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Antidote Effects of Plants of Himalayan Sub-Origin Against Arsenic Induced Toxicity

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Abstract: Arsenic is present in the environment and human all over the world are exposed to small amounts, mostly through food, water, and air. In the developing countries like Bangladesh and India, the high prevalence of contamination, the isolation and poverty of the rural population and the high cost and complexity of arsenic removal systems have imposed a programmatic and policy challenge on an unprecedented scale. Although in India, Arsenic poisoning in ground water in Gangetic basin especially the districts adjoining the river Ganges right from Eastern Uttar Pradesh, Bihar to West Bengal is the major problem of concern. Due to which, major health related problems are arising in the population.To combat the present problem, a pre-clinical study was done on Charles foster rats and sodium arsenite at the dose of 8 mg Kg^{-1} body weight per day was administered to these rats for 60 days and upon these arsenic pretreated rats, novel plant extracts of *Withania somnifera* and *Pteris longifolia* were administered for 45 days to study the antidote effects of these plant extracts. These plants not only eliminated the toxic effects of arsenic but also reversed the normal physiological activity in the animal. Thus, the present study concludes that these novel plants possesses the best bioremedial impact against arsenic induced toxicity.

Keywords: Arsenic, *Withania somnifera*, *Pteris longifolia*, Antidote, Bioremediation, Pre-clinical study.

1. Introduction

Arsenic in the present times has become one of the most important global environmental toxicant which has caused health hazards to human population through its contamination in ground drinking water with inorganic arsenic [1]. Recently in Bihar, 16 districts of lower Gangetic plains been found to have its ground water contaminated with arsenic. [2].The arsenic contamination was also observed in three districts Ballia, Varanasi and Gazipur of Uttar Pradesh in the upper and middle Ganga plain, India [3]. Approximately, 20 incidents of huge groundwater arsenic contamination have been reported from all over the world [4]. Due to groundwater contamination, a large number of populations in India and Bangladesh are suffering from arsenicosis such as melanosis, leuco-melanosis, keratosis, hyperkeratosis, dorsum, gangrene, non-petting oedema, skin cancer and skin lesions in sole and palm [5-7].

Arsenite in *in –vivo* condition are transported into cells through aquaglycoporins 7 and 9 which also transport water and glycerol.Arsenite binds to cellular sulfhydryl groups of proteins specially the vicinal ones and they interfere with the high energy generation by the hindrance of their enzymatic activity [8-10]. In the tissue, they exert toxic effects through several mechanisms such as reversible combination with sulfhydryl groups. They also inhibit numerous other

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cellular enzymes such as cellular glucose uptake, gluconeogenesis, fatty acid oxidation and product – ion of glutathione through sulfhydryl group binding [11,12]. The arsenite is also responsible for Reactive Oxygen Species (ROS) generation which lead to cell damage and cell death through the activation of oxidative sensitive signaling pathways [13].

Since last two decades, the amelioration of various heavy metal borne diseases has gained special attention to researchers. In the present investigation, amelioration against arsenic induced toxicity has been focused on two important plant extracts *Withania somnifera and Pteris longifolia*. *Withania somnifera* also known as Ashwagandha, winter cherry or Indian Ginseng has been an important medicinal herb in Ayurveda and indigenous medical systems for over 3000 years. Many studies indicate that Ashwagandha possesses antioxidant [14], antitumor [15], anti-inflammatory [16], immunomodulatory [17], antistress [18], adaptogenic [19], antiulcer [20] and rejuvenating properties [21]. *Pteris longifolia* is an fern found in Himalayan sub regions as well as around the Gangetic belt of India. It is widely used in the decoration of flower bouquets. However, no studies have been ever reported the effect of *Withania somnifera* root extract and leaf extract of *Pteris longifolia* as antidote against arsenic induced toxicity in rats.

2. Materials and Methods

2.1. Animal

Charles Foster rats (24 females), weighing 160g to 180g of 8 weeks old, were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Regd-No. 1129/bc/07/CPCSEA). The research work was approved by the IAEC (Institutional Animal Ethics Committee) with IAEC No. IAEC/2012/12/04. Food and water to rats were provided *ad libitum* (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages in small groups (2 each). The rats were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22 ± 2^{0}C with 12 h light/dark cycle.

