

Hepato- and neuro-protective effects of watermelon juice on acute ethanol-induced oxidative stress in rats



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ABSTRACT

Chronic and acute alcohol exposure has been extensively reported to cause oxidative stress in hepatic and extra-hepatic tissues. Watermelon (*Citrullus lanatus*) is known to possess various beneficial properties including; antioxidant, anti-inflammatory, analgesic, anti-diabetic, anti-ulcerogenic effects. However, there is a lack of pertinent information on its importance in acute alcohol-induced hepato- and neuro-toxicity. The present study evaluated the potential protective effects of watermelon juice on ethanol-induced oxidative stress in the liver and brain of male Wistar rats. Rats were pre-treated with the watermelon juice at a dose of 4 ml/kg body weight for a period of fifteen days prior to a single dose of ethanol (50%; 12 ml/kg body weight). Ethanol treatment reduced body weight gain and significantly altered antioxidant status in the liver and brain. This is evidenced by the significant elevation of malondialdehyde (MDA) concentration; depletion in reduced glutathione (GSH) levels and an increased catalase (CAT) activity in the brain and liver. There was no significant difference in the activity of glutathione peroxidase (GPX) in the liver and brain.

Oral administration of watermelon juice for fifteen (15) days prior to ethanol intoxication, significantly reduced the concentration of MDA in the liver and brain of rats. In addition, water melon pre-treatment increased the concentration of GSH and normalized catalase activity in both tissues in comparison to the ethanol control group. Phytochemical analysis revealed the presence of phenol, alkaloids, saponins, tannins and steroids in watermelon juice. Our findings indicate that watermelon juice demonstrate anti-oxidative effects in ethanol-induced oxidation in the liver and brain of rats; which could be associated with the plethora of antioxidant phyto-constituents present there-in.

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1. Introduction

Excessive acute or chronic alcohol consumption poses a serious health hazard and can result into several metabolic disorders in hepatic and extra-hepatic diseases [1]. Alcohol is a commonly used hepatotoxin in experimental hepatopathy. Although the pathogenesis of alcohol-induced liver disease is not clearly defined, there is evidence that ethanol-induced liver injury is due to oxidative stress that leads to fibrosis and impaired liver functions [2,3]. Alcohol overuse is also characterized by central nervous system (CNS)

intoxication symptoms, impaired brain activity, poor motor coordination, and behavioral changes [4]. Additionally, there are reports that ethanol exposure and metabolism results in cellular oxidative stress in the brain [5].

Excessive alcohol consumption commonly causes hepatic, gastrointestinal, nervous and cardiovascular injuries leading to physiological dysfunctions [6]. Cellular disturbances resulting from excessive alcohol consumption results in increased formation of oxidative stress biomarkers such as malondialdehyde (MDA); reduction in the level of reduced glutathione level and a decrease in the activities of antioxidant enzymes [7,8].

There is an increasing global interest concerning the use of medicinal plants in the prevention and treatment of different pathologies [9,10]. The beneficial effects of plants are attributed

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to the presence of secondary metabolites such as polyphenols, tannins, terpenoids, alkaloids, flavonoids [11]. Considering the central role played by free radicals in the initiation and progression of many diseases, the use of natural products with antioxidant constituents has been proposed as an effective therapeutic and/or preventive strategy against diseases and the search for potent and cost-effective antioxidants of plant origin has since increased [12]. The protective effects of antioxidant rich natural compounds against different toxicant mediated tissue injury has been identified in many studies [13–15]. In specific, numerous studies have demonstrated the protective effects of antioxidants such as pumpkin oil [16], resveratrol [17], curcumin [18], quercetin [19] and epigallocatechin-3-gallate [20] against alcohol induced tissue injury.

