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HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF *HYPTIS SUAVEOLENS* AGAINST ARSENIC-INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT: The present study was to evaluate Hepatoprotective activity of aqueous extract of *Hyptis suaveolens* whole plant (AEHS) against Sodium arsenite (2.5 mg/kg) induced hepatotoxicity in Wistar rats for a period of 28 days. Rats were divided into five groups, each with 6 rats. Except for group I, all remaining groups was served with Sodium arsenite oral administration to induce hepatotoxicity prior to standard and plant extract administration. Group II served as sodium arsenite treated group (toxin), group III served with Vitamin-E, standard (Hepatoprotective drug) Group IV and Group V Experimental rats received AEHS orally at the doses of 250 mg/kg and 500 mg/kg daily for 28 days. Hepatoprotective effects were investigated by assessment of serum biochemical enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) total bilirubin (TB), malondialdehyde (MDA), and antioxidant enzymes (SOD, CAT, GPx and GSH), along with histopathological studies. There is a significant decrease in the levels of AST, ALT, ALP and TB and an increase in the Catalase, GSH, GR and GPx levels after oral administration of AEHS and Vitamin E. The extract significantly diminished MDA levels. The results of this study are indicative of the antioxidant activity of AEHS, its potential hepatoprotective effects and its probable therapeutic properties for laboratory animals damaged by Sodium arsenite.

INTRODUCTION: The liver plays a notable role not only in the metabolism and disposition of the chemicals to which it is exposed directly or indirectly but also in the metabolism of fats, carbohydrates, proteins, and immunomodulation. The impairment of liver function is generally caused due to xenobiotics, excessive exposure to various pharmacological and chemical agents.

The environment and its compartments have been severely polluted by heavy metals. This has limited the ability of the environment to foster life and render its intrinsic values. Heavy metals are known to be naturally occurring compounds, bio-accumulation in the food chains as a result of the nondegradable state of the heavy metals results in anthropogenic activities in large quantities in different environmental compartments.

This leads to the environment's ability to foster life being reduced as human, animal and plant health become threatened. Arsenic is one of the most important metalloids and persists as organic, inorganic and elemental forms in nature. Trivalent arsenic species are most toxic than pentavalent

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arsenic compounds¹. Chronic exposure to arsenic over a period of time induces toxicity, primarily in liver but gradually in all systems of the body. Arsenic toxicity results from its ability to interact with sulfhydryl groups of enzymes and disrupt enzymes involved in cellular respiration that leads to inhibition of glycolysis and Krebs cycle and substitute phosphorus in a variety of biochemical reactions². Recent studies suggested that exposure to arsenic causes oxidative stress through increased generation of Reactive Oxygen Species and inhibition of antioxidants in the body³. In General, arsenic undergoes hepatic biomethylation to monomethylarsonic acid and dimethylarsinic acids and are potent inhibitors of GSH reductase and causes hepatotoxicity in humans and animals⁴. The quest for novel drugs leads to combat the diseases which are resistant to available pharmaceuticals are a trend of medicinal chemistry research. Traditionally, plants and terrestrial microorganisms remain the major focus of the search for new drug leads. The novelty in their chemical structure associated with such natural products offers wide scope⁵. The use of medicinal plants possessing potent antioxidant properties can help to reduce oxidative stress and hepatotoxicity caused by metals. *Hyptis suaveolens* (L.) Poit commonly known as "Wilaitituli" belongs to the family of Lamiaceae and is a common weed of roadsides and waste grounds, is an ethnobotanically important medicinal plant. All parts of this plant are being used in traditional medicine to treat various diseases. *Hyptis suaveolens* has both medicinal as well as insecticidal properties. Literature of *Hyptis* indicates that leaf extracts cure swellings, abscesses and haemorrhoids. In India, the plant is considered to be stimulant, carminative, sudorific, and lactagogue. Infusion is used in infections of the uterus; leaf juice is taken in cases of colic and stomach ache⁶. The present study was carried out to evaluate the Hepatoprotective activity of *Hyptis suaveolens* against Arsenic induced intoxication model.

MATERIALS AND METHOD:

Collection of Plant Material and Extraction: The whole plant of *Hyptis suaveolens* was collected from the forests of Maisammaguda, Secunderabad, situated in the state of Telangana (India), and shade dried and powdered mechanically. The plant specimen was authenticated by botanist of Osmania

University and authenticated voucher specimen Number 276 of the plant has been preserved in department for future reference. The dried plant powder was extracted with various solvents based on polarity using Pet ether, Chloroform, Ethyl acetate, Methanol by hot continuous extraction using Soxhlet's apparatus and method of maceration for water. All the extracts were evaporated to dryness under vacuum, dried in vacuum desiccators, and stored in the refrigerator, and the resultant crude aqueous suspension was filtered with a muslin cloth and further concentrated on a water bath by boiling for one hour at 100 °C. This was later freeze-dried. Preliminary phytochemical screening was carried out and based on the presence of phytochemical constituents, the aqueous extract was selected for screening hepatoprotective activity.

