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### *Pueraria tuberosa* potentially attenuates Arsenic induced oxidative stress mediated cardiotoxicity, blood toxicity and dyslipidemia in rats

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#### ORIGINAL RESEARCH ARTICLE

#### ABSTRACT

**Background:** The present study was carried out to evaluate protective effect of hydroalcoholic extract of *Pueraria tuberosa* (tuber) in arsenic induced cardiotoxicity in Wistar albino rats.

**Material and Methods:** Dose selection of *Pueraria tuberosa* was made on the basis of acute oral toxicity study (5, 50, 300, 1000 mg/kg body weight) as per OECD guidelines. Cardiotoxicity was produced in adult wistar rats randomly divided into six animals in six groups for 25 days. Group I rats were administered with drinking water for 30 days. Positive Control (Group II) rats were treated with sodium arsenate (1mg/kg). Group III rats were treated with sodium arsenate (1mg/kg) and Vitamin E (100 mg/kg). Group IV, V, VI rats were treated with sodium arsenate (1mg/kg) and hydroalcoholic extract of *Pueraria tuberosa* (50mg/kg, 100mg/kg, 200mg/kg). After 30 days of the treatment, blood samples were collected and analyzed for the serum parameters viz. HDL (High density lipoprotein), Total cholesterol, LDL (Low density lipoprotein), Troponin, Triglycerides and LDH. Antioxidant parameters like Malondialdehyde, catalase, Reduced glutathione and glutathione reductase were estimated. Blood parameters like Haemoglobin, Mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC), RBC, WBC, Erythrocyte sedimentation rate, DNA fragmentation, body weight, organ weight were also estimated. The heart is removed and sectioned for histopathological examination.

**Results:** The hydroalcoholic extract of *Pueraria tuberosa* (tuber) inhibits the oxidative stress hypothesis mechanism and influence of calcium homeostasis.

**Conclusion:** It was concluded that the extract of *Pueraria tuberosa* (tuber) acts on intracellular calcium ions are increased in the myocardial cells to regulate myocardial hypertrophy.

**Keywords:** Arsenic trioxide, Cardiotoxicity, blood toxicity, *Pueraria tuberosa*, DNA fragmentation.

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#### INTRODUCTION

Cardiotoxicity is defined by the National Cancer Institute as the toxicity that affects the heart. This definition includes a direct effect of the drug on the heart but also an indirect effect due to enhancement of haemodynamic flow alterations or due to thrombotic events. Cardiotoxicity is the occurrence of heart electrophysiology dysfunction or muscle damage (Akhlaghia, 2010) reported as a result of cardiotoxicity, heart is not being able to pump blood throughout body. The heart becomes weaker and is

not as efficient in pumping and circulating blood. This is due to caused by adverse effects of heavy metals intake (arsenic, mercury, lead, aluminium). Cardiotoxicity resulting from exposure to environmental toxicants and pollutants is known for a long time. For every new treatment, it will be essential to thoroughly assess toxic effects on the heart. The application of cutting-edge molecular biology approaches has provided significant and novel insights into cardiac toxicity and its mechanisms.

Mechanistic studies on cardiovascular effects of environmental toxicants and pollutants have emerged at a very fast pace. The changes myocardium to environmental toxicants involves alterations in biochemical reactions. These include alterations in ionic homeostasis such as changes in intracellular calcium concentrations, which occur in exposures to environmental toxicants. Energy metabolism is another response to environmental toxicants in the heart, resulting in decreased production and enhanced consumption of adenosine triphosphate (ATP). Alterations in enzymatic reactions are seen in cardiac toxic responses. Barbier et al. (2010) stated the signaling pathways leading to myocardial toxic responses are the focus of cardiotoxicologic research. It is activation of genes and signaling pathways is a critical response of myocardial cells to environmental toxic.

