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the Fruit and Vegetable outlet in Alice, South Africa. They were identified in the Department of Botany as common household vegetables.

Preparation of aqueous extracts of spices and their mixture

Garlic, ginger and pepper were individually sorted to remove grits and dirt, washed and thinly sliced before oven drying at 60°C for 72h. The dried spices were milled into fine powder, packed into airtight bottles and stored at -20°C until needed. From the powdered samples, 50g of each spice was individually extracted in 1000ml of distilled water at 100°C for 10min. The extract was cooled, filtered and lyophilized for 48h, each freeze-dried spice was then stored in airtight bottles at 4°C till needed.

The spice mixture was prepared by weighing equal amounts of the spices in the ratio 1:1:1 and passing the mixture through the coffee grinder of a home blender set to obtain a homogenous mix of the three. From this mixture, 50 g was taken, extracted in like manner as described above. The free-dried samples were stored in airtight bottles at 4°C until used.

Doses equivalent to 200 mg/kg of the aqueous extract of each spice, consistent with previous reports, were calculated and reconstituted in distilled water (Shanmugam *et al.*, 2010; Abdul Jawad *et al.*, 2013; Ahmadi *et al.*, 2013).

Preparation of 30% alcohol for inducing chronic alcoholism

The alcohol used to induce chronic alcoholism in the rats was prepared using absolute ethanol and distilled water. Thirty (30) ml of ethanol was taken and 70 ml of distilled water was added as earlier described (Martinez *et al.*, 1997).

Experimental

Male Wistar rats weighing 168±10 g were obtained from the University of Fort Hare Central Animal Unit. The rats were subjected to standard laboratory conditions including constant temperature (25°C±2°C), humidity (70%±4%), 12 h light/dark cycle and access to feed and water ad libitum. Approval for animal experimental procedures was given by University of Fort Hare Institutional Animal Ethics Committee and carried out as laid out by the NIH Publication (1985) on Guide for the Care and Use of Laboratory Animals.

Rats were randomly assigned into six groups consisting of six rats each. Group 1=control (standard chow + water only), Group 2=CA (chronic alcohol treated), Group 3 (CA + garlic extract); Group 4 (CA +ginger extract); Group 5 (CA + pepper extract); Group 6 (CA + mixture extract). The alcohol and spice extracts were co-

administered concurrently to the animals by oral gavage once daily for 28 d.

The experimental rats were fasted for about 12 h and anaesthetized using pentobarbital sodium at 45 mg/kg body weight intraperitoneally. Blood was obtained from the heart through cardiac puncture for the various analyses, after which the rats were euthanized. Heart, liver, kidney and brain were excised, rinsed in ice-cold saline and weighed.

Organ to body weight ratio of the rats were calculated; serum lipid and assay of serum enzymes (ALP, AST, GGT, LD and CK), were measured using the Beckman Coulter Synchron® Clinical Systems (Beckman Coulter Ireland Inc.).

Hepatic and faecal lipids were measured using Randox chemical kits as previously described (Folch *et al.*, 1957; Carr *et al.*, 1993). Lipid peroxidation in the liver, kidney, heart and brain tissues were evaluated as earlier described Okhawa *et al.* (1979), while oxidative stress was measured by assaying for SOD, GPex and Gred activities respectively using Randox chemical kits (Wooliams *et al.*, 1983; Paglia and Valentine 1967; Goldberg and Spooner 1983). The histology of the liver samples was observed using the Scanning Electron Microscope (SEM) (Khalil *et al.*, 2010).

STATISTICAL ANALYSIS

Data was expressed as means ± SEM from 6 determinations. Both descriptive and inferential statistical methods were used for data analysis. ANOVA was used to identify significant difference at P<0.05 among treatment means. Duncan's Multiple Range Test was used to segregate means that were significantly different from each other using MINITAB Student Version 12 for Windows Software.

RESULTS

Weight gain and organ/body weight of chronic alcoholic rats administered with aqueous extracts of spices

The body weight (g) in all the rats showed that repeated alcohol consumption led to significantly lower (P<0.05) mean body weight gain when compared to control rats (fig. 1A). Aqueous extracts, each of garlic, ginger, pepper or their mixture to CA rats at 200 mg/kg body weight, significantly (P<0.05) increased weight gain, with rats given the garlic extract showing the highest weight increases.