Watermelon (*Citrullus lanatus*) is one of such medicinal plant that has attracted scientific interest due to its bioactivities [21]. *C. lanatus* sp. is a natural source of antioxidants such as beta carotene [22], vitamin C [23], citrulline [24]. Watermelon with red flesh is also an excellent source of lycopene [25]. The tissue protective effects of watermelon juice have been reported [26,27]. Furthermore, the protective effects of watermelon juice against hepatotoxins such as carbon tetrachloride (CCl₄) and paracetamol has been demonstrated [23,28]. The anti-inflammatory, antioxidant, anti-ulcerogenic and anti-diabetic effects of watermelon have also been documented [29–31]. However, there is limited information on the neuroprotective and hepatoprotective effects of watermelon juice in acute ethanol-induced oxidative stress in rats.

The constituents of watermelon juice are known for their free radical scavenging activities and antioxidant effects. In the present study, the implication of oxidative stress in alcohol induced tissue damage, led to the hypothesis that watermelon juice which contains a mixture of antioxidants could be effective in ameliorating these effects. The present study evaluated the antioxidant effects of watermelon juice pre-treatment on acute ethanol-induced oxidative stress in the brain and liver of rats.

2. Materials and Methods

2.1. Chemicals

Glutathione (GSH), 5',5'-dithiobis-2-nitrobenzene (DTNB), 2-thiobarbituric acid (TBA) and hydrogen peroxide (H₂O₂) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium hydroxide (NaOH), copper(II) sulfate pentahydrate (CuSO₄·5H₂O) and potassium iodide (KI) were obtained from the British Drug Houses (Poole, Dorset, UK). All other reagents were of analytical grade.

2.2. Preparation of watermelon juice

Watermelon fruits (green skin, red flesh) were procured from a fruit vendor in a local market in Iwo, Osun state, Nigeria. Watermelon skin was peeled and the seeds removed. The mesocarp of the ripe fruit was chopped into thin slices and crushed to juice with a blender. The watermelon juice obtained was filtered through a fine mesh muslin cloth to get the fresh watermelon fruit juice. Watermelon juice was prepared fresh daily throughout the treatment period.

2.3. Experimental design

A total of 24 (twenty-four) Wistar albino rats (100–150 g) were procured from the Central Animal House, College of Medicine, University of Ibadan, Nigeria for the study. The rats were initially acclimatized for a period of 2 weeks after their purchase. They were housed in wooden cages placed in a well ventilated rat house.

Rats were provided with rat pellets and unlimited supply of water and subjected to natural photoperiod of about 12 h light:12-h dark throughout the study period. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health.

Rats were divided into 4 groups of 6 animals each. Watermelon juice was administered orally for fifteen (15) days before administration of a single oral dose of ethanol as presented below:

Group 1 (C): Control rats administered water (4 ml/kg body weight) only for 15 days.

Group II (C+W): Rats treated with watermelon (4 ml/kg body weight) only for 15 days

Groups III (E): Rats treated with a single dose of 50% ethanol (12 ml/kg) only

Group VI (E+W): Rats were pre-treated with watermelon (4 ml/kg body weight) for 15 days, followed by a single dose of 50% ethanol (12 ml/kg).

The dose of ethanol used in the present study is well documented to induce tissue toxicity and oxidative damage in rats [32]. All animals in each group were weighed before and after the experiment. At the end of the treatment, all rats were fasted for 12 h and then sacrificed by cervical dislocation. Liver and whole brain organs were excised, weighed and homogenized in 50 mmol/l Tris-HCl buffer (pH 7.4) and then centrifuged at 5000 × g for 15 min for biochemical analysis. Supernatants were immediately kept frozen until needed.

2.4. Determination of protein concentration

The protein concentration of the various samples was determined by means of the Biuret method described by Gornal et al. [33] using bovine serum albumin (BSA) as the standard.

2.5. Phytochemical analysis

The analyses for phytochemical constituents (tannins, saponins, alkaloids, phenols and steroids) were performed using standard methods [34–36].

