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Ethanol intoxicated renal oxidative stress mitigated by poly-herbal formulation-Trasina® in murine model

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Abstract

Oxidative stress has a critical role in the pathophysiology of several kidney diseases. Safe and symptomatic medication to prevent the kidney oxidative stress id highly recommended. In this study the aim and objective of this scientific study was to find out the probable protective effect of poly-herbal formulation (Trasina®) on serum and kidney antioxidant enzymes activities in ethanol intoxicated organ dysfunctions in mice model. Forty Swiss albino adult male mice were randomly divided into four groups; Group-1 served as control, Group-2 orally treated with ethanol (50% v/v), Group-3 pre-treated with herbal medicine (Trasina®) along with ethanol (50% v/v), and Group-4 only treated with poly-herbal formulation (Trasina®) without ethanol daily. Completion of six weeks treatment the animals were euthanized and kidneys were immediately removed and used fresh or kept frozen until analysis. Before sacrifice blood samples were taken to measure the antioxidant parameters i.e. super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione –S transferees (GST) from sera. Super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione–S transferees (GST) activities in serum and kidney were significantly reduced in the ethanol intoxicated mice than in the controls. Treatment with herbal medicine (Trasina®) upon ethanol intoxication significant elevated the all antioxidant activities serum and kidney. Results obtained from the present study clearly predict that treatment with poly-herbal formulation (Trasina®) might be a potent antioxidant that exerts beneficial effects on both super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione –S transferees (GST) activities in ethanol intoxicated mice and inhibit organ damage.

Keywords: antioxidant enzymes activity, kidney, ethanol, trasina®, oxidative stress, swiss albino mice

1. Introduction

Reactive oxygen species (ROS), thereby affecting the normal cellular physiology and playing a significant role in the pathological conditions [1-4]. The free radicals, apart from being involved in damaging cellular components, do play a significant role in ethanol induced organ toxicity [5, 6]. Liver is the main organ where metabolism of xenobiotics to a large extent takes place. Most of the time by-products of such metabolism makes sever toxic effects and produce cellular imbalance [7]. This could lead liver damage and emergence hepatic disorders. In very frequent oxygen containing by-product molecules damage liver cell through oxidation. They produce oxidative stress and generates enormous amount of free radicals which affects cell survival because of membrane damage through the oxidative damage of lipid, protein and irreversible DNA modification [8]. This condition destroy 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 [9]. Excessive formation and insufficient removal of free radicals lead to destructive and irreversible cell damage [10]. 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 a free radical scavengers in conditions associated with oxidative stress [11,12].

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 in order to get maximum benefit from their combined strength [13, 17].

Trasina® a marketed poly-herbal capsule composed some Indian medicinal plants classified in Ayurveda, the classic Indian system of medicine, as Medhyarasayanas or drugs reputed to improve memory and intellect. *Withania somnifera*, *Tinospora cordifolia*, *Eclipta alba*, *Ocimum* sanctum, *Picrorrhiza kurroa* and Shilajit, are the main ingredients present in Trasina® [18]. In 1997 Bhattacharya et al. reported that the said formulation has a memory-facilitating action upon animals. Sub chronic administration of Trasina for 21 days on two rodent models revelled that this medicine had simulate some biochemical features known to be associated with Alzheimer's disease (AD) [19]. Our recent study confirmed that Trasina® has no toxic effects of animals and safe for therapeutic medication. Another very recent study established that Trasina® possessed significant antistress activity and maintain normal homeostasis [20, 21]. This study stated that administration of Trasina® significantly increases anoxia tolerance time, significantly decreases immobility time and number of writhes in animals. Immobilisation stress induced changes in biochemical parameters and organs weight were

completely revert by the application of Trasina® in experimental animals [18].

Thus, the present study was designed to assess the serum and kidney antioxidant activity of poly-herbal capsule – Trasina® against ethanol induced oxidative stress in mice.

Materials and Methods

Drugs and Chemicals

Trasina®, the poly-herbal capsule was obtained from Dey's Medical Stores (Mfg.) Ltd. (Kolkata, India). Ethanol, TRIS buffer and phosphate buffer were obtained from Merck, India. All antioxidant enzyme study kits were purchase from E-mark Germany. Other chemicals were obtained from local sources and were of analytical grade.

Animals

Forty young swiss albino male mice weighing 26–28 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. Prior to 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 temperature maintained at 25 ± 2°C. Mice were allowed 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. 16/IAEC/Dey's/s/2016).

Experimental design

Healthy adult male mice were divided randomly into four experimental groups, each consisting of ten mice, that were treated as follows: Group 1 received vehicle and served as a control, Group 2 received Ethanol (50% v/v), Group 3 received Ethanol (50% v/v) along with Poly-Herbal formulation (Trasina®) (200 mg/kg) and Group 4 received only Poly-Herbal formulation (Trasina®) (200 mg/kg) for 6 weeks.

Sample collection

At the completion of the experimental period, blood was collected from the retro orbital plexus. Collected blood samples stay for 1 h in normal room temperature (27 ± 2°C) and then centrifuge at 6500 rpm for 15min to obtain clear serum. Serum was stored in aliquots at -70°C till used for estimation of various antioxidant enzymes.

