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Research Article

ETHANOL INTOXICATED HEPATIC OXIDATIVE STRESS MITIGATED BY POLY-HERBAL FORMULATION – TRASINA® IN MURINE MODEL

Hepatic Oxidative Stress Prevented by Trasina®

SOUMENDRA DARBAR[1]*, SRIMOYEE SAHA[2] AND ATISKUMAR CHATTOPADHYAY[3]

[1]Research and Development Division, Dey's Medical Stores (Mfg.) Ltd., 62, Bondel Road, Kolkata-700019, West Bengal, India
[2]Department of Physics, Jadavpur University, 188, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
[3]Faculty Council of Science, Jadavpur University, 188, Raja S C Mallick Road, Kolkata-700032, West Bengal, India

*Email: dr.soumendradarbar@deysmedical.com

ABSTRACT

Background: The main focus and aim of this scientific novel work was to find out the probable ameliorative effect of poly-herbal formulation (Trasina®) on serum and liver antioxidant enzymes activities in ethanol intoxicated organ dysfunctions in mice model.

Methods: Forty Swiss albino adult male mice were taken from our animal house and randomly break into 4 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 livers were removed immediately and used fresh or kept frozen until analysis. Blood was taken from the animals before sacrifice for measurement the antioxidant parameters first and second order enzymes i.e. catalase (CAT), super oxide dismutase (SOD), glutathione –S transferees (GST) and reduce glutathione (GSH) from sera.

Results: Activities of all antioxidant enzymes i.e. SOD, CAT, GSH and GST in serum and liver were significantly decreased 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 liver.

Conclusions: 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 catalase (CAT), super oxide dismutase (SOD), glutathione –S transferees (GST) and glutathione peroxidase (GPx) activities in ethanol intoxicated mice and inhibit organ damage.

Keywords: Antioxidant enzymes activity, Liver, Ethanol, Trasina®, Oxidative stress, Swiss albino mice

INTRODUCTION

Mammalian normal cellular activity and physiology was severely affected if body attacks by Reactive oxygen species (ROS) [1-4]. During ethanol induced organ toxicity antioxidants play a crucial role for preventing the cellular damage caused by various redox molecules [5,6]. Commonly most of the xenobiotics were largely metabolised in the liver which reduced the toxic effects of the molecules. Most of the time by-products of this type of metabolism makes sever harmful 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 creates membrane damage by free radicals, damage the membranous lipid and protein as well as modified deoxy ribonucleic acid (DNA) [8]. The generation of reactive oxygen species (ROS) and the defensive ability to counteract this deleterious effects was breaks by the ethanol which decline antioxidants functions [9]. Enormous formation and insufficient

release of free radicals lead to severe and irreversible cell damage [10]. Scientific research work reviled that when cell attacked by free radicals through oxidative damage notably decrease in various antioxidant enzymes activities such as catalase (CAT), superoxide dismutase, glutathione S-transferase (GST), and reduce glutathione (GSH) which acts as a free radical scavengers in conditions associated with oxidative stress [11,12].

Indian systems of medicine have very safe and effective for curing various diseases with no toxic effects. Now a days throughout the world people relies on herbal drugs because of its less side effects in comparison to modern synthetic medicines. Most of the chronic diseases when treated with natural medicines considerably very useful for the effective medication. Prolonged used of the herbal medicine not showed adverse side effects and reaction because its contains lots of constituents those are highly medicinal property [13,17].

Trasina® a marketed poly-herbal capsule composed of five Indian medicinal plants consistently used from the ancient time for the benefit of mankind. *Withania somnifera*, *Ocimum* sanctum, *Tinospora cordifolia*, *Picrorrhiza kurroa, Eclipta alba,* and Shilajit, are the main ingredients present in Trasina® [18]. In 1997 Bhattacharya et al. reported that the said formulation has facilitating the memory action in rodent and non-rodent. Twenty one days sub chronic administration of Trasina on two different animal 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 liver antioxidant activity of poly-herbal capsule – Trasina® against ethanol induced oxidative stress in mice.