2.2. Chemical

Sodium Arsenite (98.5%) manufactured by Biosol Laboratories Pvt. Limited, Kolkata, India was obtained from the Scientific store of Patna.

2.3. Preparation of Withania somnifera Ethanolic Extract

In the present study,dry root of *W. somnifera* were purchased from Haridwar Medicinal Store, Haridwar, Uttrakhand, India. The identity of the medicinal plant was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The collected roots of *W.somnifera* were shade dried and grinded to fine powder. The powder was then soaked in 70% ethanol for 48 hours and finally extracted with absolute ethanol using soxhlet apparatus for 6 -8 hours and the residue was concentrated and dried at 37^{0}C. The ethanolic extract dose was calculated after LD_{50} estimation and finally made to $200mgkg^{-1}$ body weight.

2.4. Preparation of Pteris longifolia Ethanolic Extract

In the present study, fresh leaves of *P.longifolia* were collected from the local pond of Patna, Bihar. The identity of the leaves of *P.longifolia* was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The collected leaves of *P.longifolia* were shade dried and grinded to fine powder. The powder was then soaked in 70% ethanol for 48 hours and finally extracted with absolute ethanol using soxhlet apparatus

for 6-8 hours and the residue was concentrated and dried at 37^0C. The dose was finally made to 400 mg kg^{-1} body weight for oral administration.

2.5. Experimental Design

The animals were grouped into 4 groups. Group 1 was Control group (n=6) to which no treatment was given and was designated as healthy control, while to the rest 3 groups (n=18) Sodium arsenite at the dose of 8mgKg^{-1} body weight was administered orally daily for 60 days. Group 2 animals at the end of the Sodium arsenite treatment were dissected for the biochemical assay. Upon these Sodium arsenite pre-treated groups, *Withania somnifera* ethanolic root extract was administered at the dose of 200 mg Kg^{-1} body weight to the Group 3 (n=6) while *P.longifolia* ethanolic leaf extracts was administered to Group 4 (n=6) at the dose of 400 mg Kg^{-1} body weight orally daily for 45 days. At the end of the entire treatment, animals were anaesthesized and dissected and their blood samples were collected and serum was extracted. The serums were then assayed for biochemical study as Liver function tests, Kidney function tests and lipid peroxidation.

2.6. Biochemical Evaluation

The Liver Function Test (LFT) as Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) were measured according to method [22], Alkaline Phosphate (ALP) by method [23] while total bilirubin activity by method [24]. The Kidney Function Test (KFT) were assayed by methods as Urea by [25,26], Uric acid by [27] and Creatinine by [28].

2.7. Lipid Peroxidation (LPO)

Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were determined by the double heating method [29]. The principle of the method was a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose, 2.5 ml of 100 g/l trichloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and incubated for 15 min at 90^0C. After cooling in tapwater, the mixture was centrifuged at 3000 rpm for 10min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube and again incubated for 15 min at 90^0C. The solution was then cooled in tap water and its absorbance was measured through Thermo Scientific UV-10 (UV –Vis) spectrophotometer (USA) at 532nm.

2.8. Statistical Analysis

Results are presented as mean ± SD and total variation present in a set of data was analysed through one way analysis of variance (ANOVA). Difference among mean values has been analysed by applying Dunnett's test. Calculations were performed with the Graph Pad Prism Program (Graph Pad software, Inc., San Diego, U.S.A.). The criterion for statistical significance was set at $P< 0.05$.

3. Results

3.1. Morbidity and Mortality

The rats after the exposure of arsenic (8 mgKg^{-1} body weight per day) for 60 days showed toxicity symptoms such as nausea, nose bleeding, lack of body co-ordination (11 percent of rats were observed with paralysis like symptoms), blackening of tongue and foot and general body weakness.

3.2 Biochemical Changes

The SGPT, SGOT, alkaline phosphatase, total bilirubin, urea, uric acid, creatinine and lipid peroxidation activity showed significant increase ($p < 0.05$) in arsenic treated group in comparison to control rat group. But, these values were significantly lowered ($p<0.05$) in *W. somnifera* and *P.longifolia* treated group. The biochemical assessment thus shows the hepatoprotective and nephroprotective activity of *W. somnifera* and *P.longifolia* (Graph fig.1-8).

