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Research Article

MITIGATING EFFECT OF HERBAL MEDICINE-LIVINA® UPON SERUM, LIVER AND KIDNEY AGAINST ETHANOL-INDUCED OXIDATIVE STRESS IN MICE

Livina® Mitigates Ethanol Intoxicated Oxidative Stress in Murine Model

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ABSTRACT

Background: The aim and objective of the study of this study were to evaluate the possible ameliorative effect of herbal medicine (Livina®) on serum, liver, and kidney antioxidant enzymes activities in ethanol intoxicated organ dysfunctions in mice.

Methods: Thirty-two Swiss albino adult male mice were randomly divided into four groups; Group-I as control, Group-IIorally treated with ethanol (50% v/v), Group-III three pre-treated with herbal medicine (Livina®) with ethanol (50% v/v), and Group-IV only treated with herbal medicine (Livina®) without ethanol daily. After six weeks the animals were euthanized and livers and kidneys were immediately removed and used fresh or kept frozen until analysis. Before the mice were killed blood samples were also collected to measure superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione –S transferees (GST) activities in sera.

Results: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione –S transferees (GST) activities in serum, liver, and kidney were significantly reduced in the ethanol intoxicated mice than in the controls. Treatment with herbal medicine (Livina®) upon ethanol intoxication significantly elevated all antioxidant activities serum, liver, and kidney.

Conclusions: In the present study our results predict that treatment with herbal medicine (Livina®) might be a potent antioxidant that exerts beneficial effects on both superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione –S transferees (GST) activities in ethanol intoxicated mice and inhibit organ damage.

Keywords: Reactive oxygen species, antioxidant enzymes activity, Oxidative stress, Herbal formulation, Ethanol, Livina®, Swiss albino mice.

INTRODUCTION

Inflammatory responses are generally developed by the involvement of reactive oxygen species (ROS), thereby affecting the normal cellular physiology and playing a significant role in pathological conditions [1]. The free radicals, apart from being involved in damaging cellular components, do play a significant role in ethanol-induced organ toxicity [2]. The liver is the main organ where the metabolism of xenobiotics to a large extent takes place. Most of the time by-products of such metabolism make severe toxic effects and produce cellular imbalance [3]. This could lead to liver damage and the emergence of hepatic disorders. In very frequent oxygen-containing by-product molecules damage liver cells through oxidation. They produce oxidative stress and generates an enormous amount of free radicals which affects cell survival because of membrane damage through the oxidative damage of lipid, protein, and irreversible DNA modification [4]. This condition destroys the balance between the production of reactive oxygen species (ROS) and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants [5]. Excessive formation and insufficient removal of free

radicals lead to destructive and irreversible cell damage. Scientific study reviled that oxidative damage is aggravated by the decrease in various antioxidant enzymes activities such as superoxide dismutase, catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPx) which acts as free radical scavengers in conditions associated with oxidative stress [6-8].

Herbal drugs, used in Indian systems of medicine are however claimed to be effective and safe in such ailments. These drugs are considered benign and are of particular value in the treatment of chronic disease requiring prolonged therapy. Plant medicines are more often used in combination rather than in a single to get the maximum benefit from their combined strength. Livina®, a multi herbal medicine is very useful as natural hepatoprotective medicine, which composes of several Indian medicinal plants such as *Solanumnigrum*, *Holarrhenaantidysentrica*, *Tephrosiapurpurea*, *Andrographispaniculata*, *Phyllanthusniruri, Tinospora cordifolia*, *Terminalia chebula*, *Asteracanthalongifolia*, *Alstonia scholaris*, *Berberisaristata*, *Chichoriumintybus*, *Picrorhizakurroa* as shown in Table 1 [9,10]. Animal experiments established that some of the plant ingredients of Livina® have potent antioxidant property but the synergistic action of all these plants has still not been evaluated. Generally, herbal medicine composed of various plant ingredients makes a synergistic action that produces optimum therapeutic effects with low toxicity and better efficacy (Figure 1). On the other hand, our previous work established that Livina® protects gastric mucosal damage and maintains mucosal lipid profile [11].

Because of this, the present study aimed to investigate whether the oral administration of herbal medicine (Livina®) at a dose of 200 mg/kg of the bodyweight being able to control the hepatic and renal oxidative stress caused by ethanol intoxication in male mice and protect the cell from redox damage.

Table 1: Composition of Multi Herbal Medicine (Livina®)

Each capsule contains:
Powder and Extractive derived from:

Sl. No.	Scientific Name	Common Name	Family	Quantity
1.	*Solanum nigrum*	Kakamachi	Solanaceae	50 mg
2.	*Holarrhena antidysenterica*	Indrayava	Apocynaceae	25 mg
3.	*Tephrosia purpurea*	Sarapunkha	Fabaceae	50 mg
4.	*Andrographis paniculata*	Kalmegh	Acanthaceae	50 mg
5.	*Phyllanthus niruri*	Bhumiamlaki	Phyllanthaceae	50 mg
6.	*Tinospora cordifolia*	Guduchi	Menispermaceae	25 mg
7.	*Terminalia chebula*	Haritaki	Combretaceae	25 mg
8.	*Asteracantha longifolia*	Kokilakshya	Acanthaceae	25 mg
9.	*Alstonia scholaris*	Saptaparna	Apocynaceae	50 mg
10.	*Berberis aristata*	Daruharidra	Berberidaceae	50 mg
11.	*Cichorium intybus*	Kasni	Asteraceae	25 mg
12.	*Picrorhiza kurroa*	Katuka	Plantaginaceae	50 mg