MATERIALS AND METHODS

Drugs and Chemicals

The poly-herbal capsule Trasina®, was taken from Dey's Medical (Kolkata, India). Ethanol, TRIS buffer and phosphate buffer were obtained from Merck, India. All antioxidant enzyme study kits were purchase from E-mark Germany. All others necessary chemicals and reagents were procured from local renounced sources and were of analytical grade.

Animals

Forty young swiss albino male mice weighing 26–28 g were randomly chosen and used for the experiment. The animals have been kept with our well ventilated Animal House with all essential environment based on the *Committee for the Purpose of Control and Supervision of Experiments on Animals* (*CPCSEA*) guidelines. The animals were acclimatized for 7 consecutive day's 12 h light and dark cycle. The animals were preserved in stainless steel (SS) cages with maintaining hygienic condition. The room was air-conditioned with temperature and humidity maintained. Mice were allowed standard chow diet (Amrut feeds, Pranav Agro, New Delhi, India) throughout the experimental period and water *ad libitum*. The entire experimental procedures were scrutinised and approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. 16/IAEC/Dey's/s/2016).

Experimental design

Healthy adult male mice were divided into four experimental groups. Cage of the each group contain ten mice The details of the group division and treatment protocols are: Group 1 received vehicle and served as a control, Group 2 received 0.5 ml of 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

After completion of the experimental period, blood sample for analysis 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 -60°C till used for estimation of various antioxidant enzymes.

After collection of blood sample, the abdomen and the thorax of the mice were opened and removed liver with proper care. The liver was washed four times in ice cold phosphate buffer saline and blotted individually on what man filter paper. The samples were then taken for homogenization for estimation of tissue antioxidant enzymes like SOD, CAT, GSH and GST.

Preparation of tissue homogenates

Small portion of liver was homogenization with a potter- Elvenhjem tissue homogenizer. Tissue was taken in phosphate buffer saline (PBS) 50 mM pH (7.4) as a homogenised medium. After the homogenization

aliquot was stores for the for estimation of total protein content, SOD, CAT, GST, GSH enzymes activities.

Determination of protein content

Serum and liver total protein levels was measured by the method of Kashyap *et al.* (Lowry assay) [22].

Determination of Lipid peroxidation

Lipid peroxidation (LPO) was measurement by using lipid peroxidation (MDA) assay kit (Sigma-Aldrich Ltd., UK) in accordance to the manufacturer's instructions. Broadly lipid peroxidation is detected by the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a colorimetric product, proportional to the MDA present. After addition of MDA-TBA adduct each sample was incubated at 90°C for 60 min, prior to cool to room temperature in an ice bath for 10 min. To conduct the reaction 200 mL mixture was transferred into a 96-well plate for analysis. The absorbance was measured at 532 nm [23].

Assay of antioxidant enzyme activities

a. Determination of Catalase (CAT) activity

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

b. Determination of Super oxide dismutase (SOD) activity

Activity of super oxide dismutase (SOD) in serum and hepatic tissue homogenate was assessed according to method of Misra *et al.* 1972 [25] with slight modification. In details 1 mL of Tris-HCl buffer, containing diethylene triaminopentaacetic acid and pyrogallol were mixed with 20 µL of liver samples. The absorbance was measured at 440 nm.

c. Determination of Glutathione –S transferees (GST) activity

GST activity of Serum and hepatic tissues was investigated by the method of Beutler *et al*.1963 [26] with slight modification. The enzymatic reaction of 1-chloro-2-4-di-nitrobenzene is neutralized by the enzyme in the presence of glutathione as a co substrate. The absorbance change was measured at 340 nm.

d. Determination of Reduce Glutathione (GSH) activity

Reduced glutathione (GSH) activity determination was based on the method of Jollow *et al.* 1974 [27]. In the main reaction 1,2-dithio-bis nitro benzoic acid (DTNB) used as substrate. After adding the substrate the yellow color developed which was immediately read at 412 nm. The activity was expressed as μmol GSH/g tissue.