After collection of blood sample, the abdomen and the thorax of the animals were opened and kidneys were removed, washed three times in ice cold saline and blotted individually on ash-free filter paper, used for preparation of tissue homogenates for estimation of tissue SOD, CAT, GSH and GST activities.

Preparation of tissue homogenates

Small portion of liver tissue was 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 protein content

Serum and kindey Total protein concentration was determined in the serum by the method of Kashyap et al. (Lowry assay) [22].

Determination of Lipid peroxidation

The process of lipid peroxidation (LPO) measurement was carried out using lipid peroxidation (MDA) assay kit (Sigma-aldrich Ltd., UK) in accordance to 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, prior to 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 [23].

Assay of antioxidant enzyme activities

Determination of Catalase (CAT) activity

CAT activity was determined by the method of Beutler *et al.* 1984 [24]. In brief, to a quartz cuvette, phosphate buffer (pH 7.0) and sample were added and the reaction was started by addition of H_2O_2. The decomposition of $H2O2$ was monitored at 240 nm.

Determination of Super oxide dismutase (SOD) activity

SOD activity in serum and kidney homogenate was assessed according to method of Misra *et al.* 1972 [25] with slight modification. In quartz cuvette, 1 mL of Tris-HCl buffer, containing diethylene triaminopentaacetic acid 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 and kidney tissues was investigated by the method of Beutler *et al* 1963 [26] 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 [27]. The assay evaluates the enzymatic reducement 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 were used to determine significant differences between groups. The values were stated as mean ± SD.

Results

Protective effect of Poly-herbal medicine (Trasina®) on serum and kidney total protein content in ethanol toxicity

Serum and kidney total protein are depicted in Table 3. Serum total protein content in the ethanol intoxicated mice was significantly lower than that of the controls (3.62±0.16 vs. 7.52 ±0.24 nmol/g).

Pre-treatment with Poly-herbal medicine (Trasina®) significantly elevated Serum total protein content compared with ethanol intoxicated mice (7.02±0.18 vs. 3.62±0.16 nmol/g). Renal total protein content in the ethanol intoxicated mice was significantly lower than that of controls (2.27±0.08 vs 5.69±0.15 nmol/g). Pre-treatment with Poly-herbal medicine (Trasina®) significantly decreased liver total protein content compared with ethanol intoxicated mice (5.18±0.17 vs. 2.27±0.08 nmol/g).

Table 1: Composition of Poly-herbal Formulation (Trasina®) Each capsule contains: Powder and Extractive derived from:

Sl. No.	Scientific Name	Common Name	Family	Quantity
1.	*Ocimum sanctum*	Tulsi	Lamiaceae	50 mg
2.	*Withania somnifera*	Ashwagandha	Solanaceae	25 mg
3.	*Picrorhiza kurroa*	Kutki	Plantaginaceae	50 mg
4.	*Eclipta alba*	Bhringraj	Asteraceae	50 mg
5.	*Tinospora cordifolia*	Guduchi	Menispermaceae	50 mg

Table 2: Protective effect of Poly-Herbal Formulation (Trasina®) on Serum and kidney total protein content in ethanol intoxicated mice.

Groups	Total Protein (mg/dL)	
	Serum	Kidney
Control	7.52 ±0.24	5.69±0.15
Ethanol (50% v/v)	3.62±0.16#	2.27±0.08#
Ethanol + Trasina® (200mg/kg)	7.02±0.18*	5.18±0.17*
Trasina® (200mg/kg)	7.41±0.25*	5.51±0.14*

Values are mean ± SD of six observations. (n=10) #significant difference from control mice ($P \leq 0.001$). *significant difference from ethanol intoxicated group ($P \leq 0.05$).

Table 3: Protective effect of Poly-Herbal Formulation (Trasina®) on Serum and kidney MDA level in ethanol intoxicated mice.

Groups	MDA (nmol/g)	
	Serum	Kidney
Control	45.92 ±1.54	32.81±0.71
Ethanol (50% v/v)	102.58±1.87#	72.05±0.89#
Ethanol + Trasina® (200mg/kg)	49.63±0.91*	38.07±0.58*
Trasina® (200mg/kg)	42.11±0.95*	35.51±0.94*

Values are mean ± SD of six observations. (n=10) #significant difference from control mice ($P \leq 0.001$). *significant difference from ethanol intoxicated group ($P \leq 0.05$).