The % organ/body weight ratio (fig. 1B) indicated that all the organs (liver, kidney, heart and brain), especially the liver of CA were significantly elevated than control rats. In contrast, CA rats administered with aqueous extract of

garlic, ginger, pepper, or a mixture of the three, exhibited significantly lower organ weights relative to untreated rats. Lowest organ weight was recorded among rats administered with the mixture extract compared to control.

Serum lipid profile of chronic alcoholic rats administered with aqueous extracts of spices

Administration of 30% ethanol for 28 consecutive days led to significant $P < 0.05$ elevation of total cholesterol and triglycerides of CA rats compared to control rats (table 1). TC and TG values in CA rats were about 2-fold and 3-fold higher than control rats, while rats administered with extracts of garlic, ginger, pepper or their mixture exhibited significant reduction compared to CA rats. Also, LDL-C was elevated and atherogenic index (AI) was increased 190-fold in CA compared to control rats. In contrast however, TC, TG, LDL-C and AI were significantly reduced in the of all the CA rats administered with extracts of garlic, ginger, pepper or their mixture competitively to control levels; with AI showing 12, 4, 1 and 3-folds for garlic, ginger, pepper and mixture respectively.

Hepatic and faecal cholesterol/triglycerides concentrations of chronic alcoholic rats administered with aqueous extracts of spices

Fig. 1C & D showed that administration of 30 % ethanol to rats for 4 weeks elevated significantly ($P < 0.05$) faecal total cholesterol and triglycerides of CA rats compared to control. Faecal excretion of both TC and TG in all the experimental rats was low compared to control in the first 3 days of the experiment. The last three days revealed significant increases in excretion of TC and TG of all the rats, with rats administered with 200 mg/kg aqueous extracts of spices showing the highest excretion of lipids. Ginger extracts gave the highest excretion of triglycerides, while pepper extract was most effective for total cholesterol. Hepatic cholesterol and triglycerides were also significantly elevated in chronic alcoholic rats (fig. 2A). Treatment of CA rats with garlic, ginger, pepper, or mixture extracts significantly ($P < 0.05$) reduced these parameters to control values, with the reduction greatest in rats treated with mixture extract.

Lipid per oxidation, antioxidant enzymes and liver function of chronic alcoholic rats administered with aqueous extracts of spices

Administration of 30 % ethanol for 4 weeks up regulated lipid per oxidation (MDA) in liver, heart, brain and kidney of CA rats compared to control (fig. 2B). Aqueous extracts of garlic, ginger, pepper or their mixture, administered to chronic alcoholic rats led to significant reduction of MDA levels of the various tissues, with the mixture extract showing the greatest effect on lipid per oxidation.

SOD, GPex and GRed were greatly inhibited in chronic alcoholic rats compared to control as shown in fig. 2C-E respectively. The administration of a 200mg/kg of any of the spice extracts was however, found to markedly increase the activities of SOD, GPex and GRed respectively in the tissues investigated.

Fig. 2F shows that chronic administration of 30% ethanol to rats for 28 d caused enzyme markers of liver function ALT, AST, GGT and LDH to rise significantly when compared with control. Aqueous extracts of garlic, ginger, pepper or their mixture administered at 200mg/kg body weight on the other hand, down regulated these enzymes to near normal values in most cases.

Liver histology of chronic alcoholic rats administered with aqueous extracts of spices

Histopathological examinations of liver sections using Scanning Electron Microscope (SEM) showed no injury in control animals (Plate 3A) while CA rats (Plate 3B) exhibited central venous congestion, fatty accumulation/infiltration, and clumping of the hepatocytes. These effects were mitigated by the administration of CA rats with 200mg/kg extracts of garlic, ginger, pepper or their mixture (Plates 3C-F) respectively.