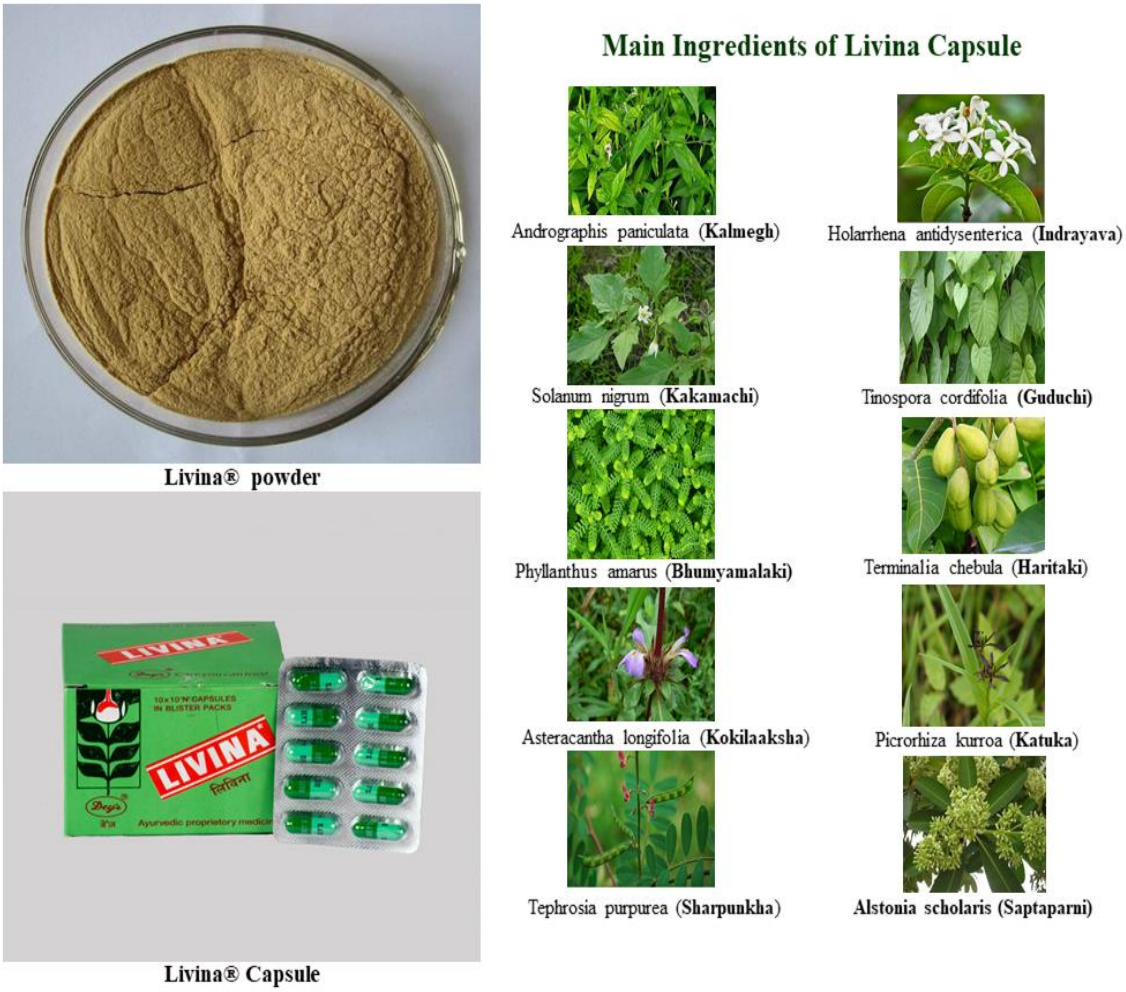

Figure 1: Herbal medicine – Livina® with different ingredients those are present in the formulation.

MATERIALS AND METHODS

Drugs and Chemicals

Livina® capsule was procured from Dey's Medical Stores (Mfg.) Ltd. (Kolkata, India). Ethanol, phosphate buffer was obtained from Merck, India. Antioxidant enzyme study kits were purchase from E-mark Germany. Other chemicals were obtained from local sources and were of analytical grade.

Animals

In this experiment 32 young Swiss male albino mice weighing 25–27 g have been randomly included for the study. The animals have been housed in healthy atmospheric conditions, normal feeding, drinking, and medical care based on the CPCSEA guidelines. Before dosing, they were acclimatized for 7 days to light from 06:00 to 18:00 h, alternating with 12 h darkness. The animals were housed in stainless steel cages in an air-conditioned room with the temperature maintained at 25 ± 2°C. Mice were allowed a standard chow diet (Amrut feeds, Pranav Agro, New Delhi, India) throughout the experiment and water *ad libitum*. The experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. 15/IAEC/Dey's/s/2016).

Experimental design

Healthy adult mice were divided randomly into four experimental groups, each consisting of eight mice, that were treated as follows: Group I received the vehicle and served as a control, Group II received Ethanol (50% v/v), Group III received Ethanol (50% v/v) along with Herbal medicine (Livina®) (200 mg/kg[DM15] [u16]) through oral route (Oral extract) and Group IV received only Herbal medicine (Livina®) (200 mg/kg) for 4 weeks.

Sample collection

At the end of the experimental period, blood was collected from the retro-orbital plexus. Collected blood samples stay for 1 h at room temperature and then

centrifuge at 6000 rpm for 10min to obtain clear serum. Serum was stored in aliquots at -70°C till used for estimation of various antioxidant enzymes.

Then the abdomen and the thorax were opened and both liver and kidneys were removed, washed three times in ice-cold saline, and blotted individually Monash-free filter paper, used for the preparation of tissue homogenates for estimation of tissue SOD, CAT, GSH, and GST activities.

Preparation of tissue homogenates

A small portion of liver and kidney tissues were weighted and homogenized separately with a potter-Elvenhjem tissue homogenizer in phosphate buffer saline (PBS) 50 mM pH (7.4) for estimation of protein content, SOD, CAT, GST, CAT enzymes activities.

Determination of Lipid peroxidation

The process of lipid peroxidation (LPO) measurement was carried out using a lipid peroxidation (MDA) assay kit (Sigma-Aldrich Ltd., UK) following the manufacturer's instructions. In this assay, lipid peroxidation is determined by the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a colorimetric product, proportional to the MDA present. To form the MDA-TBA adduct, the TBA solution (600 mL) was added into each sample and incubated at 95°C for 60 min, before cool to room temperature in an ice bath for 10 min. Each reaction mixture (200 mL) was transferred into a 96-well plate for analysis. The absorbance was measured at 532 nm [12].