The signaling pathways determine the myocardial responses to environmental toxicants and pollutants. Physiologic alterations occur both as early responses to environmental toxicants and as subsequent events in the late development of cardiomyopathy. The myocardial dysfunction that occurs in the early responses to toxicants is cardiac arrhythmia, which results from the changes in intracellular calcium concentrations and other biochemical alterations, leading to miscommunication between cells and misconduction of electricity. These changes, if not accompanied by cardiomyopathy, do not involve myocardial cell death and are reversible. The late phase of cardiac dysfunction, results from cardiomyopathy. (Mahaboob and Sujitha (2011) reported Changes in myocardial morphology take place when extensive toxic insults are imposed on the heart and toxic exposures persist on a long-term basis. Cardiac hypertrophy is observed as a consequence of long-term toxic insults. The hypertrophy is considered a protective and adaptive response. Hypertrophy leads to severe and irreversible cardiomyopathy, resulting in heart failure. From cardiac hypertrophy to heart failure, Varol et al. (2010) stated activation of mechanisms including the sympathetic nerve system and the renin-angiotensin system takes place. The response in turn activates counter regulatory mechanisms such as upregulation of atrial natriuretic peptide (ANP) expression. These responses lead to extensive biochemical, physiologic, and molecular changes, myocardial remodeling and cell death. These changes result in heart failure. Most cardiotoxicologic studies are fundamental and characteristic changes during this stage of myocardial responses to toxic insults. The targets and determining units of cardiotoxicity are cardiac cells.

*Pueraria tuberosa*, commonly known as kudzu or Indian kudzu, is a climber with woody tuberculated stem. It is a coarse, high-climbing, coiling, twining, trailing and perennial vine with large tuberous roots. The tubers are globose or pot-like, about 25 cm across

and the insides are white, starchy and mildly sweet. Woody stems grow up to 12 cm in diameter. Leaves are trifoliate and alternate, while the leaflets are egg-shaped, with round base and unequal sides. They are 18 cm long and 16 cm wide and are hairless above. Flowers are bisexual, around 1.5 cm across and blue or purplish-blue in color. The fruit pods are linear, about 2 to 5 cm long and constricted densely between the seeds. They have silky, bristly reddish-brown hair. Seeds vary from 3 to 6 in number. It is native to India, Pakistan and Nepal. The huge root can grow to the size of a human body, is the source of medicinal preparations used in traditional Chinese medicine and modern herbal products.

Sodium arsenate is the inorganic compound with the formula  $\text{NaH}_2\text{AsO}_4 \cdot \text{H}_2\text{O}$ . This particular salt is a colourless solid that is highly toxic. Sodium Arsenite exists either as an amorphous, colourless powder or as a glassy mass. Sodium Arsenite can be inhaled or absorbed through the skin. Along with its known carcinogenic and teratogenic effects, contact with the substance can yield symptoms such as skin irritation, burns, itching, thickened skin, rash, loss of pigment, poor appetite, a metallic or garlic taste, stomach pain, nausea, vomiting, diarrhea, convulsions, decreased blood pressure and headache. (Stawiarska-Pięta et al., 2012) stated severe acute arsenic poisoning leads to nervous system damage resulting in weakness, poor coordination or "pins and needles" sensations, eventual paralysis and death. Arsenic is a chemical element with symbol As. Arsenic and its compounds, especially the trioxide, are used in the production of pesticides, treated wood products, herbicides and insecticides. Stawiarska-Pięta et al., (2012) reported as arsenic is notoriously poisonous to multicellular life, although a few species of bacteria are able to use arsenic compounds as respiratory metabolites. Arsenic contamination of groundwater is a problem that affects millions of people across the world. This study was conducted to investigate some of the toxic effects of arsenic on the rat heart and the role of *Pueraria tuberosa* extract administration on these toxic changes.

## MATERIALS AND METHODS

### Chemicals

Sodium arsenate, trichloroacetic acid, formalin, sodium dihydrogen orthophosphate dehydrate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ), disodium hydrogen orthophosphate ( $\text{Na}_2\text{HPO}_4$ ) was purchased from Sigma- Aldrich Chemical Company, (St. Louis, MO), USA.