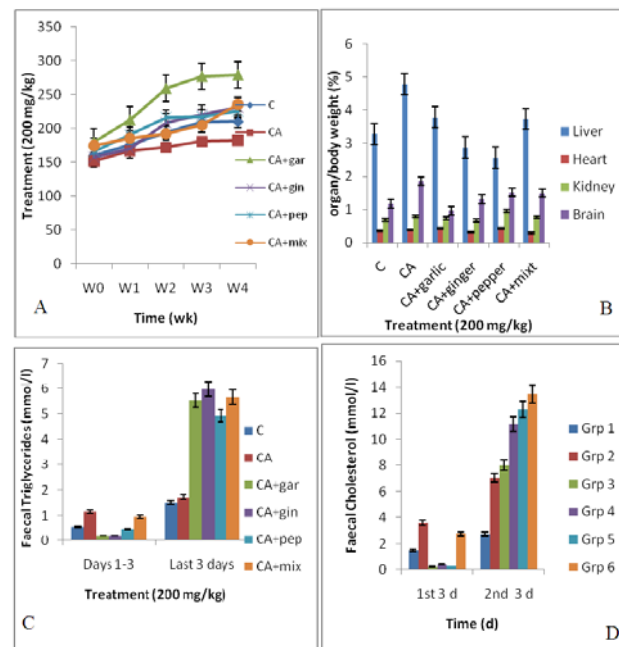
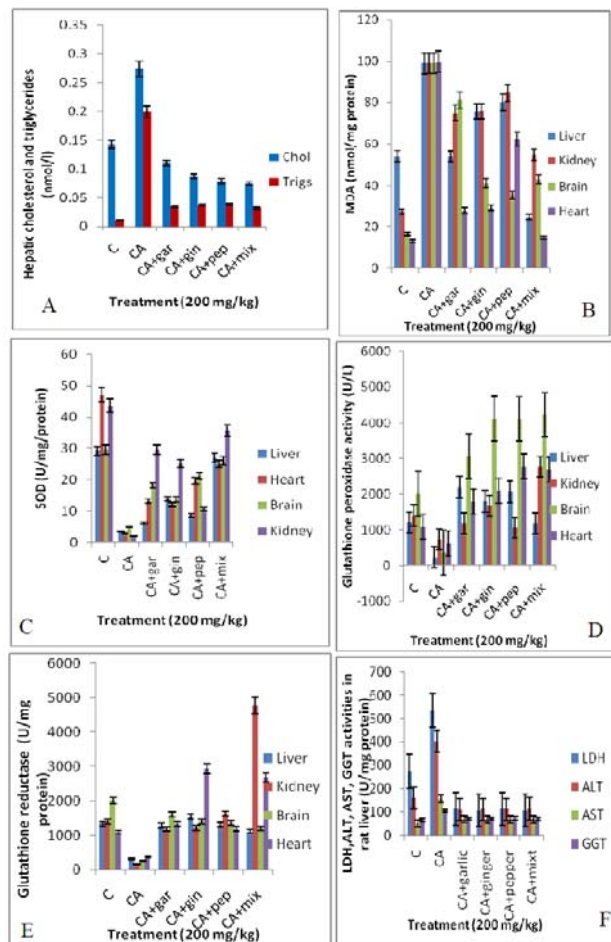


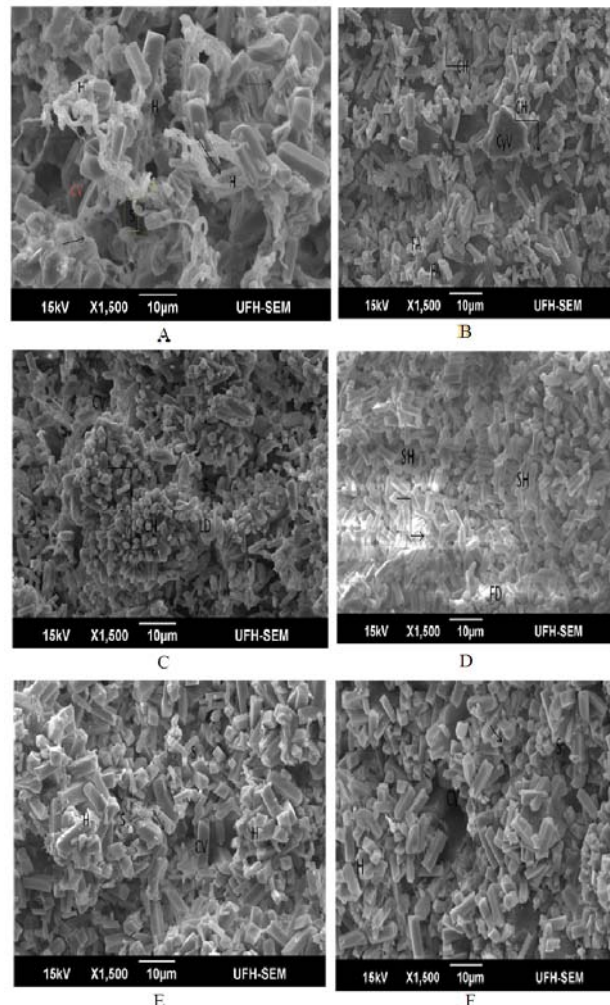
Fig. 1: Effect of aqueous extracts of garlic, ginger and pepper singly or combined on body weight, organ/body weight, faecal triglycerides and cholesterol of chronic alcoholic rats.