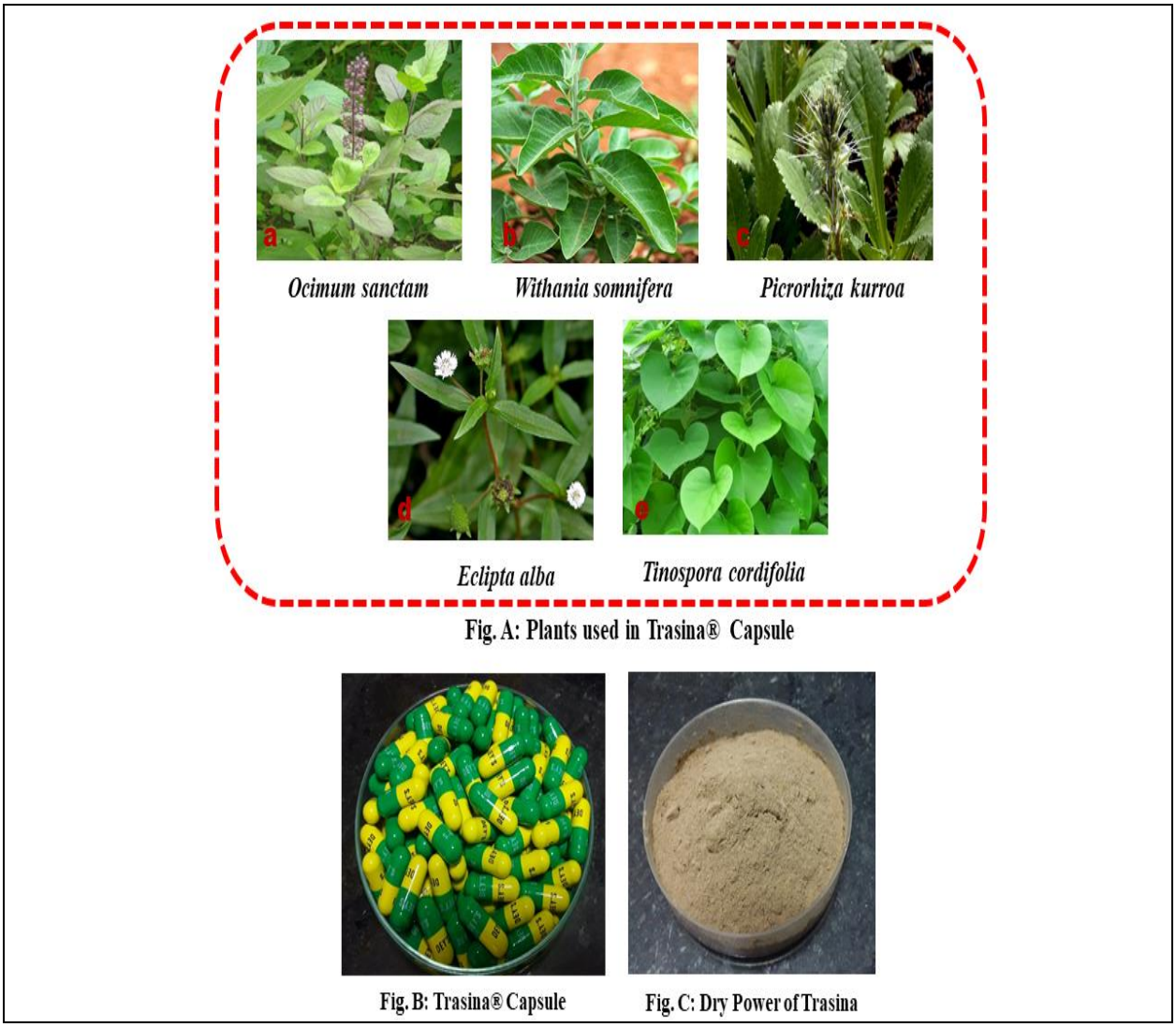

Fig 1: Poly-Herbal medicine – Trasina® with different ingredients those are present in the formulation.