2.6. Assessment of lipid peroxidation

Lipid peroxidation was determined according to the method of Varshney and Kale [37] based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA): an end product of lipid peroxidation. Briefly, 0.4 ml of the sample was mixed with 1.6 ml of Tris-KCl buffer and 0.5 ml of trichloroacetic acid (TCA, 30%). This was followed by the addition of 0.5 ml of TBA (0.75%). The reaction mixture was heated in a water bath for 45 min at 80 °C, cooled in ice and centrifuged at 3000 × g for 5 min. Absorbance of the resulting supernatant was determined at 532 nm against a reference blank of distilled water. Lipid peroxidation in units/mg protein was computed with a molar extinction coefficient of $1.56 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$.

2.7. Reduced glutathione (GSH) assay

The method of Jollow et al. [38] was used in estimating the concentration of reduced glutathione (GSH). Liver homogenates were deproteinized by the addition of 0.15 M sulphosalicylic acid (1:1, v/v). The protein precipitate was centrifuged at 4000 × g for 5 min. Thereafter, 0.5 ml of the supernatant was added to 4.5 ml of DTNB (0.001 M). At 412 nm, absorbance of the mixture was read against a blank consisting of 0.5 ml of de-proteinizing agent diluted with water (1:1) and 4.5 ml of DTNB. The concentration of reduced glu-

Table 1
Effects of watermelon juice on body and organ weight.

Group	Body weight			Tissue weight			
	Liver			Brain			
	Initial body weight (g)	Final body weight (g)	Weight gain (%)	Liver weight (g)	Relative liver weight	Brain weight (g)	Relative brain weight
C	103.68 ± 14.41	115.50 ± 8.51	11.40	2.76 ± 0.28	2.46 ± 0.31	1.39 ± 0.08	1.20 ± 0.02
C+W	109.48 ± 8.0	123.80 ± 11.68	13.08	3.15 ± 0.23 ^{a,*}	2.50 ± 0.39	1.33 ± 0.19	1.14 ± 0.11
E	128.75 ± 27.10	131.75 ± 13.72	2.30 ^{b,*}	4.67 ± 0.42 ^{a,*}	3.74 ± 0.38 ^{a,*}	1.45 ± 0.09	1.13 ± 0.10
E+W	118.20 ± 17.29	124.17 ± 12.75	5.10	3.88 ± 0.38 ^{b,*}	2.98 ± 0.29 ^{b,*}	1.49 ± 0.05	1.19 ± 0.08

[Relative kidney weight (%) = (Kidney weight (g)/Total body weight (g) × 100)]. C: control rats treated with distilled water, C+W: control rats treated with watermelon juice, E: ethanol-intoxicated rats, E+W: rats pre-treated with watermelon juice before ethanol intoxication. Data are presented as mean ± standard deviation (S.D), n = 6.

^a Value differ from control group.

^b Value differ from ethanol treated group.

* P < 0.05.

tathione is proportional to the absorbance. GSH concentration was extrapolated from calibration curve prepared with GSH standards.

2.8. Estimation of catalase (CAT) activity

Catalase (CAT) activity was determined by the method of Sinha [39] based on the reduction of dichromate (in acetic acid) to chromic acetate in the presence of H₂O₂. Briefly, the assay mixture contained 4 ml of H₂O₂ solution (800 μmol) and 5 ml of phosphate buffer (0.01 M, pH 7.0). 1 ml of diluted sample (1:10) was rapidly mixed with the reaction mixture at room temperature. 1 ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent (1:3 by volume) at 60 s intervals. The chromic acetate then produced is measured calorimetrically at 570 nm for 3 min at 60 s intervals after heating the reaction mixture in a boiling water bath for 10 min. Catalase activity expressed as μmol H₂O₂ consumed/min/mg protein.