A-bodyweight, B-organ/body weight, C-faecal triglycerides and D- faecal cholesterol of control, chronic alcoholic (CA) and spice treated CA rats. Values are means of 6 determinations \pm SEM ($P < 0.5$). CA- chronic alcoholic, gar-garlic, gin-ginger, pep-pepper, mix-mixture.



A-Hepatic cholesterol and triglycerides; B-MDA concentrations; C-SOD activity D-GPex activity, E-GRed activity and F- liver function enzymes in tissues of control, chronic alcoholic (CA) and spice treated CA rats. Values are means of 6 determinations \pm SEM ($P < 0.5$). CA- chronic alcoholic, gar-garlic, gin-ginger, pep-pepper, mix-mixture; MDA-malondialdehyde, SOD-superoxide dismutase, GPex-glutathione peroxidase, Gred-glutathione reductase.

Fig. 2: Effect of aqueous extracts of spices on hepatic lipids, lipid peroxidation and antioxidant enzymes of chronic alcoholic rats.



H-hepatocytes, CV-central vein, S-sinusoids, Black arrows-intercellular boundaries, CyV-cytoplasmic vacuoles, FD-fatty deposits, SH-shrunken hepatocytes, curved arrow-fatty infiltration, LD-lipid deposits.

Fig. 3: Effect of aqueous extracts of spices on liver histology of chronic alcoholic rats. Scanning Electron micrographs of liver of control (A), chronic alcoholic (CA) (B) and spice treated CA (C-E) rats.

Table 1: Serum lipid profile of chronic alcoholic rats treated with extracts of garlic, ginger, pepper and their mixture (mmol/l)

Group	TC	TG	LDL	HDL	AI
Control	1.28 \pm 0.12 ^a	0.45 \pm 0.36 ^a	ND ^a	1.25 \pm 0.16 ^a	0.02 ^a
Chronic Alcoholic (CA)	2.98 \pm 0.18 ^b	1.30 \pm 0.32 ^b	1.22 \pm 0.20 ^b	0.62 \pm 0.20 ^b	3.81 ^b
CA + garlic	1.70 \pm 0.09 ^c	0.41 \pm 0.02 ^a	ND ^a	1.37 \pm 0.14 ^c	0.24 ^c
CA + ginger	1.75 \pm 0.05 ^c	0.93 \pm 0.14 ^c	ND ^a	1.62 \pm 0.08 ^d	0.08 ^d
CA + pepper	1.73 \pm 0.14 ^c	1.30 \pm 0.14 ^b	ND ^a	1.70 \pm 0.02 ^d	0.02 ^a
CA + mixture	1.30 \pm 0.11 ^a	1.20 \pm 0.34 ^b	ND ^a	1.23 \pm 0.05 ^a	0.06 ^d

Values are means of 6 determinations \pm SEM. ^{a-f}Values along the same column with different superscripts are significantly ($P < 0.05$) different. ND- Not Detected, AI- atherogenic index

DISCUSSION

Chronic ethanol administration for 4 weeks produced physiological imbalances and toxicity in rats as shown by the various biochemical and histopathological results obtained. The insignificant weight gain observed in CA rats could be attributed to decreased food intake and malabsorption of nutrients (Kim *et al.*, 2006). According to Lieber (2000), high alcohol consumption affects metabolism of most nutrients negatively by making NAD unavailable for other vital processes in the body. Also, alcohol depresses the appetite, leading to poor eating habits and subsequent malnourishment. Higher weight gain in spice treated rats could therefore be an indication that the spices improved appetite or greater nutrient utilization suggestive of their protective effect against alcohol.

The observed significant increase in liver to body weights in CA rats could be due to accumulation of fat, protein and water as a result of alcohol induced cirrhosis of the liver. The reduction in liver weight as a result of administration of the spice extracts could be due to the effect of polyphenols present in the spices (Otunola and Afolayan 2013), which could have mopped up the fat deposits in the liver, thus reducing the weight.