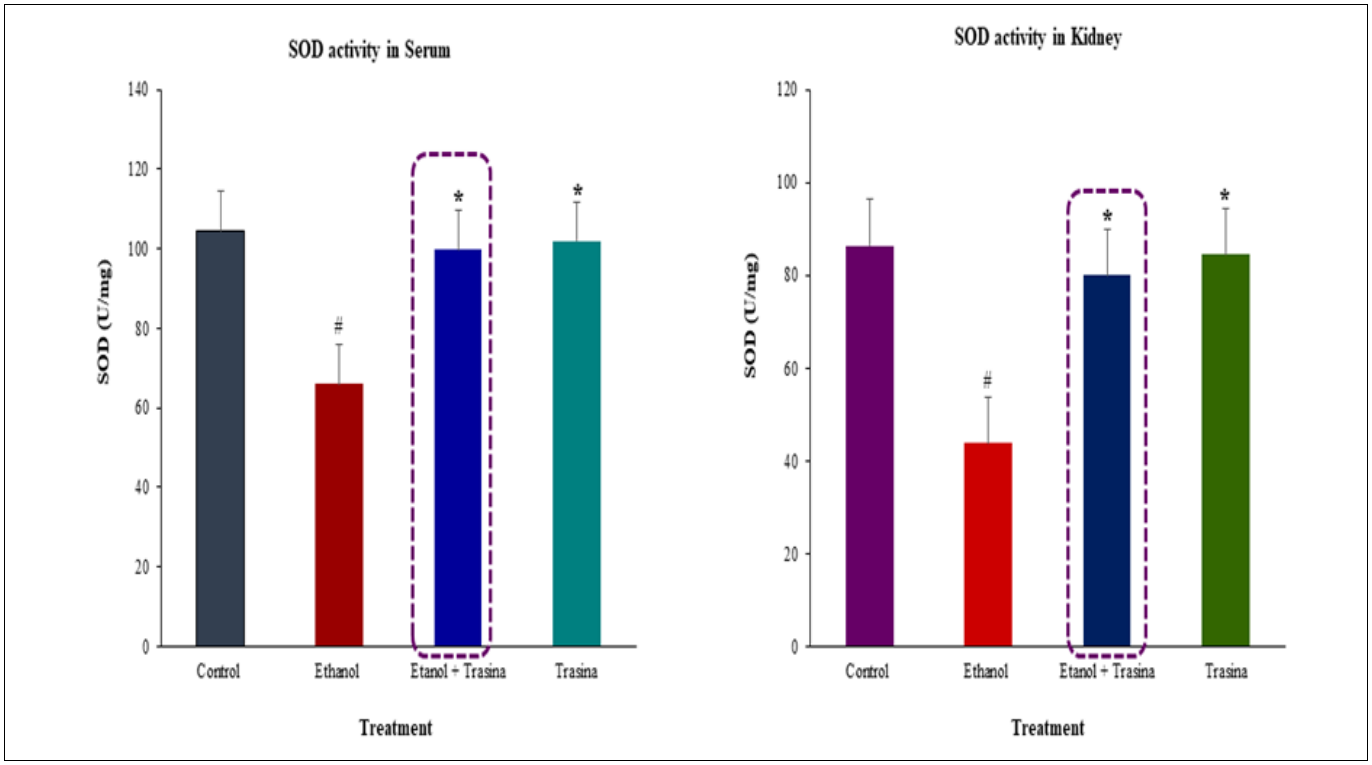

Fig 2: Effect of ethanol alone or in combination with poly-herbal medicine (Trasina®) on serum and kidney super oxide dismutase (SOD) activity. Values expressed are mean ± SE (n=10). #significantly different from control group P < 0.001 and *significantly different from ethanol treated group P < 0.001.

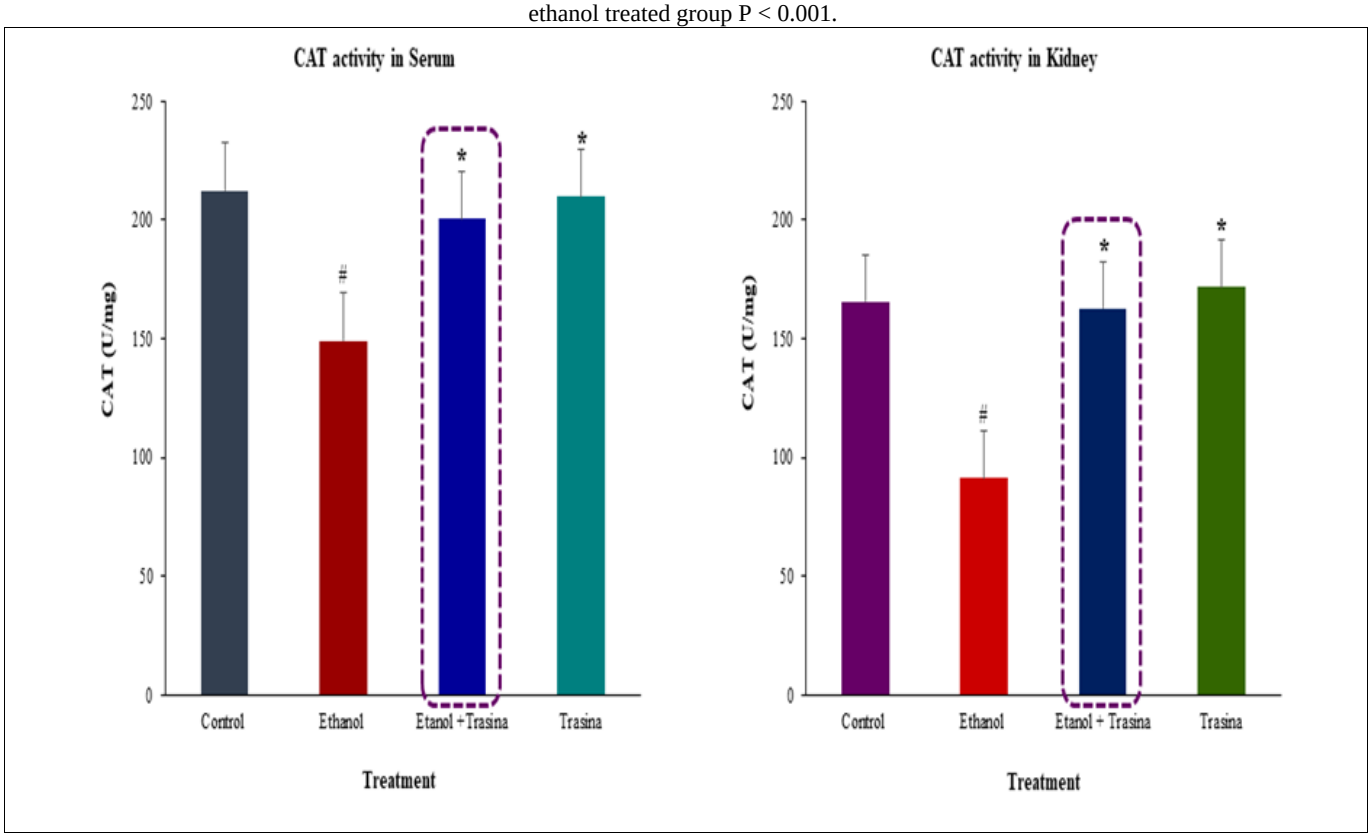

Fig 3: Effect of ethanol alone or in combination with poly-herbal medicine (Trasina®) on serum and kidney Catalase (CAT) activity. Values expressed are mean ± SE (n=10). #significantly different from control group P < 0.001 and *significantly different from ethanol treated group P < 0.001.