Statistical analysis

SPSS (version 20.0) software were used for statistical. Tukey's test were used to determine significant differences between groups with one-way analysis of variance (ANOVA; $P < 0.05$). The values were stated as mean ± SD.

RESULTS

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

Poly-herbal medicine - Trasina® composed of five Indian medicinal plants (Table 1 and Figure 1) have been effective for stress and depression. Serum and hepatic protein contain are depicted in Table 2. 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).

Hepatic total protein content in the ethanol intoxicated mice was significantly lower than that of controls (2.05±0.09 vs 5.92±0.11 nmol/g). Pre-treatment with Poly-herbal medicine (Trasina®) significantly decreased (Table 2) liver total protein content compared with ethanol intoxicated mice (5.09±0.18 vs. 2.05±0.09 nmol/g).

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

Serum and tissue (liver) 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).

Hepatic MDA content in the ethanol intoxicated mice was significantly high than that of controls (77.05±0.79 vs 35.28±0.92 nmol/g). Pre-treatment with Poly-herbal medicine (Trasina®) significantly decreased (Table 3) liver MDA content compared with ethanol intoxicated mice (39.01±0.88vs. 77.05±0.79 nmol/g).

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

Serum, and liver super oxide dismutase (SOD) activities are depicted in Figure 2**.** Serum activity of SOD in the ethanol intoxicated mice was decreased significantly 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).

Hepatic 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 (Figure 2) 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 liver CAT activity in ethanol toxicity

Serum, and liver catalase (CAT) activities are depicted in Figure 3**.** Serum CAT activity in the ethanol intoxicated mice was significantly less than that of the normal untreated animals (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).

Hepatic 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 (Figure 3) 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 liver GSH activity in ethanol toxicity

Serum, and liver 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).

Hepatic 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 (Figure 4) 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 hepatic Glutathione –S Transferees (GST) activity in ethanol toxicity

Serum and hepatic 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).

Tissue level (Liver) Glutathione –S Transferees (GST) concentration 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 (Figure 5) 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, liver is the main target organ for oxidative stress. According to the various scientific research different free radicals as Superoxide anion ($0_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), 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 [30]. When body lost the equilibrium 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. During cellular lipid oxidation malondialdehyde (MDA) is generated as an end product denoted as a marker of lipid peroxidation [32]. Treatment of mice with ethanol (50% v/v) significantly elevated lipid peroxidation which is reflected as elevated MDA levels in serum and liver tissues. The condition indicates that cell was severely affected by reactive oxygen species (ROS) leads to cellular damage. Application of poly-herbal formulation (Trasina®) significantly reduced the serum MDA level. The treatment also reduced the hepatic MDA levels which clearly indicate that normal membranous fluid level of the cell maintained by herbal medicine which plays a vital role in cell functioning. Apart from this

animals intoxicated with ethanol decreased serum and liver total protein indicated the cellular damage. Simultaneous treatment with Trasina® normalized the serum and liver protein level towards experimental animals.

The normal activity of first order anti-oxygen enzymes mainly Super oxide dismutase (SOD), Catalase (CAT) and second order enzymes Glutathione –S Transferase (GST) and Glutathione (GSH) 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 cellular homeostasis balance between antioxidant defensive system and ROS production is very crucial. In this study our aim is to determine the possible therapeutic effect of poly herbal medicine (Trasina®) upon antioxidant enzymes activities as a marker of oxidative stress. Scientific study reviled that first order antioxidant enzyme i.e. SOD is works against the superoxide radical and catalyzes its dismutation into H_2O_2, which is utilized by catalase (CAT) or glutathione peroxidase (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 liver 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 liver 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

Consumption of ethanol generates reactive oxygen species (ROS) gradually developed oxidative stress in mammalian system. Chronic administration of ethanol alter serum and liver markers and decline various essential antioxidant enzymes activities. Serum and tissue MDA level, the marker of membrane damage drastically elevated in mice after intoxication with ethanol. This may probably contribute to the additional progression of ethanol intoxication related problems and developed cellular deformities. 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.