2.9. Estimation of glutathione peroxidase (GPX) activity

Glutathione peroxidase enzyme was determined according to Rotruck et al. [40]. Briefly, 500 μl of tissue homogenates were mixed with 500 μl of assay buffer (potassium phosphate 30 mM, pH 7.0), 100 μl of sodium azide (NaN₃; 10 mM), 200 μl of reduced glutathione (GSH; 4 mM), 100 μl hydrogen peroxide (H₂O₂; 2.5 mM), and 6000 μl of distilled water. The whole reaction mixture was incubated at 37 °C for 3 min after which 0.5 ml of TCA (10%) was added and thereafter centrifuged at 3000 rpm for 5 min. 1 ml of the supernatants was added to 2 ml of K₂HPO₄ (0.3 M) and 1 ml of DTNB and the absorbance was read at 412 nm.

2.10. Statistical analysis

Data were analyzed using one-way analysis of variance and expressed as mean ± standard deviation. Statistical analyses were performed using Graph Pad Prism version 6.02, for windows (Graph Pad software, San Diego, CA). Differences between mean values of different groups were considered statistically significant at P < 0.05 and marginally significant at P < 0.1.

3. Results

3.1. Effects of watermelon juice on body and organ weights of rats exposed to ethanol

The weight gain of rats treated with ethanol decreased in comparison with control (P < 0.05), while an increase in weight was observed in watermelon pre-treated rats, suggesting the protective effect of watermelon against ethanol (Table 1). Although ethanol administration did not alter brain weight (relative to body weight),

Table 2
Phytochemical analysis of watermelon juice.

Phytochemical tests	Concentration
Alkaloids	8500 ± 424.264 (μg/ml juice)
Phenols	1400 ± 282.843 (μg/ml juice)
Saponins	12900 ± 282.843 (μg/ml juice)
Tannins	2400 ± 282.843 (μg GAE/ml juice)
Steroids	3100 ± 282.843 (μg/ml juice)

an increase was observed in relative liver weight which was normalized in watermelon pre-treated rat. There were no differences in relative organ weight between control (C) and water melon-treated control (C+W) rats.

3.2. Effects of watermelon juice on the concentration of malondialdehyde (MDA) and reduced glutathione (GSH) in rat liver and brain exposed to ethanol

Acute ethanol treatment resulted in the elevation of MDA concentration in the liver and brain of rats as well as significantly lowered glutathione (GSH) concentration (Fig. 1a and b). Treatment with watermelon juice prior to ethanol exposure significantly increased liver GSH concentration and moderately reduced (32%) MDA concentration in the liver of ethanol treated rats. Watermelon juice also significantly decreased ethanol-induced lipid peroxidation in the brain as assessed by MDA concentration while brain levels of GSH were marginally elevated

3.3. Effects of watermelon juice pre-treatment on antioxidant enzyme activities in rat liver and brain exposed to ethanol

Ethanol treatment significantly increased catalase activity in the brain and liver of rats when compared to control rats. Watermelon pre-treatment reversed this alteration in both organs (P < 0.05). The activities of glutathione peroxidase remain unaltered in both organs following acute ethanol treatment (Fig. 2a and b).

3.4. Phytochemical analysis of watermelon juice

Subjection of watermelon juice to phytochemical analysis revealed the presence of alkaloids, saponins, tannins, steroids and phenols (Table 2).

4. Discussion

Acute and chronic alcohol exposures are well documented to increase the generation of reactive oxygen species (ROS). Many investigations have revealed a decreased level of antioxidants and increased production of free radicals in animals and humans following excessive ethanol exposure [41,42]. The protective effect