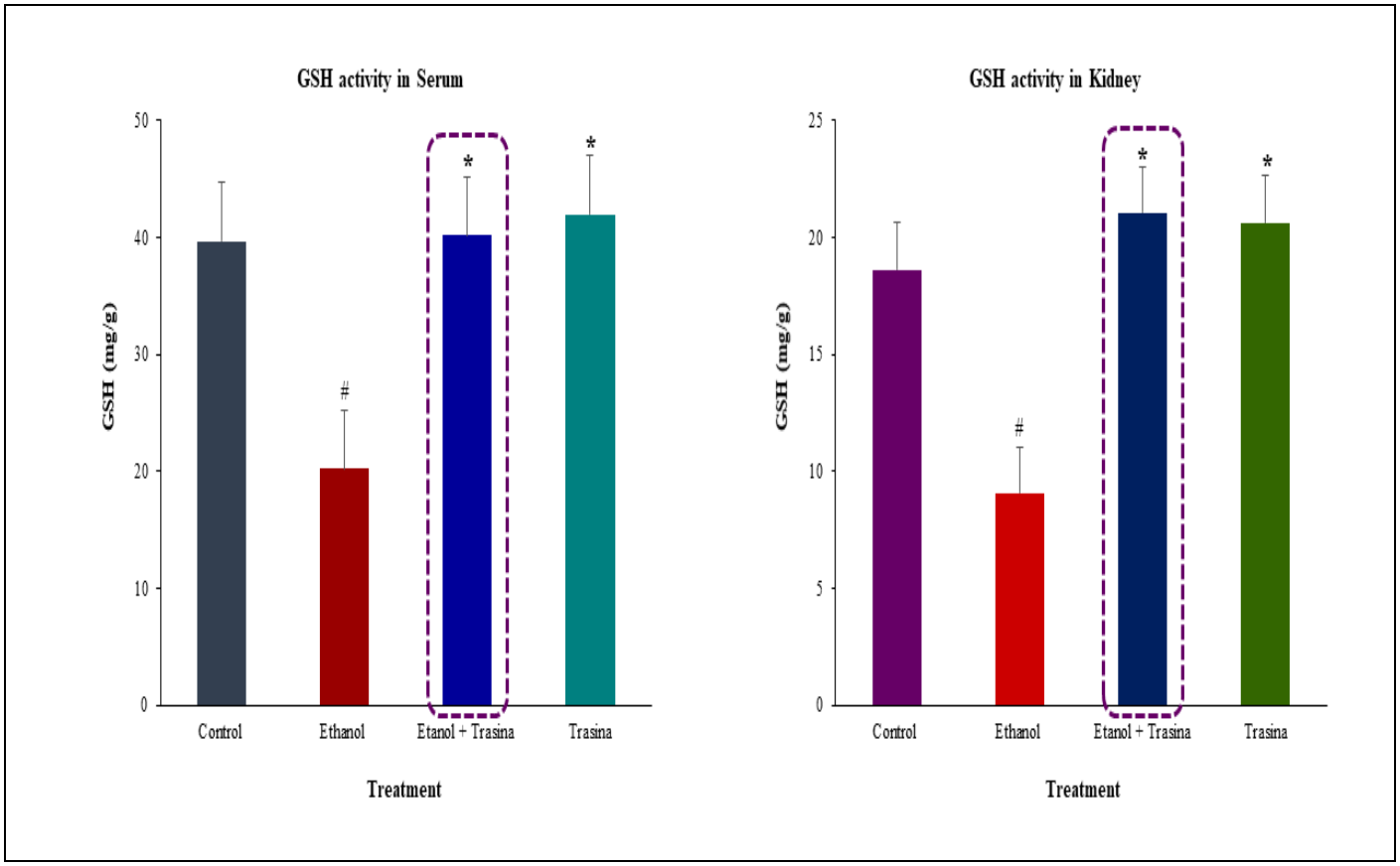

Fig 4: Effect of ethanol alone or in combination with poly-herbal medicine (Trasina®) on serum and renal glutathione (GSH) activity. Values expressed are mean ± SE (n=10). #significantly different from control group P < 0.001 and *significantly different from ethanol treated group P < 0.001.

