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Hepatoprotective Activity of Ethanolic extract *Tanacetum parthenium L* in Sodium arsenite-induced Hepatotoxicity in Wistar Albino Rats

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ABSTRACT

This study assessed the probable effects of Ethanolic extract of *Tanacetum parthenium* (EETP) whole plant as hepatoprotective compound that acts on damaged liver due to administration of Sodium arsenite ($NaAsO_2$ — 3mg/kg) in Wistar albino rats for a period of 28 days which results in alteration in Lipid peroxidation, antioxidant enzymes, biochemical factors, and liver enzyme. 30 animals were utilized for the study and are divided into five groups containing 6 rats each. Group –I treated as control received water, Group II-V were treated with sodium arsenite. Group-III received Vitamin-E (reference drug), Group IV treated with EETP 200 mg/kg dose and Group V treated with 400 mg/kg dose after hepatotoxicity induced by Sodium arsenite. Safeguarding effects of Ethanolic extract of *Tanacetum parthenium* whole plant were screened by analysis of the parameters like alanine transaminase, aspartate transaminase, alkaline phosphatase and total bilirubin, malondialdehyde, antioxidant enzymes. It was explored that administration of EETP at different doses could significantly decrease the enzymatic measures of AST, ALP, ALT, and TB concentration and increase catalase, GSH, GR and GPx levels in comparison with Sodium arsenite-induced control group. The data obtained from this experimental study prefigured the antioxidant potential of EETP and its potential hepatoprotective effects, and its beneficiary therapeutic effects on vandalization or disfigurement of liver due to Sodium arsenite induction in experimental animals.

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INTRODUCTION

The liver is having overriding importance among all organs in the body. Due to it's a distinct and considerable reconstructive ability, even if there is any moderate cell damage, it is not visible by quantifiable changes in its metabolic functions. Few of the hepatic functions are so delicate that, abnormalities start pop in by depending upon the nature and the degree of the initial damage. The etiology of the hepatic disorders were based on several factors such as biochemical, nutritional, viral, bacteriological, or environmental instability.

Nowadays, Exposure to heavy metals has become one of the most occurring problems throughout the world due to adulterated drinking water, food and polluted air. Arsenic (As) is a naturally occurring element in various forms. The toxic effect of arsenic (metalloid) depends on its form. The widespread demeanor are the trivalent (+3) and the pentavalent (+5) form. Generally, Trivalent form [arsenite (As +3)] is more toxic when compared with Pentavalent [arsenate (As+5)] (Domingo, 1995). It is omnipresent in both (organic and inorganic) forms in the mother nature. The possible ways for the Homo sapiens to arsenic exposure is either through oral route involves eating the artificial food and water or through inhalation (inhaling the polluted air) that majorly obtained due to exposure of agricultural pesticides and mining activities. The chronic poisoning caused by a higher concentration of arsenic in well (pool) water has led to a public health emergency (Alam *et al.*, 2002). Chronic arsenic exposure (Arsenicosis) through pool water raised a chance for the occurrence of peripheral vascular disease (PVD) called as a black foot disease (Tseng, 2002). When administered orally, it results in an accumulation of arsenic in major organs like the liver, kidney, heart, and lungs.

When a smaller amount of arsenic is accumulated in tissues of the muscular and neuronal region, it results in disorders like cancer, dermatitis, diabetes mellitus, hepatotoxicity, neurotoxicity, and various cardiovascular diseases. Upon absorption, immediately, Arsenic should be metabolized and is important to reduce the probable toxic effects that were generated by inhibition of roughly 200 enzymes involved in cellular energy pathways and DNA synthesis and repair, etc. (Ratnaike, 2003). Arsenic undergoes biotransformation via phase - II reactions like reduction and methylation reactions which were catalyzed by an enzyme called glutathione-S-transferase omega-1 (GSTO1) and S-Adenosyl-L-Methionine: Arsenic(III) methyltransferase enzyme (AS3MT) involving methylation of arsenic via one-carbon metabolism by S-adenosyl methionine (SAM) as a methyl donor and requiring reduced glutathione (GSH) as an electron donor in reductase reaction.