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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	190 mg
2.	*Withania somnifera*	Ashwagandha	Solanaceae	60 mg
3.	*Picrorhiza kurroa*	Kutki	Plantaginaceae	10 mg
4.	*Eclipta alba*	Bhringraj	Asteraceae	10 mg
5.	*Tinospora cordifolia*	Guduchi	Menispermaceae	10 mg

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

Groups	**Total Protein (**mg/dL**)**	
	Serum	Liver
Control	7.52 ±0.24	5.92±0.11
Ethanol (50% v/v)	3.62±0.16$^{\#}$	2.05±0.09$^{\#}$
Ethanol + Livina® (200mg/kg)	7.02±0.18*	5.09±0.18*
Livina® (200mg/kg)	7.41±0.25*	5.51±0.17*

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 liver MDA level in ethanol intoxicated mice.

Groups	**MDA (**nmol/g**)**	
	Serum	Liver
Control	45.92 ±1.54	35.28±0.92
Ethanol (50% v/v)	102.58±1.87$^{\#}$	77.05±0.79$^{\#}$
Ethanol + Livina® (200mg/kg)	49.63±0.91*	39.01±0.88*
Livina® (200mg/kg)	42.11±0.95*	34.51±0.97*

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. A: Plants used in Trasina® Capsule

Fig. B: Trasina® Capsule

Fig. C: Dry Power of Trasina

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

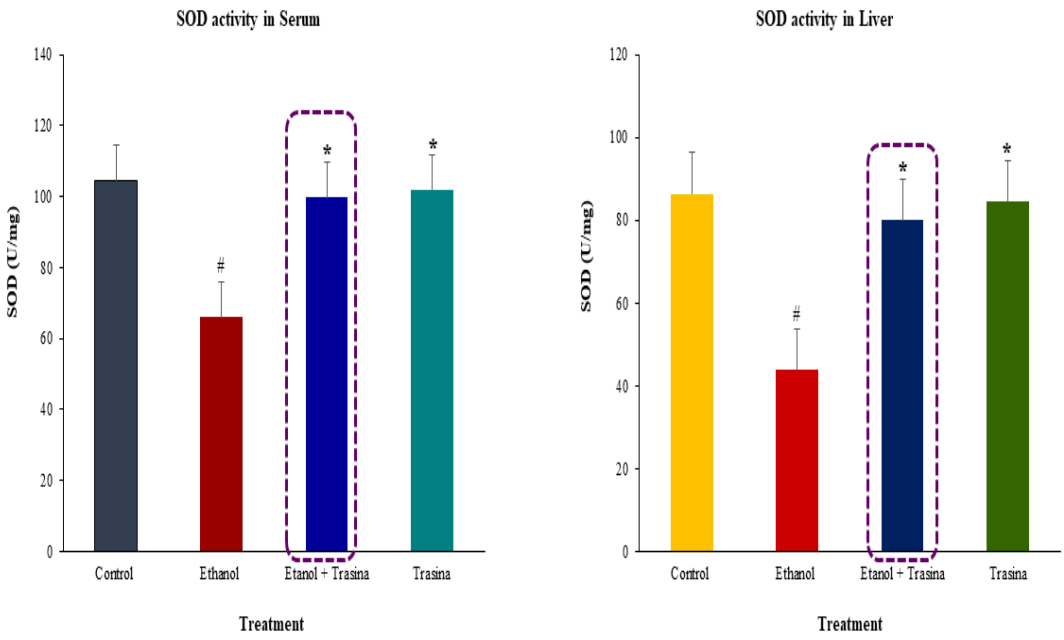

Figure 2: Effect of ethanol alone or in combination with poly-herbal medicine (Trasina®) on serum and liver super oxide dismutase (SOD) activity. Values expressed are mean ± SE (n=10). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P 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