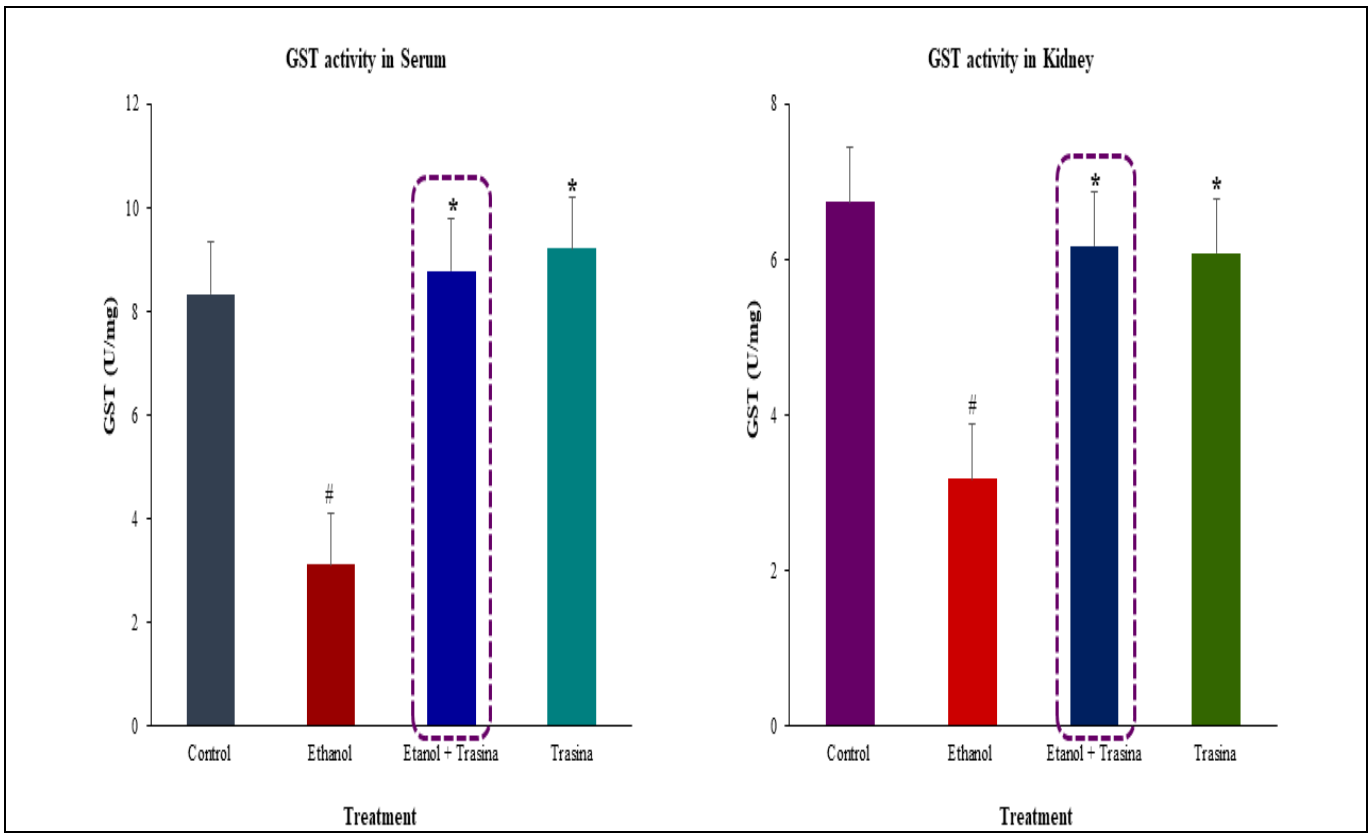

Fig 5: Effect of ethanol alone or in combination with poly-herbal medicine (Trasina®) on serum and renal glutathione –S transferase (GST) activity. Values expressed are mean ± SE (n=10). #significantly different from control group P < 0.001 and *significantly different from ethanol treated group P < 0.001.

