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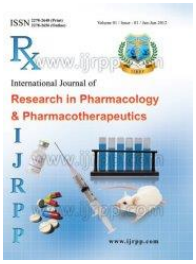

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Protective effect of ethanolic leaf extract of *ipomoea sepiaria* against sodium arsenite induced toxicity in rats

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ABSTRACT

The objective of the study is to find out the protective effect of *Ipomoea sepiaria* on Sodium Arsenite induced toxicity in Wistar rats. The liver and kidney toxicity was induced by Sodium Arsenite (8 mg/kg, p.o.) for 60 days. Ethanolic leaf extract of *Ipomoea sepiaria* (200 & 400mg/kg) was administered orally for 60 days. At the end of the entire treatment, under Pentobarbitone sodium (60mg/kg, i.p), anaesthesia, the blood was collected by retro orbital puncture and serum was separated for biochemical estimations especially for Liver (SGOT, SGPT, ALP, Bilirubin and Protein) and Kidney (Serum urea and Creatinine). The Data's were analyzed by using ANOVA. The result shows that higher dose of *Ipomoea sepiaria* reversed the biochemical parameter of both Liver functional test and the Kidney functional test in a significant manner as compared to Sodium Arsenite control. From the result it was concluded that, ethanolic leaf extract shows protective effect against Sodium Arsenite induced toxicity in rats in a dose dependent manner.

Keywords: Sodium Arsenite, *Ipomoea sepiaria*, Toxicity, Heavy Metal

INTRODUCTION

Heavy metal contamination and accumulation is a serious problem around the world due to the potential threat to food safety and its detrimental effects on human health [1]. It has also become one of the major environmental problems due to continuous industrialization and urbanization. Arsenic toxicity as one of the serious problems worldwide, its specific, reliable and safe treatment still remained mostly unknown. Although several hypotheses have been proposed, the exact mechanism of arsenic toxicity has not yet been clearly defined. Several studies suggest that higher concentrations of arsenic causes oxidative stress, increased generation of reactive oxygen species and nitric oxide, inhibition of enzyme and mitochondrial function, and induction of several

stress genes [2]. In recent years, a number of plant products, their active constituents and herbal agents have been used to protect against arsenic induced toxicity.

Ipomoea sepiaria Koenig ex. Roxb. of the family Convolvulaceae is a perennial climber growing on the bank of streams, rivers, specially over hedges. It is a glabrous or occasionally pubescent or hirsute, slender twinning with a slightly thickened or tuberous perennial root and very short stem producing annually or seasonally a number of terete villous, grayish purple branches bearing simple, cordate or ovate, variable median sized leaves, very often blotches with dull purplish patches in the centre and pink to purplish flowers in clusters on fairy long thickened clavate peduncles [3]. *Ipomoea sepiaria* known as Lakshmana in Sanskrit is a herb known to possess good antidote to arsenic, uterine tonic, aphrodisiac, cures 'tridosa' and ulcers [4], diuretic, aphrodisiac & tonic. It is also used in case of burning sensation, hyperdipsia, general debility & sterility in women [5], laxative [6] and diabetes [7]. The root powder in the dose of 1 teaspoon is administered with rice water for leucorrhoea [8]. Despite the above mentioned beneficial effect of *Ipomoea sepiaria* leaves, its efficacy in reducing metal toxicity in general and arsenic toxicity in particular, has not yet been studied. The present study was conducted to evaluate the protective effect of ethanolic leaf extract of *Ipomoea sepiaria*, against Sodium Arsinate induced toxicity in rats.

MATERIALS AND METHODS

Plant Material

The leaves of *Ipomea sepiaria* was collected from outskirts of Erode, in the month of April. The plant were identified as *Ipomea sepiaria* and authenticated by the botanist, Botanical Survey of India, Agricultural University, Coimbatore. The voucher specimen (BSI/SRC/11/72/2017-18/Sci/01211) had been deposited in the herbarium for future reference.

Preparation of Extract

The collected leaves were washed in running water to remove the adhering foreign matter and shade dried. The dried plant materials were coarsely powdered by mechanical blender. The coarse powder of *Ipomea sepiaria* leaves was soaked in 70% ethanol for 24 h followed by cold maceration for further 48 h with occasional shaking. The mixture was filtered using muslin cloth followed by removal of excess of solvent by rotatory evaporator. The dried extract of *Ipomea sepiaria* was used for the study.

Animals

Wistar albino rats of either sex weighing between 180 – 200 gms of 8 weeks were used in this study. The animals were obtained from animal house, Nandha College of Pharmacy, Erode. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines (688/PO/Re/S/02 /CPCSEA).

Sodium Arsenite Induced Toxicity [9]

Wistar albino rats were divided in to 4 groups of six each. Group I – Normal control received the vehicle 0.1% Carboxy Methyl Cellulose (CMC) solution (1ml/kg). Group 2 to 4 animals were administered orally with Sodium Arsenite at the dose of 8mg/kg once daily for 60 days. Group 3 and 4 animals were treated with *Ipomea sepiaria* ethanolic leaf extract at the dose of 200 mg & 400mg/kg respectively for 60 days. The test drugs were administered orally once daily for 60 days by suspending in 0.1% CMC solution. At the end of the entire treatment, animals were anaesthesized by Pentobarbitone sodium (60mg/kg, i.p), and the blood was collected by retro orbital puncture and serum was separated for biochemical estimations.

Biochemical Studies

The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30^{o}C for 15 min and utilized for the estimation of various biochemical

parameters namely SGOT, SGPT, SALP, serum Bilirubin and total Protein.

