EFFECTS OF GLYCOSMIS PENTAPHYLLE LEAF EXTRACT AGAINST CHRONIC ARSENICOSIS IN RATS


1Assistant Professor, BCDA College of Pharmacy & Technology, Hridaypur, Barasat, India.
2Research Scholar, Dept. of Veterinary Pharmacology & Toxicology, WBUAFS, Kolkata, India.
3Director of Medical Education, West Bengal, Kolkata, India.
4Prof. Dept. of Veterinary Pharmacology & Toxicology, WBUAFS, Kolkata, India.
5HOD of Pharmacology, R. G. Kar Medical College and Hospital, Kolkata, India.
6Ex- Prof. Dept. of Veterinary Parasitology, WBUAFS, Kolkata, India.

ABSTRACT

The present study was conducted to evaluate the effects of methanolic extract of Glycosmis pentaphylla (GP) against sodium arsenite (NaAsO₂) induced toxicosis in adult albino rats. Forty eight albino rats having body weight 150-200 gm of either sex were randomly divided into four groups viz., G₀, G₁, G₂ and G₃ each containing twelve. Sodium arsenite was administered at 4mg/kg b.w. daily in drinking water for 90 days to all rats of groups G₁, G₂ and G₃. The rats of Group G₂ and G₃ were orally treated with leaf extract of GP at 320mg/kg (1/₁₀ th of LD₅₀) and at 160mg/kg(1/₂₀ th of LD₅₀) daily from 91 st day to 120 th day. Only distilled water was given to rats of G₀ and considered as control. Blood samples were collected at different days for analysis of haemogram, biochemical parameters like ALT, AST, BUN and CRT. Tissue samples were collected to study activity of SOD, MDA, GSH, Catalase and histopathology was conducted after completion of experiment. The results reveal that extraction of G. pentaphylla leaves may have ameliorative effect in arsenicosis in rats at dose dependent manner.

KEYWORDS: Arsenic, Glycosmis pentaphylla, Haemogram, Histopathology, Rats.
INTRODUCTION

Arsenic is one of the most toxic metals derived from the natural environment. The major cause of human arsenic toxicity is from contamination of drinking water of natural geological sources rather than from mining, smelting or agricultural sources (Pesticides or fertilizers).[28]

Prolonged ingestion of arsenic contaminated water may result in the manifestations of toxicity in practically all systems of the body. The most serious concern is the potential of arsenic to act as a carcinogen. The two worst affected areas in the world are Bangladesh and West Bengal, India. In 42 districts of Southern Bangladesh and 9 adjacent districts of West Bengal, 79.9 and 42.7 million people respectively are exposed to arsenic contaminated ground water.[6]

Arsenic contamination of ground water in West Bengal comes into focus, during the mid of 1980’s in few villages of North 24 Parganas, South 24 Parganas, Nadia, Murshidabad and Burdwan. By the end of 2006, this problem spreads from few villages to 3235 villages of 79 blocks of 8 districts, Malda, Murshidabad, Nadia, North and South 24 Parganas, Howrah, Hooghly and Burdwan.[21]

Human beings as well as animals like goat, cow etc. are affected highly with arsenic. It has been reported that chevon and cow milk contained high amount of arsenic which also cause human health hazard at the arsenic prone zone.[9] Several phytoremedial studies have been conducted on arsenicosis in laboratory animals.

Medicinal plants having potent antioxidant property can help to reduce oxidative stress and hepatotoxicity caused by metals.[12]

*Glycosmis pentaphylla* (Family : Rutaceae) is a wild, oriental and small tree distributed throughout the roadsides, under large trees or on uncultivated lands in Bangladesh, Srilanka, Eastern part of India, Southern Indochina, Malaysia,, Indonesia and in Eastern Australia.[43]

*Glycosmis pentaphylla* has been found to have antioxidant, galactagogue, immune stimulant, larvicidal activity, antipyretic and hepatoprotective activities.[40] The bark of *Glycosmis pentaphylla* are used for the treatment of diabetes and gonorrhea.[14]

But reports on effects of *Glycosmis pentaphylla* on heavy metal toxicity in general and arsenic toxicity particular are scarcely available. Considering above, the present work was
undertaken to explore its effects on haematobiochemical parameters in induced arsenicosis in rats, if any, which may be useful to persons exposed with chronic arsenicosis.