The glutathione-S-transferase omega-1 enzyme reduces methylarsonate [MA(V)] and arsenate [As($^{+5}$)] to methylarsonite [MA(+3)] and arsenite [As(+3)], respectively, and the resultant toxic trivalent arsenicals formed during reduction are detoxified by S-Adenosyl-L-Methionine: Arsenic(III)methyltransferase enzyme (AS3MT) to methylarsonate [MA($^{+5}$)] and dimethylarsinate [DMA($^{+5}$)], which are less toxic pentavalent arsenicals (Lindberg *et al.*, 2007). Acute arsenic poisoning is coupled with regurgitation, abdominal pain, nausea and dreadful diarrhea (Ratnaike, 2003). Chronic quaffing of arsenite via contaminated water results in deposition of arsenite and methylarsonite [MA+3] in major organelles and tissues leading to atherosclerosis, diabetes, ischemic heart diseases, hepatotoxicity, hypertension, nephrotoxicity, and cancer of the skin, bladder, and lungs (Gurr, 2003).

Feverfew plant (*Tanacetum parthenium* L.) of Asteraceae, is a daisy-like perennial plant noticed routinely in the nurseries and along with broadsides. As conventional medicine practice (Ayurveda) point of view, *Tanacetum parthenium* is used as a remedy for curing various health issues such as arthritis and migraine. The plant is rich in various antioxidant principles like sesquiterpene lactones and various flavonoids (Pareek *et al.*, 2011). Therefore, The current study is constructed to scrutinize the probable shielding effects of the Ethanolic extract of the whole plant of *Tanacetum parthenium* on Sodium arsenite-induced hepatotoxicity and in Wistar albino rats using Vitamin — E as the reference drug (Hepatoprotective drug).

MATERIALS AND METHODS

Selection of Plant material and extraction

The whole plant of *Tanacetum parthenium* was collected from the village of Manala, Rajanna Siricilla District, situated in the state of Telangana (India) and shade dried and powdered mechanically. The plant specimen was authenticated by a botanist of Osmania University and authenticated voucher specimen Number 453 of the plant has been preserved in the department for the future reference. The completely dried plant were then milled to coarse powder mechanically and successively extracted with different organic solvents like Petroleum ether, Chloroform, Ethyl acetate and Ethanol using Soxhlet-extractor.

Method of maceration was followed for water for 72 hours. The rotary evaporator was used for concentrating the extracts, dried in vacuum desiccators, properly labelled and weighed, stored thereafter in the refrigerator until further use. Preliminary Phytochemical screening for the above plant extracts were conducted (Yada *et al.*, 2020). Based on the existence of Phytoconstituents, Ethanolic extract of Tanacetum parthenium was chosen for the evaluation of the hepatoprotective activity.

Selection of Experimental animals

Ethical committee approval for conducting the experimental study was approved from the Institu-

tional Animal Ethical Committee with an Approval no: CPCSEA/IAEC/JLS/011/11/19/13. Wistar albino rats with average body weight ranging between 150 and 250 g were selected for conducting this study. They were procured from Sanzyme Bio-analytical lab, Plot no. 8 Sys. No.542, Kothur (V), Shameerpet, R.R. dist. The rats were kept in polypropylene cages and supervised under the standard conditions (12 h light and dark cycles at 25 ± 3°C and 35-60 % humidity). Standard pelletized feed and tap water were provided *ad-libitum*.

Experimental Methodology

For conducting the experimental study, 30 rats will be assigned into 5groups and each group with 6 rats. Treatment will be carried for 28 days.