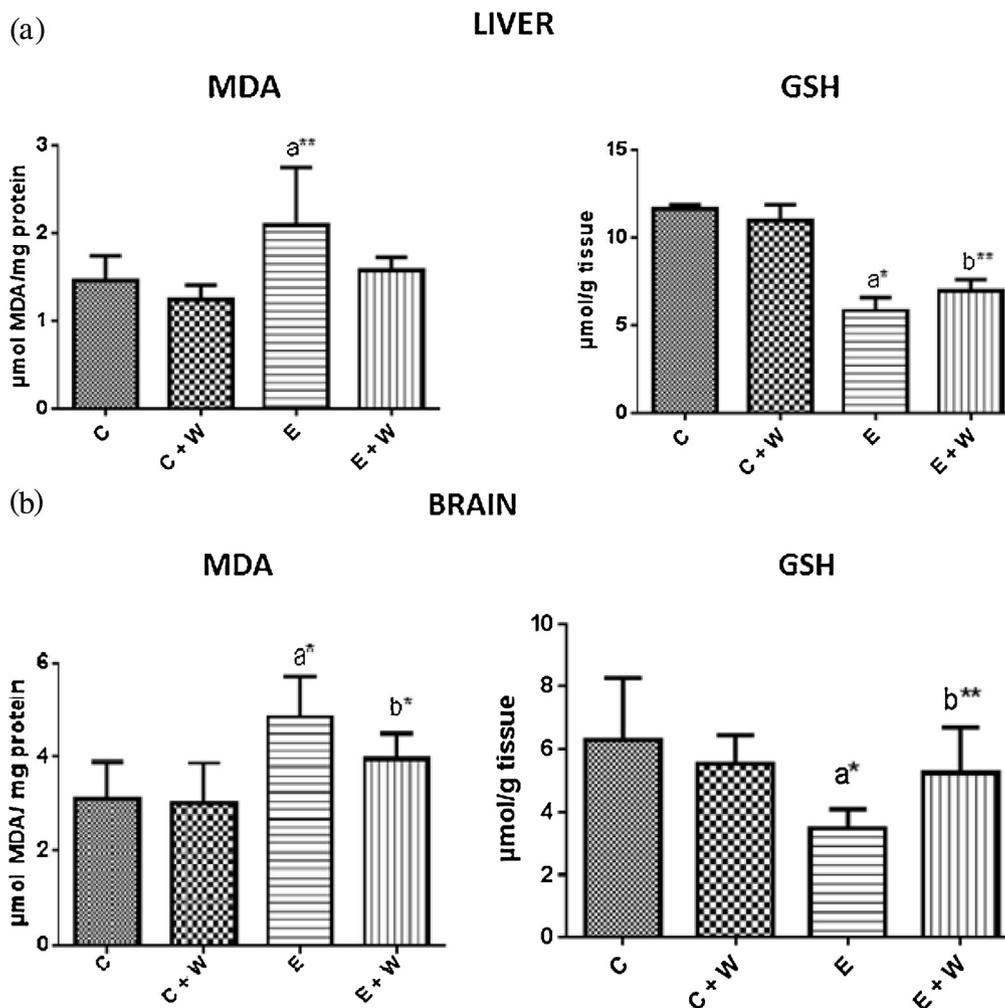


Fig. 1. (a) Effects of watermelon juice on MDA and GSH concentrations in the liver of experimental rats. MDA: malondialdehyde, GSH: reduced glutathione, C: control rats treated with distilled water, C+W: control rats treated with watermelon juice, E: ethanol-intoxicated rats, E+W: rats pre-treated with watermelon juice before ethanol intoxication. Data are presented as mean \pm standard deviation (S.D); $n=6$. ^aValue differ from control rat group; ^bvalue differ from ethanol treated group; * $P<0.05$; ** $P<0.1$. (b) Effects of watermelon on MDA and GSH concentrations in the brain of experimental rats. GSH: reduced glutathione, MDA: malondialdehyde, C: control rats treated with distilled water, C+W: control rats treated with watermelon juice, E: ethanol-intoxicated rats, E+W: rats pre-treated with watermelon juice before ethanol intoxication. Data are presented as mean \pm standard deviation (S.D); $n=6$. ^aValue differ from control rat group; ^bvalue differ from ethanol treated group; * $P<0.05$; ** $P<0.1$.

of antioxidants against alcohol-induced liver injury in many studies further supports the involvement of oxidative stress [43,44]. As reported previously, watermelon exhibits good antioxidant activity in vitro [45]. In addition, the hepatic-protective effect of watermelon on oxidative stress in rats has been demonstrated [46]. However, the action of watermelon juice against liver and brain oxidative stress induced by ethanol overdose has not been reported so far.