**MATERIALS AND METHODS**

**Chemicals:** All chemicals and kits used in this present study were obtained from Bangalore Geni (India), Congent (India), Merck (Germany), Rankem (India) and Sigma Chemicals (USA).

**Experimental Animals:** Forty eight white albino rats of either sexes having body weight 150-200 gm were procured from registered animal breeder. They were caged in polypropylene cages and were acclimatized in experimental animal room for seven days before starting the experiment. The animals were maintained with standard pellet feed and provided drinking water *ad libitum*. The Institutional Animal Ethics Committee approved the technical programme and the no. is EC/235/2013/CPCSEA.

**Preparation of Glycosmis pentaphylla leaf extract**

The plant was identified by Dr. Subir Bandyopadhyay, Botanist of BSI (Botanical Survey of India, Howrah, Kolkata) and specimen no. of *Glycosmis pentaphylla* was 1 and the voucher specimen was kept at the Dept. of Pharmacology and Toxicology, West Bengal University of Animal & Fishery Sciences, Kolkata, West Bengal, India.

Fresh plant leaves of *Glycosmis pentaphylla* were collected from local areas. Then the leaves were washed with distilled water, cut into pieces and room shade dried for 7 days. The dried leaves were pulverized into coarse powder in a grinding machine. The powder was extracted by soxhlet apparatus using methanol (99%). Then the collected condensed solution (extraction of leave powder) was kept in room temperature for 2 to 3 days and was dissolved in water (Tripple distilled water) for use.

**Determination of LD$_{50}$ of Glycosmis pentaphylla**

Healthy albino rats (either sex) were used to determine LD$_{50}$ of *Glycosmis pentaphylla* leaf extract as per the method described by Ghosh, 2008$^{[13]}$ and it was found to be 3200 mg/kg. Two dose levels i.e. $\frac{1}{10}^{th}$ and $\frac{1}{20}^{th}$ of LD$_{50}$ of *Glycosmis pentaphylla* were selected (320 and 160 mg/kg) for the present research work.

**Experimental Design:** Forty eight animals were randomly divided into four groups having twelve rats in each viz., G$_0$, G$_1$, G$_2$ and G$_3$. Rats in group G$_0$ were given feed and water *ad*
Each rat in groups G₁, G₂ and G₃ were treated with sodium arsenite (4mg/kg) daily in drinking water for 90 days and methanolic extract of *Glycosmis pentaphylla* was administered at 320 and 160 mg/kg dissolved in distilled water to animals of groups G₂ and G₃ respectively from 91 to 120 days. Animals of group G₁ were considered as experimental control/untreated control group.

**Collection of samples**

Blood samples were collected on day 0, 14, 28, 42, 60, 90 and 120 from animals of each group. Tissue samples were collected on day 0, 90 and 120 after sacrificing four animals in each group.

**Blood**

Pooled blood samples were collected from the tail vein of 4 rats of each group according to procedure of Brown[3] and kept 1ml blood in EDTA treated test tube for haemogram and 2ml into pre-marked centrifuge glass test tubes immediately after collection and was kept at room temperature for 1hr without agitation to clot with a view to collect serum. The harvested sera were kept at -20°C until used for biochemical parameters.

**Tissue**: The rats were sacrificed by maintaining standard protocol using higher dose of ketamine. Pieces of liver, kidney, heart, spleen, lung and intestine from each rat were collected and fixed in 10% buffered formalin for histopathological examination, while some part of liver, kidney and heart were used for antioxidant status.