4. Discussion

In the present scenario Arsenic in South East sub-continent region of Asia has created major health related problems through contamination in underground drinking water. Among possible target organs of heavy metals like arsenic, the liver, kidney and central nervous system appear to be the most sensitive ones. Having been absorbed through the alimentary tract, most of the metals form durable combination with the protein thionein, forming metallothionein, which plays an important role in the further metabolism of these metals [30,31]. The kidney and liver are considered to be the most susceptible organs for metals, because these organs contain most of the metallothionein binding toxic metals [32-36]. These toxic metals also produce free radicals such as lipid peroxides [37] and they encounter with biomembranes and sub-cellular organelles [38-48].

The abnormal high levels of serum SGPT, SGOT, ALP and total bilirubin in the present study are probably because of the induced liver dysfunction and denotes damage to the hepatic cells. The significant increase in the levels is a direct measure of hepatic injury and they show the status of the liver as there may be cellular leakage and loss of functional integrity of hepatocytes. Furthermore, consequence there was significant increase in the levels of the lipid peroxidation denotes oxidative stress produced by the arsenic leading to high degree of degeneration in the liver cells. But, after administration of *W.somnifera* and *P.longifolia,* it shows significant decrease in the serum LFT levels denotes their hepatoprotective activity. The hepatoprotective effect of *W.somnifera* have been well documented which also proves its antioxidant activity [49,50]. But, no studies till date have reported any bioremedial impact of *P.longifolia* on animals. Lipid peroxidation amelioration by *W.somnifera* and its free radical scavenging activity have been well documented [51-55].

The significant increase in the serum urea, uric acid and creatinine denotes the high degree of degeneration in the nephrocytes. But, after administration of *W.somnifera* and *P.longifolia* there was significant restoration in their levels denotes their nephroprotective effect. Very least documents support the nephroprotective activity of *W.somnifera* [56]. Thus, these two novel plants could play vital role to combat arsenic induced hepatotoxicity and nephrotoxicity by normalizing the functions of these two metabolic organs liver and kidney. Furthermore, they scavenge the oxidants denotes their antioxidant properties.

5. Conclusions

In the present time, the study on search for antidote against arsenic induced toxicity is very least shows lack of awareness in this field. The present study is indeed a novel work, which deciphers for the first time *Withania somnifera* and *Pteris longifolia* as the antidote against arsenic induced toxicity. These novel plants *W.somnifera* and *P.longifolia* show not only hepatoprotective and nephroprotective activity but also antioxidant activity which eliminates the arsenic from the body in *in-vivo* system.

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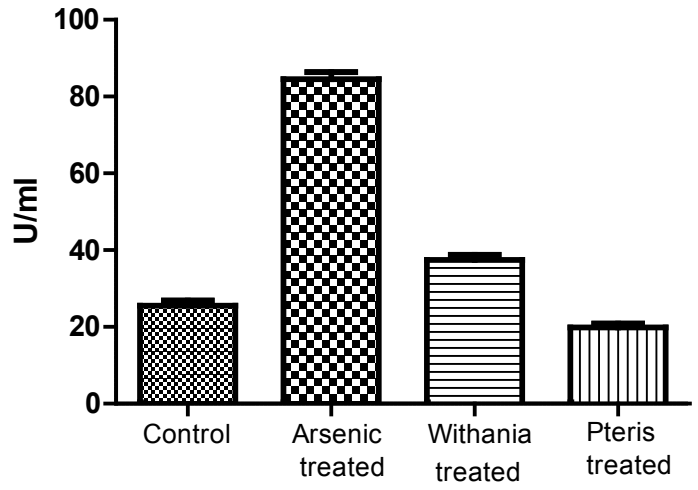

Fig.1: Effect of *W. somnifera* and *P.longifolia* on Arsenic induced toxicity showing SGPT activity (*n*=6, values are mean± S.D)