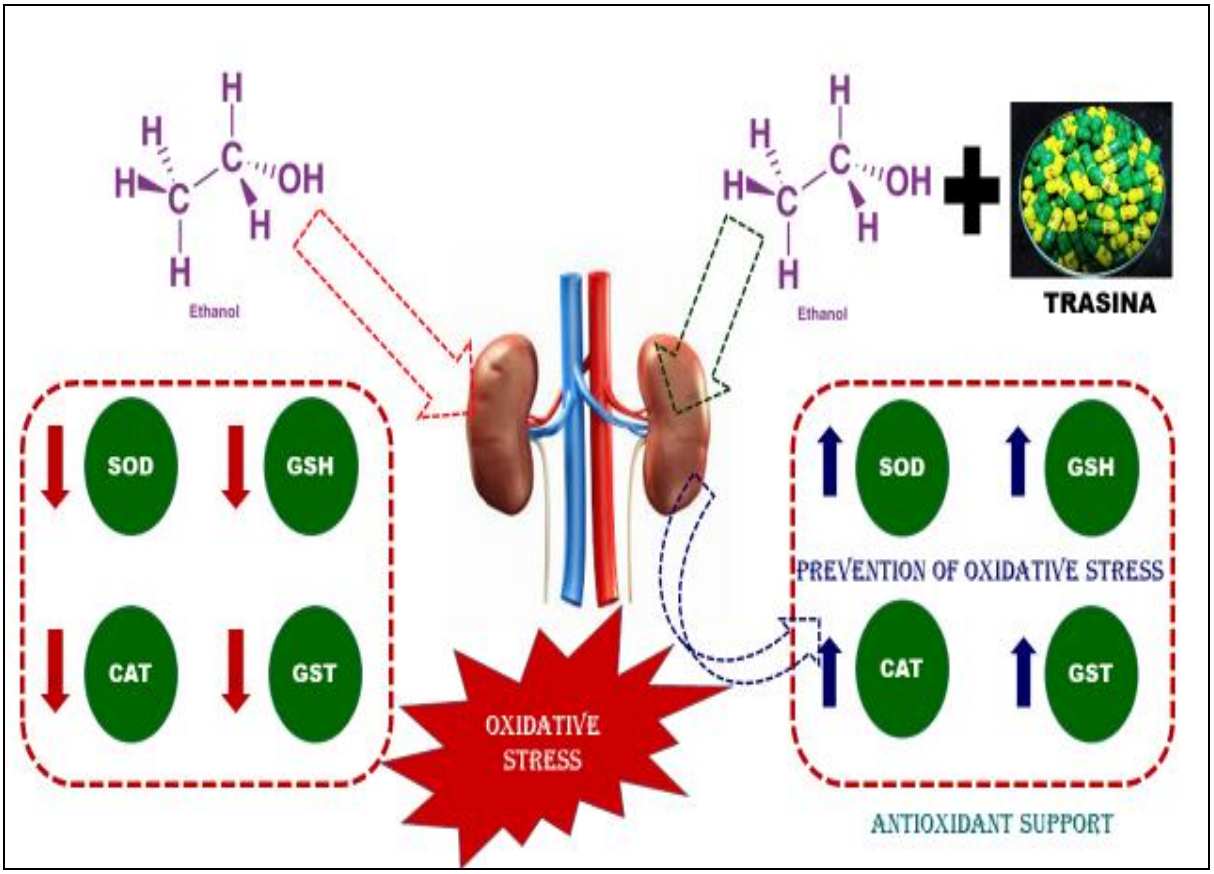

Fig 6: Safe and symptomatic prevention of ethanol induced oxidative stress by Trasina®

Protective effect of Poly-herbal medicine (Trasina®) on serum and kidney MDA content in ethanol toxicity

Serum and tissue (kidney) lipid peroxidation (MDA levels) are depicted in Table 3. Serum MDA content in the ethanol intoxicated mice was significantly higher than that of the controls (102.58±1.87 vs. 45.92 ±1.54 nmol/g). Pre-treatment with Poly-herbal medicine (Trasina®) significantly reduced Serum MDA content compared with ethanol intoxicated mice (49.63±0.91vs. 102.58±1.87 nmol/g).

Renal MDA content in the ethanol intoxicated mice was significantly high than that of controls (72.05±0.89 vs 32.81±0.71 nmol/g). Pre-treatment with Poly-herbal medicine (Trasina®) significantly decreased liver MDA content compared with ethanol intoxicated mice (38.07±0.58 vs. 72.05±0.89 nmol/g).

Protective effect of Poly-herbal medicine (Trasina®) on serum and kidney SOD activity in ethanol toxicity

First order antioxidant enzyme Super oxide dismutase (SOD) activities of serum, and kidney are depicted in Figure 2**.** Serum SOD activity in the ethanol intoxicated mice was significantly less than that of the controls (66.02 ± 2.31 vs. 104.62 ± 3.62 U/mg protein). Pre-treatment with Poly-herbal medicine (Trasina®) significantly increased Serum SOD activity compared with ethanol intoxicated mice (99.65 ± 2.01 vs. 66.02 ± 2.31 U/mg protein).

Renal super oxide dismutase (SOD) activity in the ethanol intoxicated mice was significantly less than that of controls (43.85 ± 1.84 vs 86.27 ± 1.59 U/mg protein). Pre-treatment with Poly-herbal medicine (Trasina®) significantly increased hepatic SOD activity compared with ethanol intoxicated mice (80.02 ± 2.21 vs. 43.85 ± 1.84 U/mg protein).

Protective effect of Poly-herbal medicine (Trasina®) on serum and kidney CAT activity in ethanol toxicity

Serum, and kidney catalase (CAT) activities are depicted in Figure 3**.** Serum CAT activity in the ethanol intoxicated mice was significantly less than that of the controls (149.26 ± 6.85 vs. 212.36 ± 5.98 U/mg protein). Pre-treatment with Poly-herbal medicine (Trasina®) significantly increased Serum CAT activity compared with ethanol intoxicated mice (200.55 ± 5.71 vs. 149.26 ± 6.85 U/mg protein).

Renal catalase (CAT) activity in the ethanol intoxicated mice was significantly less than that of controls (91.26 ± 2.64 vs 165.48 ± 6.22 U/mg protein). Pre-treatment with Poly-herbal medicine (Trasina®) significantly increased hepatic CAT activity compared with ethanol intoxicated mice (162.65 ± 4.78 vs. 91.26 ± 2.64 U/mg protein).

Protective effect of Poly-herbal medicine (Trasina®) on serum and kidney GSH activity in ethanol toxicity

Serum, and kidney reduced glutathione (GSH) activities are depicted in Figure 4**.** Serum GSH activity in the ethanol intoxicated mice was significantly less than that of the controls (20.20 ± 0.61 vs. 39.67 ± 0.88 U/mg protein). Pre-treatment with Poly-herbal medicine (Trasina®) significantly increased Serum GSH activity compared with ethanol intoxicated mice (40.19 ± 1.51 vs. 20.20 ± 0.61U/mg protein).