**Haemogram**: Haemoglobin, total RBC, total WBC, PCV and differential count were determined as per standard method.[7]

**Biochemical parameters**: Serum AST and ALT activity[44], BUN[52] and CRT[50]., were measured.

**Tissue Biochemical (anti oxidant status)**: Reduced glutathione (GSH) [20], superoxide dismutase (SOD) [27], MDA [38], Catalase [1] activity in liver, kidney and heart tissues were determined.

**Statistical analysis**: The results were expressed as mean ± SE. The data were analysed statistically by using Univariate General Linear Model with two ways ANOVA in SPSS10 version of software.
RESULTS AND DISCUSSION

Fig. 1: Effect of arsenic on haemoglobin (gm/dl) level in rat after daily single oral administration of sodium arsenite at 4mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 2: Effect of arsenic on total RBC count (X10^6/µl) in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 3: Effect of arsenic on total WBC count (X10^3/µl) in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.
Fig. 4: Effect of arsenic on PCV(%) level in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 5: Effect of arsenic on total Lymphocyte count (X10³/µl) in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 6: Effect of arsenic on total Neutrophil count (X10³/µl) in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.
Fig.7: Effect of arsenic on total ALT(IU/L) activity in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig.8 : Effect of arsenic on total AST(IU/L) activity in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig.9 : Effect of arsenic on total BUN(mg/dl) level in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.
Fig. 10: Effect of arsenic on total CRT (mg/dl) level in rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 11: Effect of arsenic on total (SOD ng/min/mg protein) in heart of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 12: Effect of arsenic on total MDA (nM of MDA/mg protein) in heart of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.
Fig. 13: Effect of arsenic on total GSH (mg/mg protein) in heart of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 14: Effect of arsenic on total Catalase (µmol/min/mg protein) in heart of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 15: Effect of arsenic on SOD activity of liver (SOD ng/min/mg protein) of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.
Fig. 16: Effect of arsenic on total MDA (nM of MDA/mg protein) in liver of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 17: Effect of arsenic on GSH (mg/mg protein) in liver of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 18: Effect of arsenic on Catalase (µmol/min/mg protein) in liver of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.
Fig. 19: Effect of arsenic on SOD activity of kidney (SOD ng/min/mg protein) of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 20: Effect of arsenic on MDA (nM of MDA/mg protein) in kidney of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

Fig. 21: Effect of arsenic on GSH (mg/mg protein) in kidney of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.
Fig. 22: Effect of arsenic on Catalase (µmol/min/mg protein) in kidney of rat after daily single oral administration of sodium arsenite at 4 mg/kg for consecutive 90 days and ameliorative effect by *Glycosmis pentaphylla* leaf extract after 90 days onwards.

**Histopathological Findings**

Fig. 23: Cross section of heart shows congested blood vessels in the myocardium in arsenicosis induced rats (H&E 10X).

Fig. 24: Cross section of intestine shows excessive mucous secretion in arsenicosis induced rats (H&E 10 X).

Fig. 25: Cross section of lungs shows local pneumonic lesion in arsenicosis induced rats (H&E 10 x).
Fig. 26: Cross section of lungs shows cellular infiltration and proliferation of peribronchial lymph nodes in *G. pentaphylla* treated rats (H & E 10X).

Fig. 27: Cross section of spleen shows degeneration of the lymphocytes in the germinal foci of white pulp in arsenicosis induced rats (H & E 10X).

Fig. 28: Cross section of liver shows massive fatty changes at the periphery and of the lobule in arsenicosis induced rats (H & E 10X).

Fig. 29: Cross section of liver shows coagulative changes around the veins in *G. pentaphylla* (1/10 of LD₅₀) treated rats (H & E 40X).