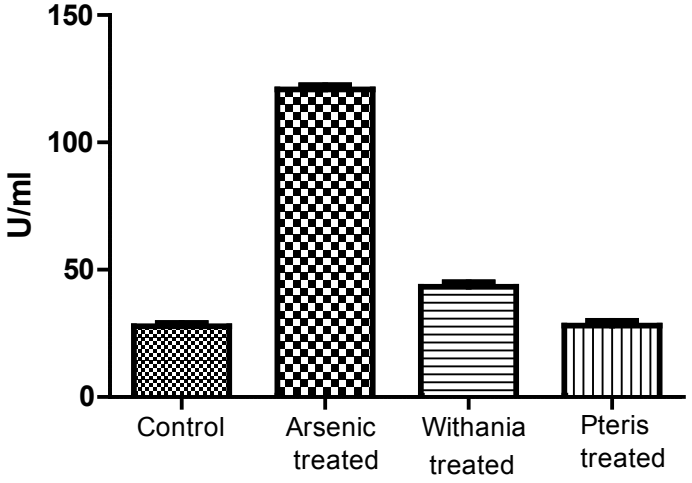

Fig.2: Effect of *W. somnifera* and *P.longifolia* on Arsenic induced toxicity showing SGOT activity (*n*=6, values are mean± S.D)

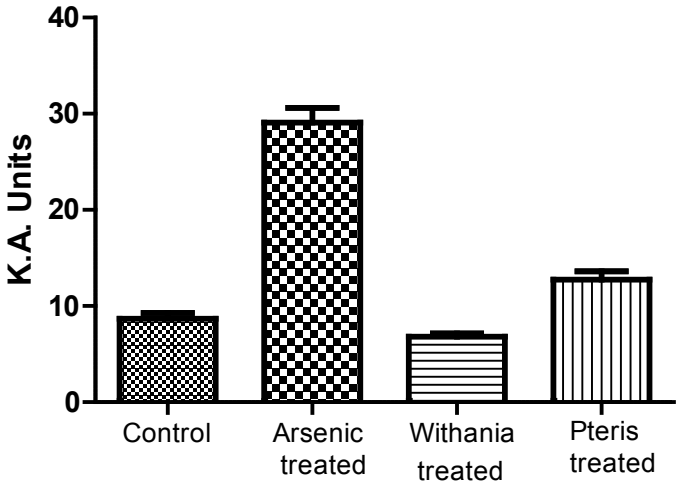

Fig.3: Effect of *W. somnifera* and *P.longifolia* on Arsenic induced showing alkaline phosphatase activity (*n*=6, values are mean± S.D)

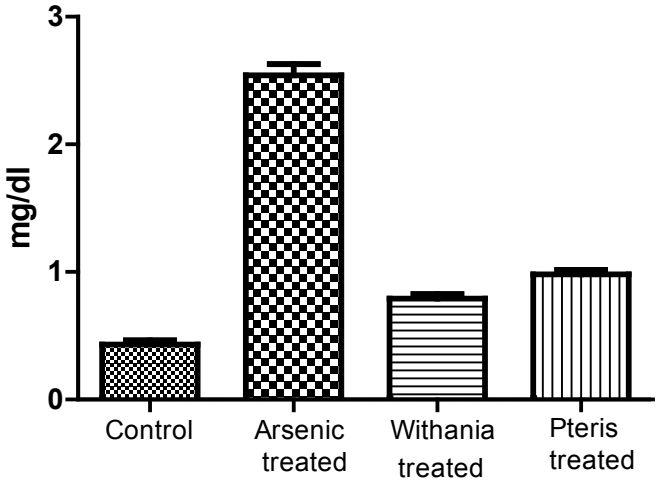

Fig.4: Effect of *W. somnifera* and *P.longifolia* on Arsenic induced toxicity showing total bilirubin activity (*n*=6, values are mean± S.D)

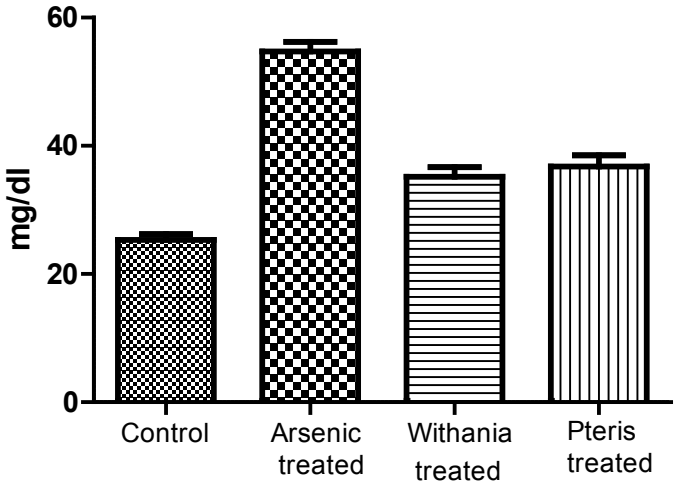

Fig.5: Effect of *W. somnifera* and *P.longifolia* on Arsenic induced toxicity showing urea activity (*n*=6, values are mean± S.D)