Experimental Animals: An ethical approval for this experimental study was obtained from the Institutional Animal Ethical Committee with Approval no: CPCSEA/IAEC/JLS/011/11/19/13). Wistar albino rats with an average body weight from 150 to 200 g were utilized in 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 housed in polypropylene cages and maintained under 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 the study 30 rats will be assigned into 5 groups, and each group contains 6 rats. Treatment will be carried for 28 days. Group I: control will be given with water p.o. Group II: Sodium arsenite (2.5 mg/kg) p.o Group III: Sodium arsenite + vit E (100 mg/kg) p.o. Group IV: Sodium arsenite + Aqueous extract of *Hyptissuaveolens* Linn. (250 mg/kg) p.o Group V: Sodium arsenite+ Aqueous extract of *Hyptissuaveolens* Linn. (500 mg/kg) p.o. At the end of the experiment, rats were fasted overnight. The bodyweight of the rats was measured. The animals were sacrificed on the 28th day by carbon dioxide inhalation through the euthanasia chamber & blood was immediately collected by the carotid bleeding method. Blood was centrifuged using Remi centrifuge at 4000 rpm for 15 min & the resultant serum was collected and stored at -20 °C

till analysis. Livers were dissected rapidly a part of these tissues was minced and then homogenized with phosphate buffer using a tissue homogenizer. Homogenates were centrifuged at 10,000 x g for 15 min at 4 °C, and the resultant supernatant was removed and stored at -80 °C until used for antioxidant enzyme activities and lipid peroxidation (MDA) assays.

Biochemical Assessment: Total bilirubin ⁷, Serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ⁸, alkaline phosphatase (ALP) ⁹ were measured calorimetrically.

Biochemical Estimation of Markers of Oxidative Stress: Reduced glutathione ^{10, 11} (GSH) level was determined in the liver and kidney tissue homogenates, enzymatic antioxidant catalase (CAT) ¹² activity, Glutathione peroxidase was assayed according to the procedure of Hafeman *et al.*,

method ¹³ with some modifications. Malondialdehyde ¹⁴ (MDA) level was analyzed by estimating the produced thiobarbituric acid reactive substances (TBARS), Glutathione reductase activity was measured according to previous reports ¹⁵.

Statistical Analysis: The obtained results were analyzed for statistical significance using one-way ANOVA followed by a Dunnett test using the graph pad statistical software to compare different experimental groups. P-values < 0.001 were considered statistically significant.

RESULTS AND DISCUSSION:

Body Weight Estimation: The body weight of rats from toxin group were significantly (p<0.0001) decreased when compared with normal control group. Treatment with extract of *Hyptis suaveolens* has shown dose-dependant protection against Arsenic intoxication in rats **Table 1**.

TABLE 1: EFFECT OF AQUEOUS PLANT EXTRACT OF *H. SUAVEOLENS* ON BODY WEIGHT IN SODIUM ARSENITE INDUCED OXIDATIVE STRESS 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±2.41 [#]	254.41±0.312 [#]	-9.5
III	Sodium arsenite + Vit E (2.5mg/kg+ 100 mg/kg) p.o.,	244.91±0.126 [#]	215.51±0.32 ^{***}	2.79
IV	Sodium Arsenite+ AEHS (2.5mg/kg+250mg/kg) p.o.,	218.3±0.11 ^{***}	219.53±0.868 [*]	4.72
V	Sodium Arsenite+ AEHS (2.5mg/kg+500mg/kg) p.o.,	224.3±1.87 [*]	218.49±0.588 ^{**}	3.92

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one way analysis of variances (ANOVA). Values are expressed as mean ± SEM. *p< 0.05, **p< 0.01, and ***p< 0.001 as compared to Sodium arsenite treated group (Group II) [Groups III to VI were compared with Group II], # p< 0.001 as compared to Control group (Group I) [Group II was compared with Group I].

TABLE 2: EFFECT OF AQUEOUS PLANT EXTRACT OF *H. SUAVEOLENS* ON ORGAN WEIGHT IN SODIUM ARSENITE-INDUCED OXIDATIVE STRESS 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+ AEHS (2.5mg/kg+250mg/kg) p.o.,	8.1 [*]
V	Sodium Arsenite+ AEHS (2.5mg/kg+500mg/kg) p.o.,	8.34

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one-way analysis of variances (ANOVA). Values are expressed as mean ± SEM. *p< 0.05, **p< 0.01, and ***p< 0.001 as compared to Sodium arsenite treated group (Group II) [Groups III to VI were compared with Group II], # p< 0.001 as compared to Control group (Group I) [Group II was compared with Group I].