Group I

Control will be given with water p.o

Group II

Sodium arsenite (3mg/kg) p.o

Group III

Sodium arsenite+ vitamin E (100mg/kg) p.o

Group IV

Sodium arsenite + Ethanolic extract of *Tanacetum parthenium* (200mg/kg) p.o

Group V

Sodium arsenite + Ethanolic extract of *Tanacetum parthenium* (400mg/kg) p.o

Except for Group I, remaining group animals were treated with Sodium arsenite to induce the Hepatotoxicity in rats. Group III, IV and V hepatotoxic rats will be treated with the standard drug and test administration for screening the efficacy. At the end of the experiment, rats were fasted overnight. The Rats were weighed (pre and post-treatment with Standard and test). At the end of the experimental study, on 28th day The animals were subjected to sacrification by carbon dioxide inhalation using euthanasia chamber & blood was promptly withdrawn by carotid bleeding method.

Resultant blood was centrifuged with the help of Remi centrifuge at 4000 rpm for about fifteen minutes & the resultant serum was collected and stored at -20°C temperature till analysis. Livers were went on dissection, a part of these tissues were minced and then homogenized with phosphate buffer using a tissue homogenizer. Homogenates were subjected to centrifugation at 10,000 x g for about 15minutes at 4 °C temperature and the resultant supernatant was kept aside and stored at -80°C, used for performing the analysis of antioxidant enzyme activities and lipid peroxidation (MDA)assays.

Biochemical Assessment

Enzymes like Serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were calorimetrically estimated according to the method of Reitman and Frankel while serum alkaline phosphatase (ALP) level was measured according to the method described by Belfeld (Belfield and Goldberg, 1971). Total bilirubin concentration (TB) was determined calorimetrically using Schmidt and Eisenburg method. (Schmidt and Eisenburg, 1975)

Biochemical estimation of markers of oxidative stress

Reduced glutathione (GSH) level was assessed in the liver tissue homogenates by following Ellman method (Ellman, 1959), enzymatic antioxidant catalase (CAT) activity was also assessed according to Aebi *et al.*, method (Aebi, 1984), Glutathione peroxidase was assayed according to the procedure of Hafeman *et al.*, method (Rotruck *et al.*, 1973) with some modifications. Malondialdehyde (MDA) level was predicted by analysing the produced thiobarbituric acid reactive substances (TBARS) using the method of Buege and Aust (Buege and Aust, 1978), Glutathione reductase activity was carefully measured according to previous procedures (Mavis and Stellwagen, 1968).

Statistical analysis

The experimental results obtained were subjected to analysis for statistical significance using one way ANOVA followed by Dunnet test using the graph pad prism statistical software for correlation between different experimental groups. P-values < 0.001 were considered statistically significant.

RESULTS AND DISCUSSION

Estimation of Bodyweight

From the experimental data obtained, (Table 1) it is evident that there was marked cutback in the bodyweight of Group-II rats received the sodium arsenite (toxin) when compared with the control group. Treatment with Ethanolic extract of Tanacetum parthenium whole plant is shown dose-dependent protection against Sodium arsenite intoxication in rats. Values were expressed as Mean ± SEM, (n=6). Using t-test, an inter group deviation between various groups was analyzed by graph pad Prism software & Data were assessed by using One way ANOVA. $^{*}p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$ as compared to Sodium arsenite treated toxin group (Group II) [Groups III to V were compared with Group II], $^{\#}p < 0.001$ as compared to Control group (Group I) [Group II (sodium arsenite treated control) was compared with Group I (normal control)].

Table 1: Effect of Ethanolic extract of *T. parthenium* whole plant on Bodyweight in Sodium arsenite induced Hepatotoxicity in Rats

S. No	Group	Initial weight(gm)	Weight After treatment (gm)	Change in body weight(gm)
I	Control	188.83±0.845	190.98±0.394	-2.15
II	Sodium arsenite treated (2.5mg/kg) p.o.,	244.91±0.126$^{\#}$	254.41±0.312$^{\#}$	-9.5
III	Sodium arsenite + Vit E (2.5mg/kg+ 100 mg/kg) p.o.,	218.3±0.11***	215.51±0.32***	2.79
IV	Sodium Arsenite + EETP (2.5mg/kg+200mg/kg) p.o.,	226.08±0.19*	220.75±0.476*	5.33
V	Sodium Arsenite + EETP (2.5mg/kg+400mg/kg) p.o.,	224.105±0.07**	219.21±0.458**	4.89