Fig. 30: Cross section of liver shows focal degenerative changes in the hepatocytes in *G. pentaphylla* (1/20 of LD₅₀) treated rats (H & E 10X).
The level of haemoglobin, total RBC count and PCV did not alter on respective days for group G0 animals but the above values significantly (p<0.05) decreased till 90 days with respect to ‘0’day value in G1, G2 and G3 groups. The values significantly (p<0.05) decreased on day 120 in group G1 but increased in G2 and G3 treated with Glycosmis pentaphylla leaf extract (Fig.1,2 and 4).

It is also evident from figures 3,5,6 that WBC, lymphocyte and neutrophil counts significantly decreased (p<0.05) till 90 day in G1, G2 and G3 compared to ‘0’day but the value significantly (p<0.05) decreased on day 120 in group G1 but increased in G2 and G3 treated with Glycosmis pentaphylla leaf extract.

Arsenic is a toxic element for human and livestock causing serious health hazards. Biochemical and haemogram indices are the reliable parameters for assessment of the health status of humans and animals in arsenic toxicity. A significant increase in WBC count in arsenicosis rats has been observed in the present study which is in agreement with the findings of. [4]

It is observed from Fig.12, 16 and 20 that MDA of heart, liver and kidney did not alter significantly (p<0.05) in group G0 at different days compared to its ‘0’day value. Again those values significantly (p<0.05) increased on day 90 for all G1, G2 and G3 groups but decreased on day 120 for group G2 and G3 animals and increased for group G1 animals.

It is also found from Figs. 11, 15, 19, 14, 18, 22, 13, 17, 21 that SOD activity, catalase and GSH level of heart, liver, kidney, did not alter significantly (p<0.05) in group G0 at different days compared to its day ‘0’ value whilst the activity was decreased significantly (p<0.05) on day 90 and day 120 in arsenic treated animals (group G1) but the activity was increased on
day 120 in rats of groups G2 and G3 administered with leaf extract of *G. pentaphylla* at two dose levels.

Arsenic causes toxicity through its interaction with sulfhydryl groups of proteins and enzymes (to denature the proteins and enzymes within the cells) and also through an increase of ROS in the cells, consequently causing cell damage.\[^4\] It has been reported that arsenic induced hematotoxicity is associated with As induced oxidative stress, imbalance of antioxidant system, increased lipid peroxidation resulting heme dysfunction through influencing heme biosynthesis pathway.\[^{23,29,51,47}\] The present results also showed that exposure to As significantly increase the oxidative stress which is supported with the increase level of lipid peroxidation and decreased level of non enzymic and enzymic antioxidants. But leaf extract of *G. pentaphylla* has significantly improved all these altered parameters in arsenic intoxicated rat.

Increased oxidative stress represents an imbalance between intracellular production of free radicals and the cellular defense mechanisms; notably, MDA is one of the most important markers of oxidative stress.\[^4\] Extensive research demonstrated that arsenic causes oxidative stress in a dose and time-dependent manner\[^{25}\] and increase the levels of MDA, deplete GSH and decrease activities of antioxidant enzymes such as SOD and CAT. The leaf extract of GP was found to produce a significant less lipid peroxides than arsenic-treated rats.

Section of heart of group G1 animals revealed that blood vessels were congested in the myocardium with focal loss of striations of myocardial muscles and no necrotic changes were observed (Fig.23). Section of heart of groups G2 and G3 animals did not reveal any significant changes apart from congestion of myocardial vessels following treatment with *Glycosmis pentaphylla* leaf extract at two dose levels.

Section of intestine of group G1 animals showed an excessive mucous secretion (Fig.24). On the other hand the lymphocytic proliferation of Payer’s patches and increased in Globet cells activity were indicative of local defence mechanism of the intestine following oral dose of *Glycosmis pentaphylla* leaf extract at 320 and 160 mg/kg for 30 days in G2 and G3 animals.