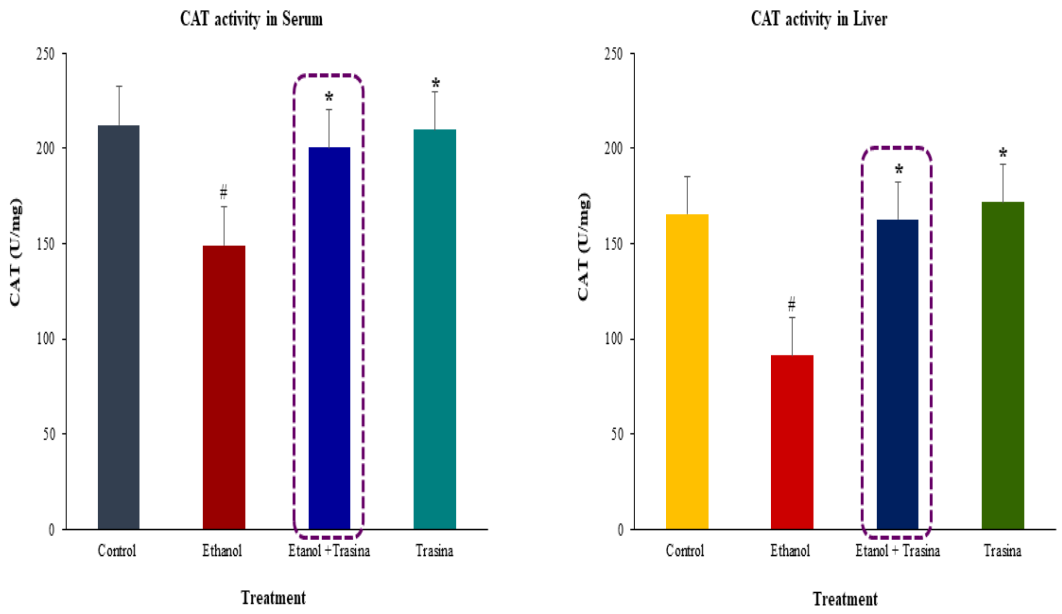

Figure 3: Effect of ethanol alone or in combination with poly-herbal medicine (Trasina®) on serum and liver Catalase (CAT) activity. Values expressed are mean ± SE (n=10). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P < 0.001$.