Assay of antioxidant enzyme activities

Determination of Catalase (CAT) activity

CAT activity was determined by the method of Beutler et *al.* 1984 [13]. In brief, to a quartz cuvette, phosphate buffer (pH 7.0) and sample were added and the reaction was started by the addition of H_2O_2. The decomposition of $H_2O_2$was monitored at 240 nm.

Determination of Superoxide dismutase (SOD) activity

SOD activity in serum, liver, and kidney homogenate was assessed according to the method of Misra*et al.* 1972 [14] with slight modification. In quartz cuvette, 1 mL of Tris-HCl buffer, containing diethylenetriaminopentaacetic acid (DTPA) and pyrogallol were mixed with 20 µL of kidney samples. The difference in the absorbance was measured at 440 nm.

Determination of Glutathione –S transferees (GST) activity

GST activity of Serum, liver, and kidney tissues was investigated by the method of Beutler*et al*1963 [15] with slight modification. 1-chloro-2-4-di-nitrobenzene is neutralized by the enzyme in the presence of glutathione as a cosubstrate. The change in absorbance was measured at 340 nm.

Determination of Glutathione peroxidase (GPx) activity

The GPx activity determination was based on the method of Alin*et al.* 1965 [16]. The assay evaluates the enzymatic replacement of H_2O_2 by GPx by way of depletion of reduced glutathione that is restored from oxidized glutathione in a coupled enzymatic reaction by glutathione reductase. The decrease in absorbance was determined at 340 nm.

Statistical analysis

All analyses were carried out using the SPSS software, version 20.0. A one-way analysis of variance (ANOVA; $P < 0.05$) and Tukey's test was used to determine significant differences between groups. The values were stated as mean ± SD.

RESULTS

Effect of Herbal medicine (Livina®) on serum, kidney, and liver MDA content in ethanol toxicity

Serum, kidney, and liver MDA levels are depicted in Table 2. Serum MDA content in the ethanol intoxicated mice was significantly higher than that of the controls (101.25±1.56 vs. 47.26 ±1.02 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased Serum MDA content compared with ethanol intoxicated mice (48.25±0.99 vs. 101.25±1.56 U/mg protein).

Hepatic MDA content in the ethanol intoxicated mice was significantly less than that of controls(75.28±0.88 vs 34.88±0.95 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal MDA content compared with ethanol intoxicated mice (38.92±0.91vs. 75.28±0.88 U/mg protein).

Renal MDA content activity in the ethanol intoxicated mice was significantly reduced than that of the controls

(59.61±0.77 vs. 28.57±0.75 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal MDA content compared with ethanol intoxicated mice (30.27±0.81vs. 59.61±0.77 U/mg protein). The catalase (CAT) activities in the sera, liver, and kidneys of the Herbal medicine (Livina®) plus ethanol intoxicated mice were almost normalized as the control group, act as an antioxidant.

Effect of Herbal medicine (Livina®) on serum, kidney, and liver SOD activity in ethanol toxicity

Serum, kidney, and liver superoxide dismutase (SOD) activities are depicted in Figure 2, 4 & 6**.**Serum SOD activity in the ethanol intoxicated mice was significantly less than that of the controls (61.85 ± 2.67 vs. 102.64 ± 3.92 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased Serum SOD activity compared with ethanol intoxicated mice (100.69 ± 1.58 vs. 61.85 ± 2.67 U/mg protein).

Hepatic super oxide dismutase (SOD) activity in the ethanol intoxicated mice was significantly less than that of controls (44.85 ± 1.74 vs 85.27 ± 1.98U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal SOD activity compared with ethanol intoxicated mice (81.02 ± 2.51 vs. 44.85 ± 1.74 U/mg protein).

Renal superoxide dismutase (SOD) activity in the ethanol intoxicated mice was significantly reduced than that of the controls (34.28 ± 1.62 vs. 72.89 ± 2.24 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal SOD activity compared with ethanol intoxicated mice (68.51 ± 3.27 vs. 34.28 ± 1.62 U/mg protein). The superoxide dismutase (SOD) activities in the sera, liver, and kidneys of the Herbal medicine (Livina®) plus ethanol intoxicated mice were almost normalized as the control group, act as an antioxidant.

Effect of Herbal medicine (Livina®) on serum, kidney, and liver CAT activity in ethanol toxicity

Serum, kidney, and liver catalase (CAT) activities are depicted in figure 2, 4 & 6**.**Serum CAT activity in the ethanol intoxicated mice was significantly less than that of the controls (156.29 ± 7.18 vs. 225.17 ± 6.38 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased Serum CAT activity compared with ethanol intoxicated mice (202.05 ± 5.74 vs. 156.29 ± 7.18 U/mg protein).

Hepatic catalase (CAT) activity in the ethanol intoxicated mice was significantly less than that of controls(101.26 ± 3.69 vs 175.48 ± 6.01 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal CAT activity compared with ethanol intoxicated mice (170.65 ± 5.88 vs. 101.26 ± 3.69 U/mg protein).

Renal catalase (CAT) activity in the ethanol intoxicated mice was significantly reduced than that of the controls (89.62 ± 5.21 vs. 122.05 ± 4.29 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal CAT activity compared with ethanol intoxicated mice (120.47 ± 5.91 vs. 89.62 ± 5.21 U/mg protein). The catalase (CAT) activities in the sera, liver, and kidneys of the Herbal medicine (Livina®) plus ethanol intoxicated mice were almost normalized as the control group, act as an antioxidant.

Effect of Herbal medicine (Livina®) on serum, kidney, and liver GSH activity in ethanol toxicity

Serum, kidney, and liver reduced glutathione (GSH) activities are depicted in Figure 3, 5 & 7**.**Serum GSH activity in the ethanol intoxicated mice was significantly less than that of the controls (19.62 ± 0.67 vs. 38.91 ± 0.96 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased Serum GSH activity compared with ethanol intoxicated mice (36.07 ± 1.01 vs. 19.62 ± 0.67 U/mg protein).