The elevation of TG, LDL-C, AI and lowering of HDL-C in CA rats observed in this study could be as a result of changes in lipid metabolism or hypercholesterolemia caused by enhanced cholesterol synthesis and alcohol-induced toxicity in the liver. This agrees with the report of Kim *et al.* (2006) that rats fed alcohol containing diets developed hyperlipidemia as a result of long term alcohol intake. The marked reduction in serum lipids and elevation of HDL-C in CA rats given extracts of garlic, ginger, pepper and their mixtures suggest that the spices possess hypocholesterolemic properties as observed earlier (Dammodara *et al.*, 2007).

The observed significant elevation of liver function enzymes, ALT, AST, GGT and LDH in CA rats are indicative of alcohol-induced pathological changes and oxidative stress in the liver. Clinically, measurement of these enzymes is widely used as markers of damage or injury to the liver. The reduction in the levels of these enzymes by the aqueous extracts of garlic, ginger, pepper or their mixture implies that the spice extracts are not toxic to liver cells but may rather have hepatoprotective properties.

The synergistic effect of combining the three spices is highlighted by the fact that extract of the mixture was more effective in lowering the elevated enzyme activities. Our results agree with the findings of (Kim *et al.*, 2006; Kanchana and Jayapriya 2013; Rong *et al.*, 2012) in which herbal extract mixtures, livomax and curcumin treatment respectively reduced serum marker enzymes elevated by ethanol toxicity.

Measurement of lipid per oxidation using the level of malondialdehyde (MDA) is a well-established indicator of oxidative stress and cellular injury. Alcohol induced oxidative stress in the hepatic, cardiac, renal and brain tissues of chronic alcoholic rats in this study was made evident by increased lipid per oxidation as shown by the MDA levels in these organs. The marked reduction in MDA levels in the groups administered with extracts of the spices could be attributed to the protective roles of the spices which repaired the damage caused by inhibiting lipid per oxidation.

SOD, GPex and GRed are enzymatic antioxidants that are part of the initial cellular line of defense against oxidative injury. According to Arhumolzi *et al.* (2012), reactive oxygen species alter the defense mechanism through down regulating activities of these enzymes. In this study, the significant decrease in the activities of SOD, GPex and GRed recorded could be attributed to oxidative inactivation of the enzymes brought about by increased generation of ROS since ethanol has been reported to deplete antioxidant enzymes through generation of oxidants and the inhibition of mitochondrial glutathione transporter (Rasjasree *et al.*, 2009; Shanmugam *et al.*, 2011; Mallikarjuna *et al.*, 2010).

In this study, chronic ethanol consumption altered the activities of the antioxidant enzymes (SOD, GPex and GRed) and promoted the generation of ROS led to liver injury. The observed elevation of SOD, GPex and GRed in CA rats administered with extracts of garlic, ginger, pepper or their mixture is an indication of their potent antioxidant properties. These three spices have been reported to possess antioxidant properties due to the presence of polyphenolics such as phenols, flavonoids and tannins (Krishnamurthy 2012) which protects against all types of oxidative stress. These results suggest that extracts of these spices have antioxidant properties and can prevent oxidative stress caused by chronic alcohol consumption.

These observations agree with reports that *Cnidioscolus aconitifolius* leaf extract (Adaramoye *et al.*, 2011), *solanum nigrum* fruit extract (Arulmozhi *et al.*, 2012), curcumin (Rong *et al.*, 2012), and cacao polyphenol supplementation (Suzuki *et al.*, 2013) respectively, impact positively on hepatic damage caused by chronic alcoholism in rats. The scanning electron microscope (SEM) confirmed the biochemical findings of this study that alcohol toxicity led to fatty liver and oxidative stress which were repaired and attenuated by extracts of garlic, ginger, pepper and their mixture.

CONCLUSION

This study suggests that aqueous extracts of any of garlic, ginger, pepper or their mixture possess hypolipidemic properties, successfully ameliorated ethanol induced lipid

peroxidation singly or synergistically, ameliorated oxidative stress and liver injury induced by chronic alcohol administration in rats, thus confirming the antioxidant properties of the spices. These effects are mainly due to their polyphenol content and free radical scavenging properties, which protects against oxidative stress by promoting antioxidant status.

This study also reports for the first time the synergistic additive and potential benefit of combining the three spices-garlic ginger and pepper. Further studies on the extracts of these spices are needed to isolate the compound(s) responsible for these actions and elucidate the mechanism by which the spices and their mixture exert protective effect against chronic alcohol induced oxidative stress.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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