Alcohol metabolism results in oxidative and nitrosative stress via elevation of NADH/NAD⁺ redox ratios, induction of nitric oxide synthase (NOS) and NADPH/xanthine oxidase [47,48]. Although the liver is the main organ for ethanol oxidation, the extent of mitochondria DNA (mtDNA) depletion in extra-hepatic tissues such as brain and muscles is about the same as in the liver, suggesting that ethanol-induced free radical formation could be as high in brain and muscles as in the liver [49].

Lipid peroxidation, a primary mechanism of cell membrane destruction and cell damage is a common feature of both acute and chronic alcohol consumption [50–52]. The presence of a high concentration of oxidisable fatty acids and iron in liver significantly contributes to ROS production. Furthermore, the abundance of polyunsaturated fatty acids (PUFAs) and redox active transition metal ions in the brain in addition to its high oxygen usage makes

it highly susceptible to oxidative damage [53,54]. In the present study, acute ethanol exposure significantly elevated the malondialdehyde (MDA) levels in the liver and brain indicating enhanced peroxidation and breakdown of the antioxidant defense mechanisms. Watermelon juice treatment significantly reversed these alterations causing a significant decrease in MDA levels in both organs, suggesting its protective effects against ethanolic-induced oxidative damage.

Glutathione (GSH) is highly abundant in all cell compartments and it's the major soluble antioxidant. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism [55]. Glutathione detoxifies hydrogen peroxide and lipid peroxide by donating electron to hydrogen peroxide to reduce it to water and oxygen protecting macromolecules such as lipids from oxidation. In this study, the decrease in the reduced glutathione level in the ethanol group is connected with ethanol induced oxidative stress and direct conjugation of GSH with acetaldehyde and other reactive intermediates of alcohol oxidation. The result of the present study agrees with the finding of Pinto et al. [41] who reported that acute ethanol treatment caused reduction in the glutathione levels in different tissues. The significant increase ($P<0.1$) in the glutathione levels in the liver and brain of watermelon juice treated rats prior to ethanol-administration may be