Hepatic reduced glutathione (GSH) activity in the ethanol intoxicated mice was significantly less than that of controls (9.06 ± 0.45 vs 19.62 ± 0.82 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal GSH activity compared with ethanol intoxicated mice (21.05 ± 1.31 vs. 9.06 ± 0.45 U/mg protein).

Renal reduced glutathione (GSH) activity in the ethanol intoxicated mice was significantly reduced than that of the controls (6.87 ± 0.49 vs. 14.95 ± 1.12 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal GSH activity compared with ethanol intoxicated mice (14.11 ± 0.99 vs. 6.87 ± 0.49 U/mg protein). The reduced glutathione (GSH) activities in the sera, liver, and kidneys of the Herbal medicine (Livina®) plus ethanol intoxicated mice were almost normalized as the control group, act as an antioxidant.

Effect of Herbal medicine (Livina®) on serum, kidney, and liver Glutathione –S Transferees (GST) activity in ethanol toxicity

Serum, kidney, and liver Glutathione –S Transferees (GST) activities are depicted in Figure 3, 5 & 7**.**Serum GSH activity in the ethanol intoxicated mice was significantly less than that of the controls (4.11 ± 0.47 vs. 8.92 ± 0.81 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased Serum GSH activity compared with ethanol intoxicated mice (8.47 vs. 2.58 ± 0.47 U/mg protein).

Hepatic Glutathione –S Transferees (GST) activity in the ethanol intoxicated mice was significantly less than that of controls(3.28 ± 0.39 vs 6.35 ± 0.81 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal GSH activity compared with ethanol intoxicated mice (6.11 ± 0.24 vs. 3.28 ± 0.39 U/mg protein).

Renal Glutathione–S Transferees (GST) activity in the ethanol intoxicated mice was significantly reduced than that of the controls (1.16 ± 0.12 vs. 4.28 ± 0.27 U/mg protein). Pre-treatment with Herbal medicine (Livina®) significantly increased renal GSH activity compared with ethanol intoxicated mice (4.68 ± 0.31 vs. 1.16 ± 0.12 U/mg protein).The Glutathione –S Transferees (GST)activities in the sera, liver, and kidneys of the Herbal medicine (Livina®) plus ethanol intoxicated mice were almost normalized as the control group, act as an antioxidant.

Table 2: The effect of Multi Herbal Medicine (Livina®) on Serum, liver and kidney MDA level in ethanol intoxicated mice.

Groups	**MDA (**nmol/g**)**		
	Serum	Liver	Kidney
Control	47.26 ±1.02	34.88±0.95	28.57±0.75
Ethanol (50% v/v)	101.25±1.56#	75.28±0.88#	59.61±0.77#
Ethanol + Livina® (200mg/kg)	48.25±0.99*	38.92±0.91*	30.27±0.81*
Livina® (200mg/kg)	43.28±1.32*	32.67±0.94*	27.51±0.69*

Values are mean ± SD of six observations. #significant difference from control mice ($P \leq 0.001$). *significant difference from ethanol intoxicated group ($P \leq 0.05$).