Renal reduced glutathione (GSH) activity in the ethanol intoxicated mice was significantly less than that of controls (9.06 ± 0.35 vs 18.62 ± 0.84 U/mg protein). Pre-treatment

with Poly-herbal medicine (Trasina®) significantly increased hepatic GSH activity compared with ethanol intoxicated mice (21.05 ± 1.44 vs. 9.06 ± 0.35 U/mg protein).

Protective effect of Poly-herbal medicine (Trasina®) on serum and kidney Glutathione –S Transferees (GST) activity in ethanol toxicity

Serum and tissue (kidney) Glutathione–S Transferees (GST) activities are depicted in Figure 5**.** Serum GSH activity in the ethanol intoxicated mice was significantly less than that of the controls (3.11 ± 1.02 vs. 8.32 ± 0.99 U/mg protein). Pre-treatment with Poly-herbal medicine (Trasina®) significantly increased Serum GSH activity compared with ethanol intoxicated mice (8.77 ± 1.51 vs. 3.11 ± 1.02 U/mg protein).

Renal Glutathione–S Transferees (GST) activity in the ethanol intoxicated mice was significantly less than that of controls (3.18 ± 0.41 vs 6.74 ± 0.79 U/mg protein). Pre-treatment with Poly-herbal medicine (Trasina®) significantly increased hepatic GSH activity compared with ethanol intoxicated mice (6.17 ± 0.25 vs. 3.18 ± 0.41 U/mg protein).

Discussion

Chronic intake of alcohol generates reactive oxygen species (ROS) which produced cellular damage in every mammalian species. During ethanol intoxication, kidney is the another main target organ apart from liver where huge amount of ROS generation takes place which developed oxidative stress as a result damage the targeted tissue. According to the various scientific research different free radicals as hydroxyl radical ($OH^{\bullet}$), Superoxide anion ($0_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) are the major ROS generated during normal redox reaction in our body developed cytotoxic effects. These ROS molecule are generally neutralized by the defensive action of the endogenous antioxidant system, primarily composed of glutathione [28], superoxide dismutase [29], glutathione peroxidase and catalase [36]. When body lost the balance between ROS production and antioxidant defensive system 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 [31]. During the cellular oxidative stress lipid peroxidation is commonly used as marker. The level of MDA, which is generated as an end product during the oxidation of lipids, was used as a marker of lipid peroxidation [32]. Treatment of mice with ethanol (50% v/v) significantly increased lipid peroxidation as shown by elevated MDA levels in serum and kidney. This situation suggests the induction of oxidative stress in cells. Application of poly-herbal formulation (Trasina®) significantly reduced the serum MDA level. The treatment also reduced the renal MDA levels which clearly indicate that this herbal medicine maintain the normal fluidity of cell membrane which plays a vital role in cell functioning. Apart from this animals intoxicated with ethanol decreased serum and kidney total protein indicated the cellular damage. Simultaneous treatment with Trasina® normalized the serum and kidney protein level towards experimental animals.

The normal activity of anti-oxygen enzymes mainly Super oxide dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Glutathione –S Transferase (GST) are commonly inhibited by intoxication of ethanol resulting oxidative damage. Sever oxidative stress suppress the normal cellular functions and gradually damage the cellular activity. To maintain the balance between ROS production and antioxidant defensive system very vital for homeostasis. In this study our aim is to determine the possible therapeutic effect of poly herbal medicine (Trasina®) upon SOD, GPx, GST and CAT enzyme activities as a marker of oxidative stress. Scientific study reviled that SOD is an enzyme against the superoxide radical and catalyzes its dismutation into H_2O_2, which is utilized by CAT or GPx [33]. On the other hand GST catalyzes the conjugation of several substrates to the thiol group of glutathione, transforming toxic materials into less toxic forms [34, 35]. In the present study, oral administration of ethanol (50% v/v) on mice significantly reduced the serum and kidney antioxidant enzyme activities as compared to control untreated animals which supported the previous experiment that chronic consumption of ethanol generate free radicals which reduced the antioxidant enzymes activities. Generation of reactive oxygen species (ROS) within the cell decreased the cellular performance by changing the antioxidant enzymes actions. Treatment with poly herbal medicine (Trasina®) at a dose of 200 mg/kg/day on mice those are intoxicated with ethanol, significantly elevated serum and kidney the SOD, GPx, GST and CAT enzyme activities. From this result it is clear that this herbal medicine (Trasina®) inhibit the free radical production within the cell which indicate that synergistic action of various plants compounds in a single medicine may potent to prevent cellular oxidative stress and boost the cell for their normal function.

Conclusion

Daily intake of ethanol generates reactive oxygen species (ROS) which gradually developed oxidative stress in experimental mice. Long term consumption of ethanol alter serum and kidney markers and reduced antioxidant enzymes activities. Serum and tissue MDA level, the marker of membrane damage drastically elevated in mice after ethanol intoxication. This may probably contribute to the additional progression of ethanol intoxication related problems. Treatment with poly-herbal medicine (Trasina®) normalized the serum and tissues antioxidant enzymes activities by suppression of extensive ROS generation during ethanol intoxication. Thus Trasina® capsule composed of various medicinal herbs may be a potent drug which sound for prevention of cellular oxidative stress.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgement

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