Table 2: Effect of Ethanolic extract of *T. parthenium* whole plant on Organweight in sodium arsenite induced Hepatotoxicity in Rats

S. No	Group	Liver (gm)
I	Control	9
II	Sodium arsenite treated (2.5 mg/kg) p.o.,	7.6$^{\#}$
III	Sodium arsenite + Vit E (2.5mg/kg+ 100 mg/kg) p.o.,	8.45***
IV	Sodium Arsenite+ EETP (2.5mg/kg+200mg/kg) p.o.,	8.04*
V	Sodium Arsenite + EETP (2.5mg/kg+400mg/kg) p.o.,	8.28**

Table 3: Effect of Ethanolic extract of *T. parthenium* in Serum Biochemical Parameters for Hepatoprotective activity in sodium arsenite-induced Oxidative stress in Rats

S. No	Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dl)
I	Control	9.40±0.221	11.43±0.155	124.38±0.958	0.29±0.037
II	Sodium arsenite treated (2.5 mg/kg) p.o.,	19.33±0.159$^{\#}$	21.15±0.664$^{\#}$	281.93±0.806$^{\#}$	1.71±0.031$^{\#}$
III	Sodium arsenite + Vit E (2.5mg/kg + 100 mg/kg) p.o.,	10.62±0.232***	12.77±0.162***	134.31±0.731***	0.93±0.035***
IV	Sodium Arsenite+ EETP (2.5mg/kg + 200mg/kg) p.o.,	15.84±0.249*	17.11±0.210*	175.34±0.515*	1.23±0.02*
V	Sodium Arsenite + EETP (2.5mg/kg + 400mg/kg) p.o.,	13.69±0.193**	14.88±0.228**	161.13±0.388**	1.02±0.027**

Estimation of Organ weight

Liver from toxin group rats were separated and weighed and shown a significant reduction in weight when compared with a normal control group. Treatment with Ethanolic extract of *Tanacetum parthenium* whole plant to Group IV and Group V has shown a dose-dependent protection. (Table 2) Values were expressed as Mean ± SEM, (n=6).

Using t-test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. $^{*}p$<0.05, $^{**}p$<0.01, and $^{***}p$<0.001 in comparison to Sodium arsenite administered toxin group (Group II) [Groups III to V were compared with Group II], $^{\#}p$<0.001 as compared to Control (Group I) [Group II (sodium arsenite treated control) was compared with Group I (normal control)].

Table 4: Effect of Ethanolic extract of *T.parthenium* on Antioxidant Parameters in sodium arsenite Induced Oxidative stress in rat Liver

S. No	Group	MDA (nm/gm)	GSH (μg/mg)	Catalase (K/min)	GR (u/ml)	GPx (μg/mg)
I	Control	164.86±0.96	32.6±0.98	25.26±0.527	23.93±0.516	36.01±0.28
II	Sodium arsenite treated (2.5mg/kg) p.o.,	430.28±0.244	12.96±0.29	11.89±0.339	11.61±0.26	22.22±0.58
III	Sodium arsenite + Vit E (2.5mg/kg + 100 mg/kg) p.o.,	169.46±0.93	31.59±0.24	19.22±0.40	21.91±0.45	31.68±0.33
IV	Sodium Arsenite + EETP (2.5mg/kg + 200mg/kg) p.o.,	195.39±0.86	27.50±0.123	18.16±0.09	18.37±0.145	28.72±0.149
V	Sodium Arsenite + EETP (2.5mg/kg + 400mg/kg) p.o.,	181.43±0.26	29.65±0.344	18.53±0.302	19.31±0.178	30.02±0.07