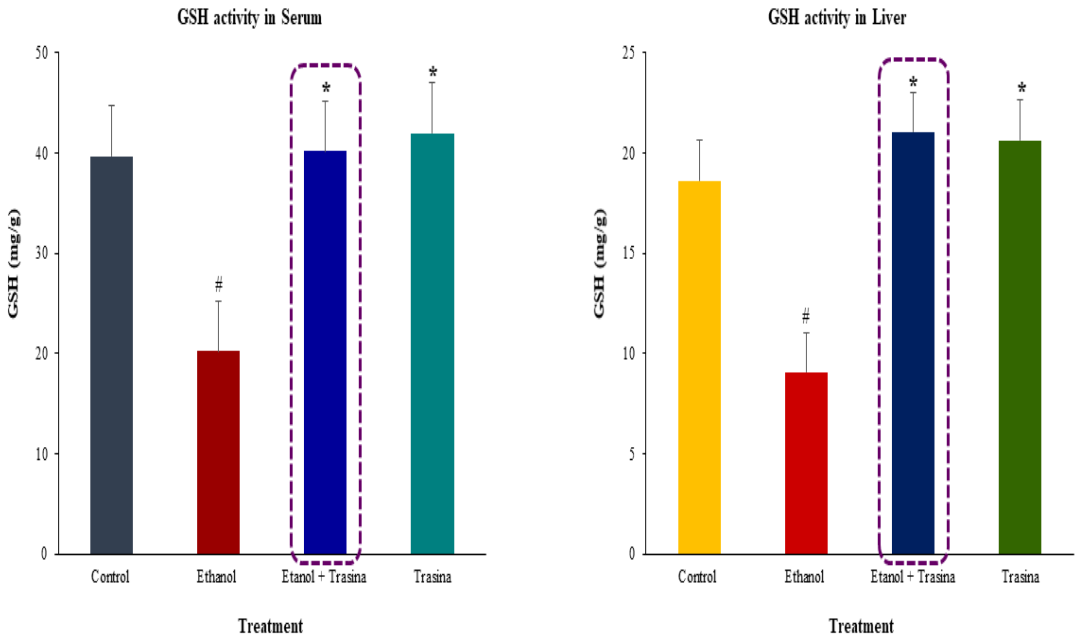

Figure 4: Effect of ethanol alone or in combination with poly-herbal medicine (Trasina®) on serum and hepatic glutathione (GSH) activity. Values expressed are mean ± SE (n=10). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group $P < 0.001$.

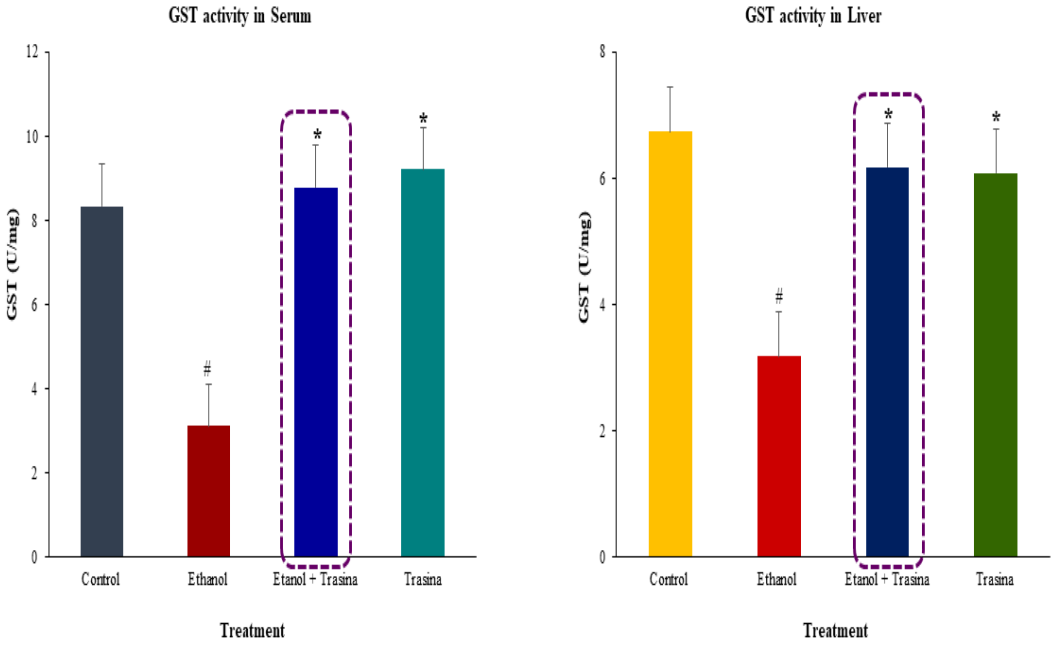

Figure 5: Effect of ethanol alone or in combination with poly-herbal medicine (Trasina®) on serum and hepatic glutathione –S transferase (GST) activity. Values expressed are mean ± SE (n=10). #significantly different from control group $P < 0.001$ and *significantly different from ethanol treated group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