Organ Weight Estimation: The liver weight of rats from the toxin group was significantly (p<0.0001) decreased when compared with normal control group. Treatment with extract of *Hyptis suaveolens* prior to intoxication by sodium arsenite has shown a dose-dependent protection **Table 2**.

Biochemical Estimation of ALP, AST, ALT and Total Bilirubin: Oral administration of Sodium arsenite to Wistar albino rats for 28 days resulted in significant increase (P<0.001) in serum activities of AST, ALT, ALP, and total bilirubin concentration.

Interestingly, the levels of all these parameters showed significant improvement toward their normal levels seen in the control rats received the vehicle when aqueous plant extract and Vitamin-E

were concomitantly administered with Sodium arsenite **Table 3**.

Biochemical Estimation of Markers of Oxidative Stress: The effects on some components of the oxidative system in the liver of Wistar albino rats after 21 days of exposure to Aqueous plant extract and Sodium arsenite were represented in (Table-4). There was a significant depletion of GSH level, GPx, Catalase and Glutathione reductase activities with significant concurrent elevation ($P < 0.05$) in the MDA level in the liver tissues of orally exposed rats to Sodium arsenite when compared with the other experimental groups. Vitamin-E, plant extract administration along with Sodium arsenite ameliorate the deleterious effects produced by Arsenic via improvement of antioxidant status, causing elevation of GPx, GSH, GR and Catalase levels and a significant decrease ($P < 0.05$) in the

levels of MDA when compared with Sodium arsenite treated group. Notably, enhanced antioxidant level in the extract-treated groups than normal indicates the high antioxidant potential of Aqueous plant extract and is able to inhibit the free radical generation, which could further reduce the oxidative threat caused by arsenic, which could mitigate the consumption of endogenous enzymatic and non-enzymatic antioxidants and increased their levels and markedly reduces the hepatic and renal LPO. In view of the present study, it can be concluded that Aqueous plant Extract of *Hyptis suaveolens* played an important role as an antioxidant, which includes free radical scavenging and metal-chelating property and thereby improved the detrimental state of liver cells, which unraveled its use as a possible mitigator/attenuating agent in arsenic-induced hepatotoxicity.

TABLE 3: EFFECT OF AQUEOUS PLANT EXTRACTS OF *H. SUAVEOLENS* 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+ AEHS (2.5mg/kg+250mg/kg) p.o.,	14.65±0.121*	17.11±0.210	170.58±0.762	1.13±0.034
V	Sodium Arsenite+ AEHS (2.5mg/kg+500mg/kg)	12.95±0.183	14.05±0.129	152.43±0.896	1.008±0.016

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one way analysis of variances (ANOVA). Values are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ as compared to Sodium arsenite treated group (Group II) [Groups III to VI were compared with Group II], # $p < 0.001$ as compared to Control group (Group I) [Group II was compared with Group I].

TABLE 4: EFFECT OF AQUEOUS PLANT EXTRACT OF *H. SUAVEOLENS* ANTIOXIDANT PARAMETERS IN SODIUM ARSENITE-INDUCED OXIDATIVE STRESS IN RAT LIVER

S. no.	Group	MDA	GSH	Catalase	GR	GP _x (µg/mg)
		(nm/gm)	(µg/mg)	(K/min)	(u/ml)	
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+ AEHS (2.5mg/kg+250mg/kg) p.o.,	192.45±0.94	28.66±0.207	18.47±0.055	18.67±0.065	29.24±0.433
V	Sodium Arsenite+ AEHS (2.5mg/kg+500mg/kg) p.o.,	177.07±0.508	30.20±0.433	18.82±0.089	19.84±0.075	30.31±0.414

Values are expressed as mean ±SEM, n=6. Using t-test, the intergroup variation between various groups was conducted by graph pad Prism software & Data were analyzed by using one way analysis of variances (ANOVA). Values are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ as compared to Sodium arsenite treated group (Group II) [Groups III to VI were compared with Group II], # $p < 0.001$ as compared to Control group (Group I) [Group II was compared with Group I]

CONCLUSION: *Hyptis suaveolens* has strong antioxidant activity, and this activity may be responsible for the Hepatoprotective activity against Arsenic induced liver damage. However, further studies on the active compounds and their biochemical mechanisms, which may attribute to the Antioxidant and Hepatoprotective effects of *Hyptis suaveolens* are necessary to be done.

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