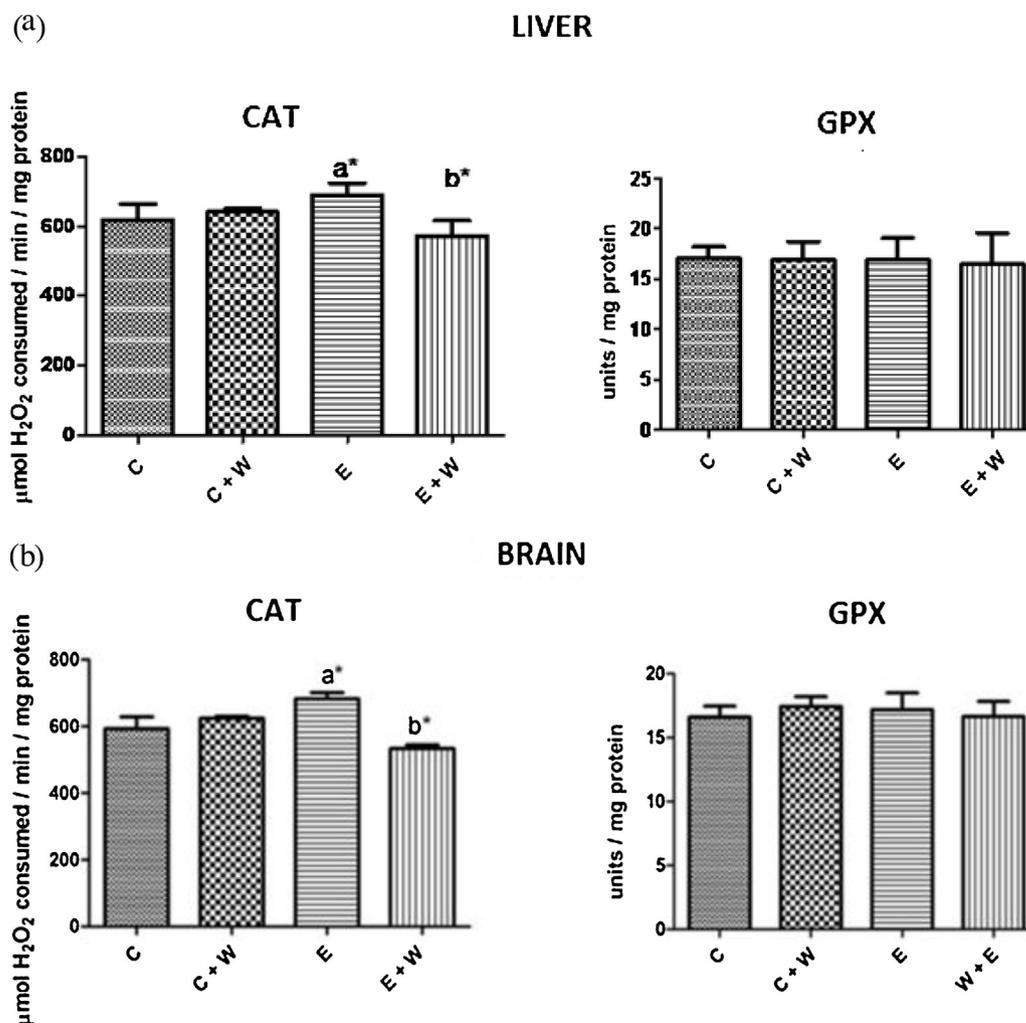


Fig. 2. (a) Effects of watermelon juice pre-treatment on antioxidant enzyme activities in rat liver exposed to ethanol. GPX: glutathione peroxidase, CAT: catalase, C: control rats treated with distilled water, C+W: control rats treated with watermelon juice, E: ethanol-intoxicated rats, E+W: rats pre-treated with watermelon juice before ethanol intoxication. Data are presented as mean \pm standard deviation (S.D), $n=6$. ^aValue differ from control rat group; ^bvalue differ from ethanol treated group; * $P<0.05$. (b) Effects of watermelon juice pre-treatment on antioxidant enzyme activities in rat brain exposed to acute ethanol. GPX: glutathione peroxidase, CAT: catalase, C: control rats treated with distilled water, C+W: control rats treated with watermelon juice, E: ethanol-intoxicated rats, E+W: rats pre-treated with watermelon juice before ethanol intoxication. Data are presented as mean \pm standard deviation (S.D), $n=6$. ^aValue differ from control rat group; ^bvalue differ from ethanol treated group; * $P<0.05$.

due to the direct ROS—scavenging effect of water melon juice or an increase in GSH synthesis.

Catalase contributes to ethanol oxidation, by oxidizing a small amount of ethanol in the presence of a hydrogen peroxide (H_2O_2) generating system to form acetaldehyde [56]. Inhibition of brain catalase activity in mice inhibits many of the pharmacologic effects of ethanol [57]. Increased catalase activity in the present study following acute ethanol exposure suggests elevated ethanol oxidation and formation of oxidising product—acetaldehyde. Our result is in accordance with the study of Oh et al. [58] who demonstrated a significantly higher CAT activity after ethanol treatment. Glutathione peroxidase (GPX) is another enzymic anti-oxidant that acts as a defense against oxidative stress. The lack of significant effect in GPX activity observed in our study after ethanol treatment may be due to the duration of exposure to ethanol. Our result is in line with the study of Yang et al. [59] who observed no difference in GPX activities in rats hepatocyte exposed to varying concentrations of ethanol at an incubation time of 12 h.