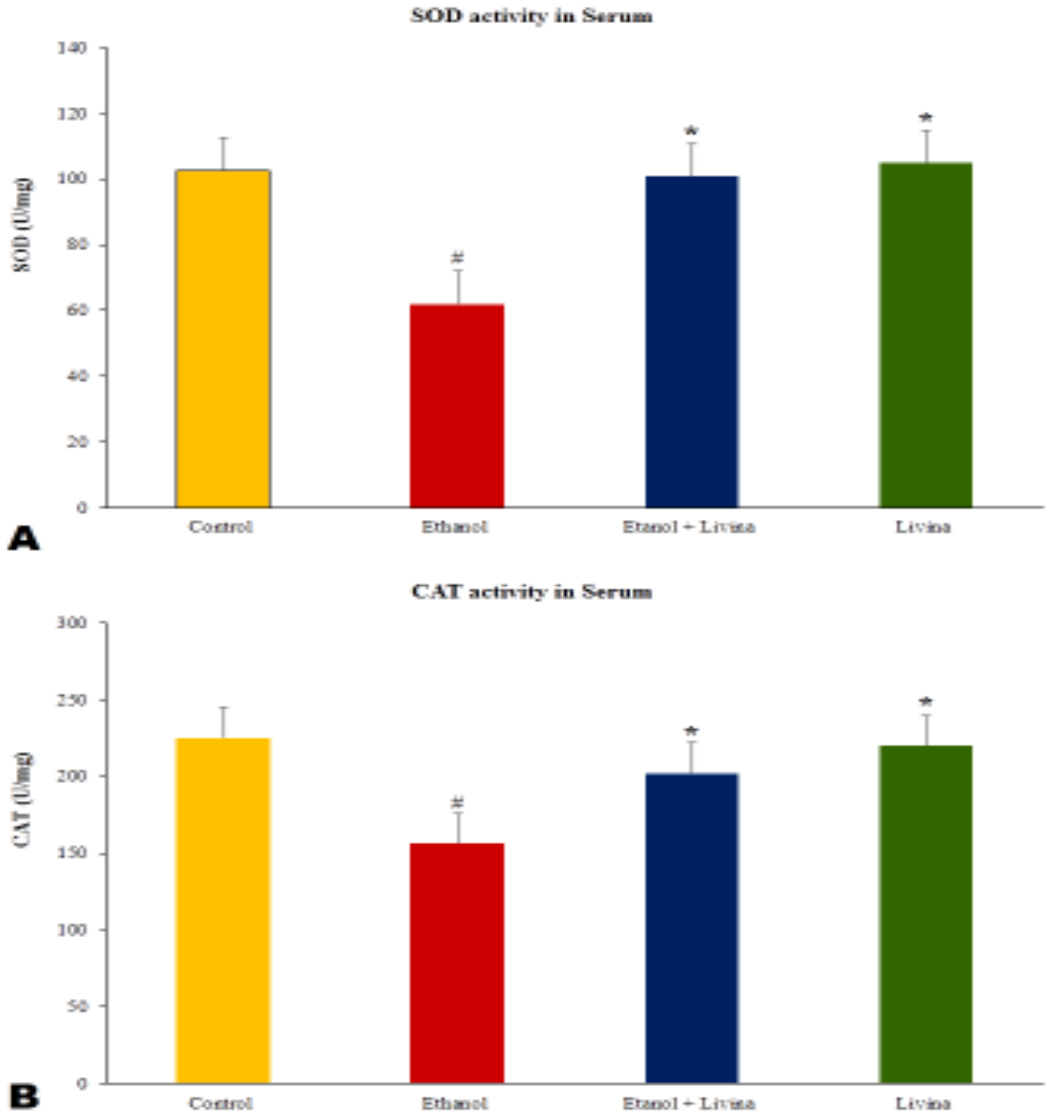

Figure 2: Effect of ethanol alone or in combination with herbal medicine (Livina®) on serum super oxide dismutase (SOD) and catalase (CAT) activity. Values expressed are mean ± SE (n=8). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P < 0.001$.

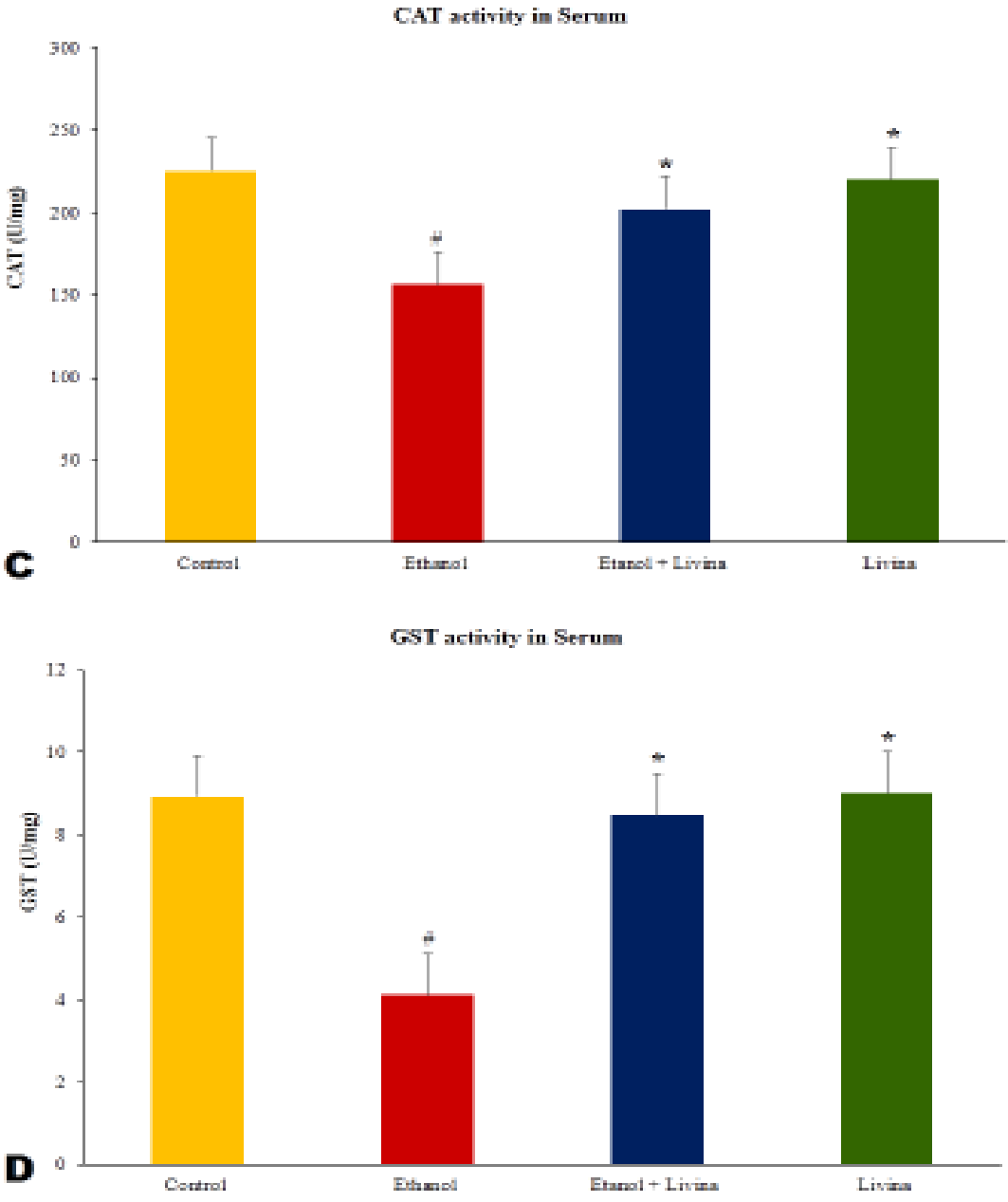

Figure 3: Effect of ethanol alone or in combination with herbal medicine (Livina®) on serum glutathione (GSH) and glutathione –S transferase (GST) activity. Values expressed are mean ± SE (n=8). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P < 0.001$.