The local pneumonic lesion was evident in the lungs of arsenic treated animals (G1) (Fig.25) whilst pneumonic lesions expressing respiratory trouble with cellular infiltration and proliferation of peribronchial lymphnodes suggest the defence mechanism in animals of group G2 and G3 (Fig.26).
Section of spleen of group G₁ animals showed the follicular proliferation of the lymphocytes of the white pulp extending to the red pulp. Degeneration of the lymphocytes in the germinal foci of white pulp was found (Fig. 27). Section of spleen of group G₂ and G₃ animals showed moderate follicular lymphocytic proliferation infiltrating the red pulp.

It may be observed that *G. pentaphylla* leaf extract acted as scavenger of superoxide and hydroxyl radical. The results also showed that arsenic exposure to rats caused a significant reduction in GSH level and decreased activities of SOD suggesting arsenic-induced oxidative stress. The treatment with *G. pentaphylla* extract was able to restore the activities of SOD catalase and increased GSH level.

Figures 7, 8, 9 and 10 showed the activity of ALT, AST and level of BUN and CRT in different groups of animals. These values are higher till 90 days in arsenic treated animals suggesting some damage of both liver and kidney which is corroborated with the findings of Charles.[⁴] *G. pentaphylla* plays an important role to reduce ALT, AST, BUN and Creatinine level The increase in liver marker enzyme (AST and ALT) is responsible for the hepatotoxicity in arsenicosis which was improved by *G. pentaphylla* administration.

Section of liver of arsenic treated (G₁) group animals showed massive fatty changes at the periphery and of the lobule of the liver (Fig. 28).

Hepatocytes showed coagulative changes around the veins of the liver lobules of group G₂ animals (Fig. 29) and Focal degenerative changes in the hepatocytes were noticed in the liver parenchyma and kuffer cells of group G₃ animals (Fig. 30). Histopathology of liver shows the intensity of damage is reduced in *Glycosmis pentaphylla* treated groups (Fig. 29 and 30).

The section of liver shows a toxic activity in group G₁ animals (Fig. 28) and localized in the peripheral area of the lobule, while a significant residual necrobiotic changes persist in the liver parenchyma even after treatment with GP leaf extract at 320 mg/kg and 160 mg/kg (Fig. 29, 30).

The significant increase in urea and creatinine values in arsenicosis in rats suggest renal impairment which may be corrected by *G. pentaphylla* treatment. It is transpired from histopathological findings that section of kidney of the experimental control group (G₁) was damaged by arsenic (Fig. 31) which was actually excreted out through the kidney resulting partially damaged Bowmen capsule in the cortex as well as loops of Henley’s. The toxic
activity in the cortex of group G2 animals was reduced. Its mild toxic activity was marked in the proximal tubules of Henley during excretion of the toxic substances. After treated with *G. pentaphylla* leaf extract of 160 mg/kg for 30 days, it had been found that the degenerative changes in the kidney was reduced and a repairative activity in the form of focal interstitial nephritis was noticed.

Section of kidney of group G1 animals showed a few atropic Bowmen capsules in the cortex.

Section of kidney of group G2 and G3 animals showed reduce degenerative changes.

Increased level of creatinine was reported by Faires \(^{10}\) in arsenic intoxicated cattle. Patel and Kalia \(^{39}\) also obtained higher level of serum creatinine in arsenic induced rats. Nandi *et al.* \(^{35}\) and Rana *et al.* \(^{42}\) also suggested that arsenic is a potential nephrotoxic agent – Arsenic acts on renal capillaries, tubules and glomerule to cause several renal damage. \(^{24}\)

Arsenic is rapidly and extensively accumulated in liver where it inhibits NAD linked oxidation of pyruvate or α-ketoglutarate. This occurs by complexation of trivalent arsenic with vicinal thiols necessary for oxidation of this substrate.\(^{49}\) Toxic effect of arsenic on hepatic parenchymal cells reflected by elevation of liver enzymes AST and ALT in blood.

*G. pentaphylla* plays an important role to reduce ALT, AST, BUN and Creatinine level.

**CONCLUSION**

*Glycosmis pentaphylla* leaf extract may have some ameliorative effects in chronic arsenicosis in rats.

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