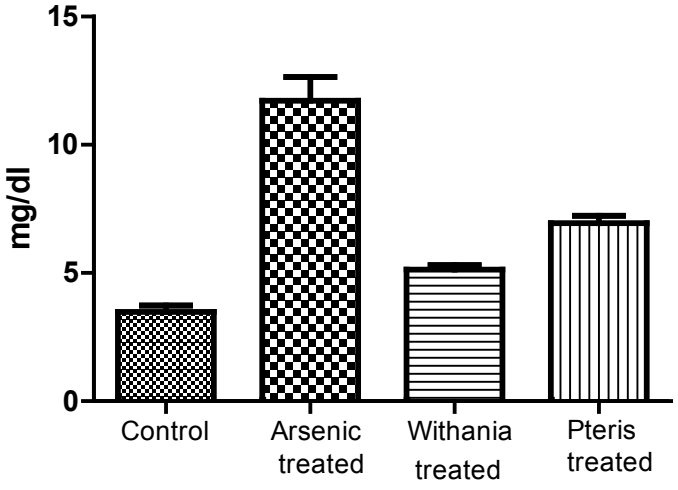

Fig.6: Effect of *W. somnifera* and *P.longifolia* on Arsenic induced toxicity showing Uric Acid activity (*n*=6, values are mean± S.D)

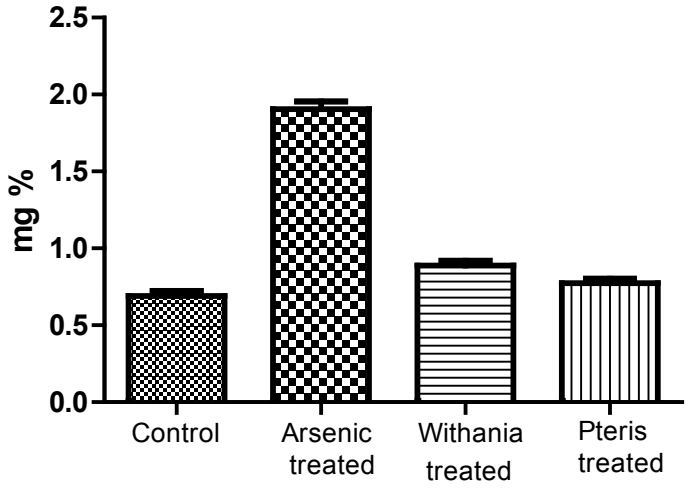

Fig.7: Effect of *W. somnifera* and *P.longifolia* on Arsenic induced toxicity showing Creatinine activity (*n*=6, values are mean± S.D)

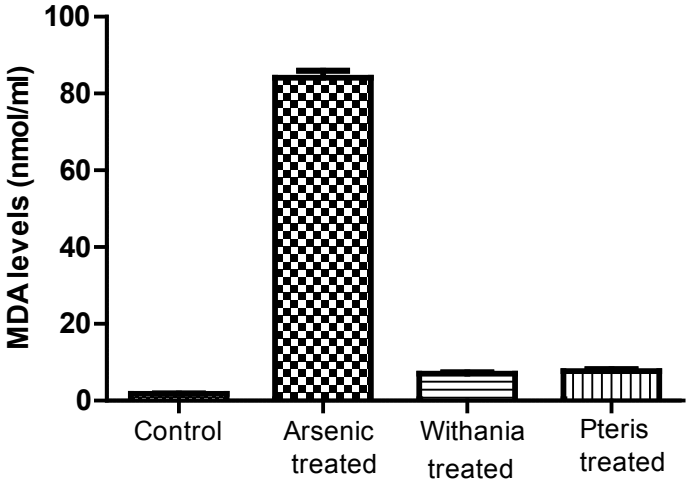

Fig.8: Effect of *W. somnifera* and *P.longifolia* on Arsenic induced toxicity showing lipid peroxidation activity (*n*=6, values are mean± S.D).