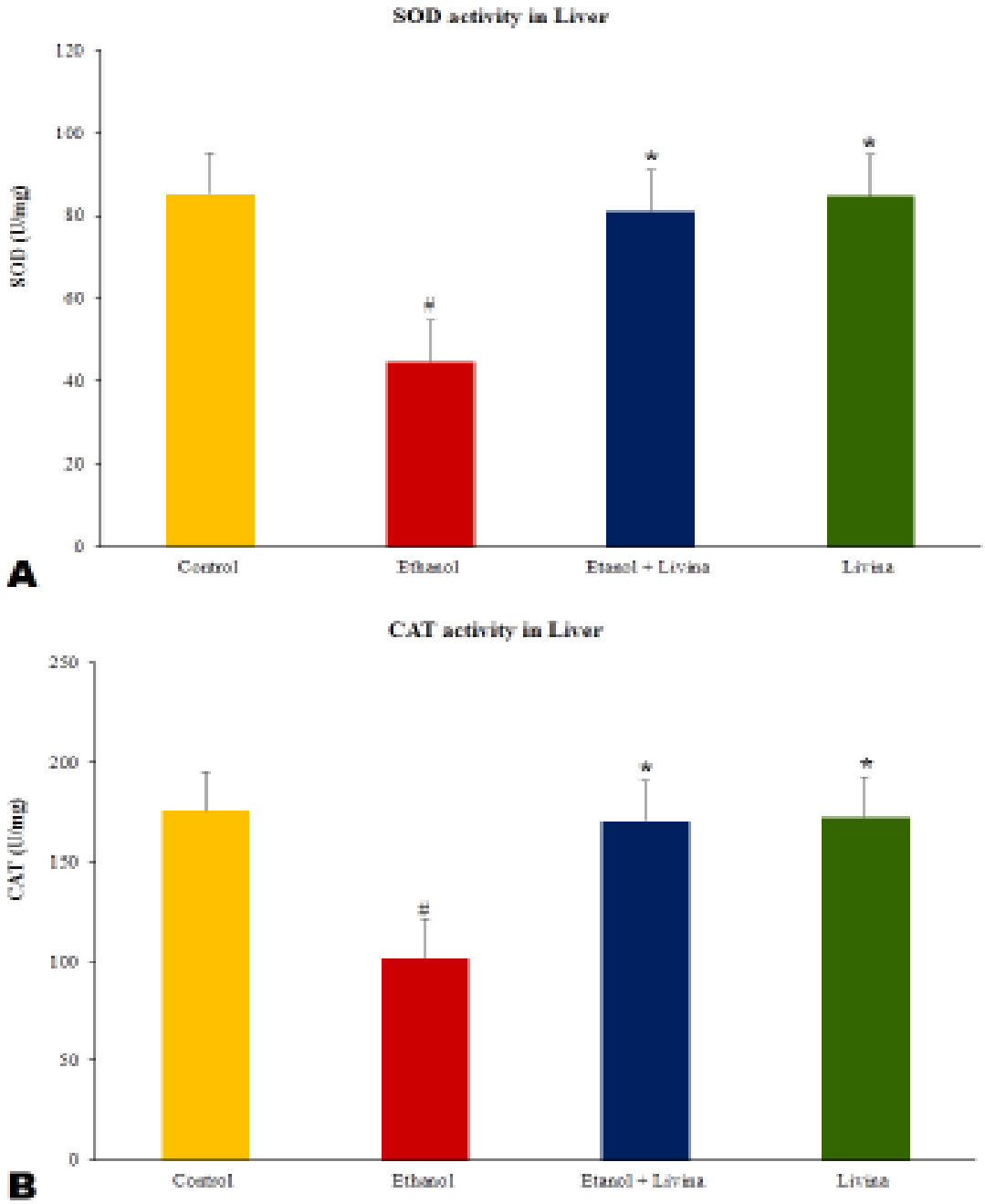

Figure 4: Effect of ethanol alone or in combination with herbal medicine (Livina®) on hepatic super oxide dismutase (SOD) and catalase (CAT) activity. Values expressed are mean ± SE (n=8). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P < 0.001$.

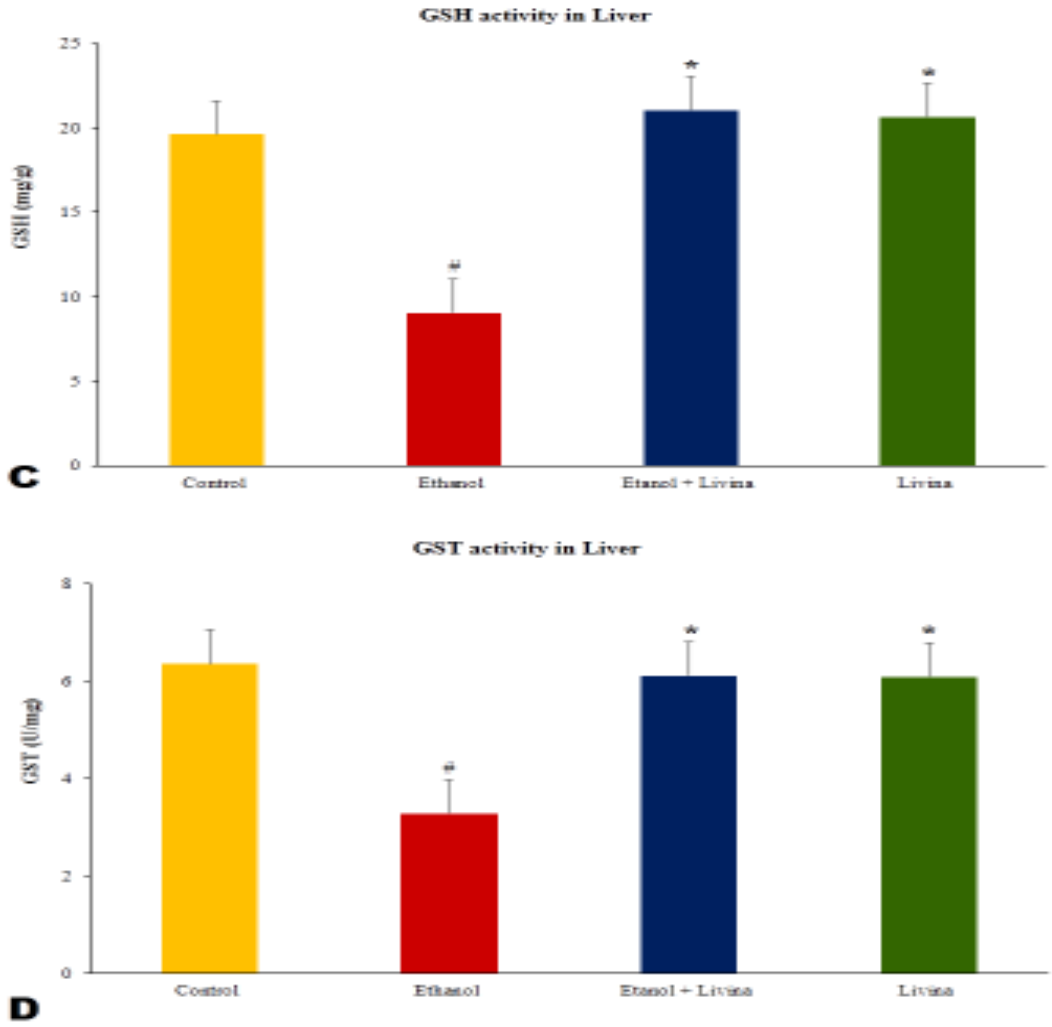

Figure 5: Effect of ethanol alone or in combination with herbal medicine (Livina®) on hepatic glutathione (GSH) and glutathione –S transferase (GST) activity. Values expressed are mean ± SE (n=8). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P < 0.001$.