Biochemical Estimation of ALP, AST, ALT, and total bilirubin

It was observed, the empirical data (Table 3) revealed that induction of Sodium arsenite to rats resulted in chronic hepatic damage which is evidenced by raised serum levels of AST, ALT, ALP and Total bilirubin concentration. Disfiguration of hepatocytes resulted in alteration of functional transition, led to enhanced membrane permeability and leakage of enzymes into the outer cellular environment as an outcome. Treatment with Ethanolic extract of *Tanacetum parthenium* whole plant largely inflected the severity of Sodium arsenite-induced hepatic damage and was documented as Enzymatic levels returned to normalcy levels in treated rats (standard and plant extract). It was shown that the Ethanolic extract of *Tanacetum parthenium* whole plant can stabilize liver cell membranes (Normalize the membrane permeability) and prevent the leakage of enzymes. Values were expressed as Mean ± SEM, (n=6). Using t-test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were assessed by using One way ANOVA. $^{*}p< 0.05$, $^{**}p< 0.01$, and $^{***}p< 0.001$ as compared to Sodium arsenite treated toxin group (Group II) [Groups III to V were compared with Group II], $^{\#}p < 0.001$ as compared to Control group (Group I) [Group II (sodium arsenite treated control) was compared with Group I (normal control)].

Estimation of markers of Oxidative stress

Antioxidant enzymes are sensitive to serious devastation to cells. The findings of this study (Table 4), reported an elevation in levels of lipid peroxidation product (MDA), the reduction in Glutathione reductase (GR), Glutathione (GSH), Glutathione peroxidase (GPx), and Catalase (CAT) levels. It was revealed that Damage to the liver following the administration of Sodium arsenite is mainly due to lipid peroxidation induced by the free radicals, derived from Sodium arsenite. Therefore, antioxidant potential and the inhibition of free radical generation are quite important in preventing Sodium arsenite-induced hepatotoxicity. Endogenous antioxidants like glutathione peroxidase (GPx), catalase (CAT) can frame a shared backing system against reactive oxygen species (ROS).

In Sodium arsenite-induced liver impairment, disturbance in the balance between the production of ROS and antioxidant defense system pop in due to the oxidative stress ultimately causes cellular dysfunction and causes liver damage and necrosis. GSH, a Non-enzymatic antioxidant can judge tissue sensitivity to oxidative damage. Glutathione reductase (GR) catalyzes the metabolism via reduction process of glutathione disulfide to the sulfhydryl form of glutathione (GSH), is a crucial molecule in holding out against oxidative stre and maintaining the reducing environment of the cell Depleted levels of liver GSH, results in the increased sensitivity of

liver towards the chemical substances like Sodium arsenite. Come back of the experimental animals to normal after being damaged by Sodium arsenite in the groups administered with Ethanolic extract of *Tanacetum parthenium* whole plant and the standard drug might be due to the ability to stimulate these antioxidant enzymes to counteract the ROS produced by Sodium arsenite.

The present investigation reports a significant increase in MDA in the liver of Sodium arsenite treated rats which suggest that peroxidative injury maybe occurs in the liver. The extract treated animals, showed a significant reduction in MDA, which indicate that Ethanolic extract of Tanacetum parthenium whole plant is having, potential to inhibit the oxidative damage of liver tissues. Values were expressed as Mean $\pm$ SEM, (n=6). Using t-test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were assessed by using One way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ as compared to Sodium arsenite treated toxin group (Group II) [Groups III to V were compared with Group II], $^{\#}p < 0.001$ as compared to Control group (Group I) [Group II (sodium arsenite treated control) was compared with Group I (normal control)].

CONCLUSIONS

From the findings of the current experimental framework, it was evident that the Ethanolic extract of *Tanacetum parthenium* whole plant has antioxidant potential and can shield liver against disfigurement due to free radicals produced as the result of Sodium arsenite metabolism. However, further studies on the isolation of active constituents and their mechanistic studies responsible for the Antioxidant and Hepatoprotective effects of *T. parthenium* are necessary to be done.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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