The deleterious effect of acute ethanol exposure on metabolism was exhibited by diminished weight gain in rats. Prior treatment with watermelon juice ameliorated the weight gain decrease in ethanol-group. The toxicity of ethanol is related to the product

of its metabolic oxidation. Acetaldehyde and acetate, produced from the oxidative metabolism of alcohol are capable of forming adducts with cellular macromolecules, causing oxidative damage and affecting metabolic processes [56,60]. The observed increase in liver weight (relative to the body weight) in ethanol-treated rats in comparison with control could imply increased formation of protein adducts and impaired protein secretion, which has been proposed to play a role in hepatomegaly. Pre-treatment of ethanol intoxicated rats with watermelon significantly ($P<0.05$) reduced relative liver weight.

Alcohol exposure reduces brain weight, although the degree of brain atrophy correlates with the rate and amount of alcohol consumed over a lifetime [61]. Although a single dose of ethanol treatment induced oxidative stress in the brain no significant difference was observed in brain weight in the present investigation.

Antioxidant agents of natural origin have attracted special interest because of their health promoting effects [62–64]. The phytochemical tests carried out on watermelon juice used in this study showed the presence of alkaloids ($8500 \pm 424.264 \mu\text{g/ml}$ juice), saponins ($12900 \pm 282.843 \mu\text{g/ml}$ juice), tannins ($2400 \pm 282.843 \mu\text{g GAE/ml}$ juice), steroids ($3100 \pm 282.843 \mu\text{g/ml}$ juice) and phenols ($1400 \pm 282.843 \mu\text{g/ml}$ juice). The anti-oxidat

activities of these bioactive compounds have been reported [65–69].

Watermelon juice is a rich source of phenolics, α tocopherol, carotenoids such as beta carotene and lycopene, and vitamin C [70,71]. Vitamin C, a water soluble antioxidant, has demonstrated cardio-protective action in a rat model of ethanol-induced injury. The beneficial effects of vitamin C are attributed mainly to its antioxidant properties. Combined treatment of vitamin C with other antioxidants was also proven to be protective against CCL₄ induced toxicity in the liver and kidney by preventing oxidative stress [72]. Watermelon juice is an excellent source of lycopene, having about 40% higher lycopene content than raw tomatoes [16,17]. Studies have attributed the antioxidant properties of water melon juice to its high lycopene content [73,74]. There is a compelling evidence for the antioxidant role of lycopene in animal models of toxicant induced toxicities. Lycopene induces enzymes of the cellular antioxidant defense systems by activating the antioxidant response element transcription system [75]. An *in vitro* study demonstrated that lycopene treatment of alcohol-treated HepG2 cells led to a reduction in alcohol-induced oxidative stress, down-expression of CYP2E1, decreased free radical formation (hydrogen peroxide) and restoration of mitochondrial glutathione levels [76]. β -Carotene supplementation also decreased ethanol-induced oxidative stress and prevented liver damage [77]. Ethanol-induced lipid peroxidation was inhibited in ethanol-treated rats that received a natural β -carotene isomer mixture of *Dunaliella bardawil* [78].

The protective effects demonstrated by watermelon juice in the present study may be due to the antioxidant effects of some of the active constituents or their synergism. However, further studies are required to reach this conclusion.

5. Conclusion

The results of our study suggest that watermelon juice pre-treatment boosted antioxidant status and offered some protection against acute alcohol induced oxidative injury in rat's brain and liver. Future research work will be focused on the establishing the mechanism of antioxidant action of watermelon juice in conferring protection against ethanol induced toxicity.

Competing interests

The authors declare that they have no competing interests.

Transparency document

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