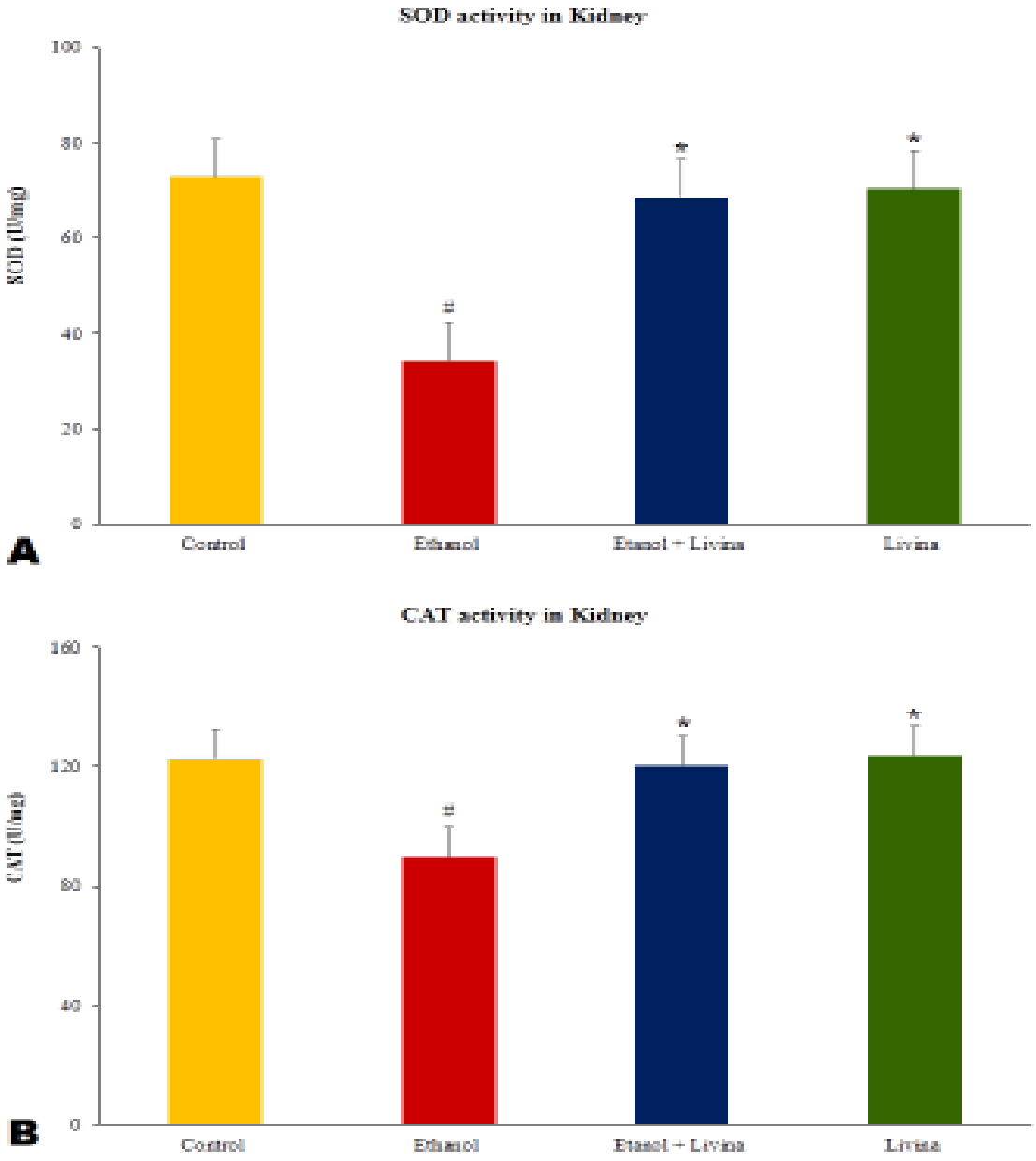

Figure 6: Effect of ethanol alone or in combination with herbal medicine (Livina®) on renal super oxide dismutase (SOD) and catalase (CAT) activity. Values expressed are mean ± SE (n=8). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P < 0.001$.