SERUM HEPATOSPECIFIC MARKERS

Serum Glutamate Oxaloacetate Transaminase (SGOT) & Serum Glutamate Pyruvate Transaminase (SGPT) [10]

0.05 ml of serum with 0.25 ml of substrate (aspartate and α-ketoglutarate for SGOT; alanine and α - keto glutarate for SGPT, in phosphate buffer at pH 7.4) was incubated for an hour in case of SGOT and 30 min. for SGPT. 0.25 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 505 nm in UV spectrophotometer. Activities were expressed as IU/L.

Alkaline Phosphate (ALP) [11]

Alkaline phosphatase activity was assayed using disodium phenyl phosphate as substrate. The colour developed was read at 510 nm in UV spectrophotometer after 10 min. Activities of ALP was expressed as IU/L.

Serum Bilirubin [12]

Diazotised sulphonilic acid (0.25 ml) reacts with bilirubin in diluted serum (0.1 ml serum + 0.9 ml distilled water) and forms purple colored azobilirubin, which was measured at 540 nm in UV spectrophotometer. Activities of total bilirubin were expressed as mg/dl.

Total Protein [13]

Biuret reagent (1.0 ml) reacts with serum (10 µL) and the colour developed was read at 578 nm in *uv-vis* spectrophotometer. Activities of total protein were expressed as mg/dl.

Kidney Function Tests

Serum urea and Creatinine

Serum urea was determined using diacetylmonoxime (DAM) reagent (Modified Berthelot methodology) and Serum creatinine was determined by alkaline picric acid method using standard diagnostic kits.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's'*t*' – test using graph pad version I. P values <0.05 were considered significant.

RESULT & DISCUSSION

Effect on Liver Function Test

Table 1 represents the protective effect of *Ipomea sepiaria on* Liver function test against the Sodium Arsenite induced toxicity. The hepatic enzymes SGOT, SGPT, and total bilirubin were increased and total protein was decreased in Sodium Arsenite alone exposed animals when compared to control groups. *Ipomea sepiaria* at 200mg/kg significantly reversed the levels of SGOT, SALP ($P<0.05$), and SGPT, Total Bilirubin, Total Protein ($P<0.01$) against the Sodium Arsenite intoxicated animals. *Ipomea sepiaria* at higher dose of 400mg/kg showed more significant reverse in the levels of SGOT, SALP ($P<0.01$), and SGPT, Total Bilirubin, Total Protein ($P<0.001$) against the Sodium Arsenite intoxicated animals.

Effect on Kidney Function Test

Table 2 represents the protective effect of *Ipomea sepiaria* on Kidney function test against the Sodium Arsenite induced toxicity. The serum Urea and Creatinine were increased in the Sodium Arsenite induced toxicity in rats compared to vehicle control. Ethanolic leaf extract of *Ipomea sepiaria* at 200 mg/kg, significantly decrease the serum urea($P<0.01$) and serum Creatinine ($P<0.05$) as compared to sodium Arsenite control. Ethanolic leaf extract of *Ipomea sepiaria* at 400 mg/kg significantly ($P<0.001$) decrease both the serum urea and Creatinine as compared to sodium arsenite control.

RESULT

Table: 1. The table shows the effect ethanolic leaf extract of *Ipomea sepiaria* on liver function against Sodium Arsenite induced toxicity in rats.

Groups	Drug Treatment	Liver Function Test				
		SGOT (IU/L)	SGPT(IU/L)	SALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
I	**Vehicle Control CMC (1ml/kg)**	138.23±4.74	85.45±4.05	245.52± 6.22	0.57±0.04	16.22±0.93
II	**Sodium Arsenite (8 mg /kg)**	305.42 ±5.19	155.70±6.24	386.45 ±6.90	6.66±0.42	32.22±1.42
IV	*Ipomea sepiaria* **(200mg/kg)**	252.72±5.04*	106.35±3.33**	322.00±7.70*	3.63±0.09**	22.65±0.54**
III	*Ipomea sepiaria* **(400mg/kg)**	191.32±6.20**	90.78±4.46***	299.35±5.80**	2.06±0.07***	19.55±0.94***

Values are in mean ± SEM (n=6),
*P<0.05 , **P<0.01, ***P<0.001 Vs Sodium Arsenite Control

Table: 1. The table shows the effect ethanolic leaf extract of *Ipomea sepiaria* on kidney function against Sodium Arsenite induced toxicity in rats.

Groups	Drug Treatment	Kidney Function Test	
		Serum Urea (mg/dl)	Serum Creatinine (mg/dl)
I	**Vehicle Control CMC (1ml/kg)**	36.50±2.88	0.35±0.02
II	**Sodium Arsenite (8 mg /kg)**	61.52 ±3.56	1.72±0.06
IV	*Ipomea sepiaria* **(200mg/kg)**	44.24±2.30**	1.09±0.03*
III	*Ipomea sepiaria* **(400mg/kg)**	40.05±2.22***	0.64±0.02***

Values are in mean ± SEM (n=6),
*P<0.05 , **P<0.01, ***P<0.001 Vs Sodium Arsenite Control

CONCLUSION

Protective effect of ethanolic leaf extract of *Ipomea sepiaria* was studied against Sodium arsenite induced toxicity in rats. From the result it was concluded that *Ipomea sepiaria* protected the organs like liver and kidney damage induced Sodium Arsenite in rats. further studied may be focused on the exact mechanism and the phyto-constituents responsible for its protective activity against the heavy metals.

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