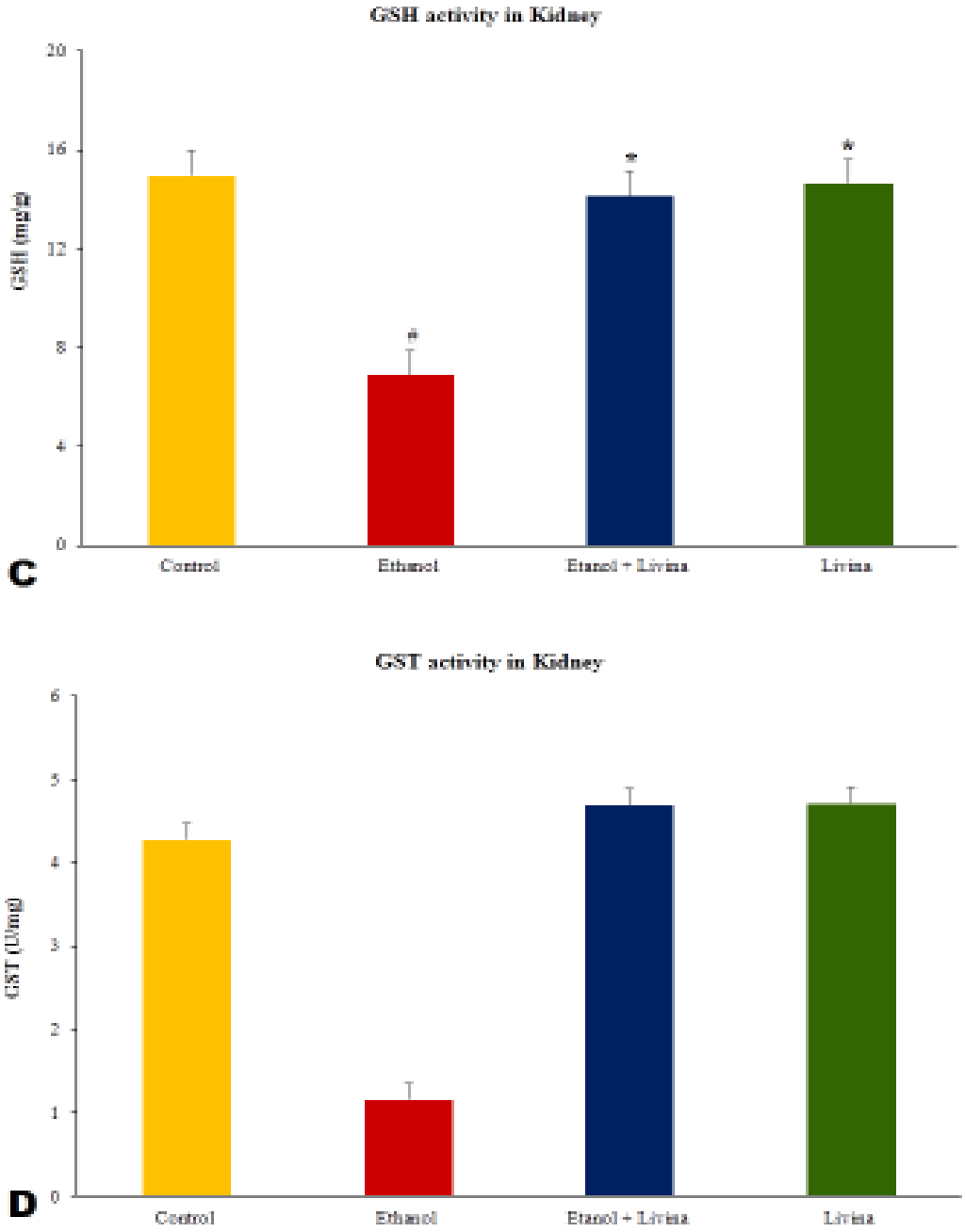

Figure 7: Effect of ethanol alone or in combination with herbal medicine (Livina®) on renal glutathione (GSH) and glutathione –S transferase (GST) activity. Values expressed are mean ± SE (n=8). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P < 0.001$.

DISCUSSION

Consumption of ethanol produces reactive oxygen species (ROS) in every mammalian cell. The liver and kidney are the two target organs for oxidative stress during ethanol intoxication. Various free radicals as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) are the major ROS generated during normal redox reaction in our body produce cytotoxic effects. They are generally neutralized by the defensive action of the endogenous antioxidant system, primarily composed of glutathione [17], superoxide dismutase [18], glutathione peroxidase, and catalase [19]. The imbalance between the generation and neutralization of ROS can create severe oxidative stress-induced damage; consequently, ROS accumulation may cause protein oxidation leading to the disruption of cell membranes, organelles, and loss of function [20].

Lipid peroxidation is commonly used as a marker for the induction of oxidative stress in cells. The level of MDA, which is generated as an end product during the oxidation of lipids, was used as a marker of lipid peroxidation [21]. Treatment of mice with ethanol (50% v/v) increased lipid peroxidation as shown by elevated MDA levels in serum, liver, and kidney tissues. This situation suggests the induction of oxidative stress in cells. The application of multi herbal medicine (Livina®) significantly reduced the serum MDA level. The treatment also reduced the hepatic and renal MDA levels which indicate that this herbal medicine maintains the normal fluidity of the cell membrane which plays a vital role in cell functioning.

Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH), and Glutathione–S Transferase (GST) are the most common antioxidant enzymes inhibited by ethanol intoxication during oxidative damage. In the present study, we aim to determine the possible therapeutic effect of herbal medicine (Livina®) upon SOD, GPx, GST, and CAT enzyme activities as an indicator of oxidative stress. Scientific study reviled that SOD is an enzyme against the superoxide radical and catalyzes its dismutation into H2O2, which is utilized by CAT or GPx[22]. On the other hand, GST catalyzes the conjugation of several substrates to the thiol group of glutathione, transforming toxic materials into less toxic forms [23,24]. In the present study, oral administration of ethanol (50% v/v) on mice significantly reduced the antioxidant enzyme activities as compared to control untreated animals which supported the previous experiment that chronic consumption of ethanol generates free radicals which reduced the antioxidant enzyme activities. Generation of reactive oxygen species (ROS) within the cell decreased the cellular performance by changing the antioxidant enzyme's actions. Treatment with multi herbal medicine (Livina®) at a dose of 200 mg/kg/day on mice who are intoxicated with ethanol, significantly elevated the SOD, GPx, GST, and CAT enzyme activities. This herbal medicine (Livina®) inhibits the free radical production within the cell which indicates that synergistic action of various plant compounds in a single medicine may potent to prevent cellular oxidative stress and boost the cell for their normal function.

CONCLUSION

Chronic consumption of ethanol is accompanied by increased serum, hepatic, and renal tissue oxidative stress, which is characterized by a reduction in the antioxidant enzyme's activities and glutathione levels that correlate with the increase in MDA in the tissues. This may probably contribute to the additional progression of ethanol intoxication-related problems. Treatment with multi herbal medicine (Livina®) normalized the serum and tissue antioxidant enzyme activities by suppression of extensive ROS generation during ethanol intoxication. So, Livina® capsule composed of various medicinal herbs may be a potent drug that sounds for the prevention of cellular oxidative stress.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgment

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Conflict of Interest

We declare that we have no conflict of interest.

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