### Experimental animals

Thirty six Wistar albino rats weighing between 200-250 g of either gender were used in this study. The animals were allowed access to pellet diet and water *ad libitum* for two weeks prior to the experiments, for their acclimatization. All of the animals were kept in ventilated cages at 28-30°C, and 12/12 h light/dark

cycles. All procedures were conducted as per guidelines of the committee for the purpose of control and supervision of experimental animals. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2013-14/MPCOL/18).

### Experimental Design

These animals were randomly divided into six groups of 6 animals each. Vitamin E was dissolved in oil and was also administered to control group. The total water consumption in each animal of all the groups was monitored. Approximately the water consumption in all the groups was observed to be the same.

**Group I:** Normal control animals were given standard diet and tap water for 30 days.

**Group II:** Sodium arsenate (1 mg/kg, p.o.) for 30 days.

**Group III:** Vitamin E 100mg/kg orally + Sodium arsenate (1 mg/kg) for 30 days by oral route.

**Group IV:** HEPT 50mg/kg orally + Sodium arsenate (1 mg/kg) for 30 days by oral route.

**Group V:** HEPT 100mg/kg orally + Sodium arsenate (1 mg/kg) for 30 days by oral route.

**Group VI:** HEPT 200mg/kg orally + Sodium arsenate (1 mg/kg) for 30 days by oral route.

After 24 hrs of the last treatment, rats were anesthetized; blood was collected and centrifuged for the estimation of serum parameters like LDH, total cholesterol, LDL, HDL and triglycerides. Some amount of blood was collected in heparinized tubes for the estimation of hematological parameters like hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), RBC, WBC, erythrocyte sedimentation rate (ESR). The whole heart tissues were isolated, weighed and then used for the estimation of antioxidant parameters like malondialdehyde (MDA) catalase (CAT), glutathione reductase (GR), reduced glutathione (GSH) and for further processing of DNA fragmentation. The hearts were stored in 10% buffer formalin and later used for histopathological studies. Body weight and heart weight were calculated.

### Tissue Homogenate

The heart of each of the sacrificed rats was removed, weighed and individually homogenized in ice cold phosphate buffer solution (0.1 M, pH 7.4) to give a 10 % (w/v) heart homogenate, which was centrifuged at 5000 rpm for 20 min. The supernatant was used for the estimation of anti-oxidant parameters like MDA, catalase, GSH and GR.

### Malondialdehyde

MDA level were assessed according to the method of Niehaus and Samuelsson (1968). The level of MDA in the supernatant was determined

spectrophotometrically by measuring thiobarbituric acid- reactive substances with a maximum absorbance at 532 nm. Briefly, 0.5mL of the sample was mixed with 3mL of 1% phosphoric acid and 1mL of 0.6% TBA solution. The mixture was heated in a boiling water bath for 45 min and cooled to the room temperature. Then, 4mL of n-butanol was added, and mixture was vortexed and centrifuged at 3000×g for 10 min. The absorbance of butanol phase (supernatant) was measured at 532 nm. Tissue MDA content was expressed as nmol/mg protein.

### Catalase

Cardiac catalase activity was measured by the method of Aebi, 1984. A 5% tissue homogenate was prepared with 0.1M phosphate buffer (pH 7.4) with 1% triton X-100. The catalase activity was measured by calculating the rate of degradation of H<sub>2</sub>O<sub>2</sub>, the substrate of the enzyme. Changes in absorbance were recorded at 240nm in kinetic U.V - Visible spectrophotometer and the activity was calculated in terms of K/min.

### Reduced glutathione (GSH)

The GSH content in heart was estimated by the method of Ellman, 1959. 1.0 ml sample of homogenate was precipitated with 1.0 ml of (4%) sulfosalicylic acid. The samples were kept at

4°C for 1 h and then centrifuged at 1200 × g for 20 min at 4°C. The total volume of 3.0 ml assay mixture contained: 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1 mol; pH 7.4) and 0.2 ml DTNB (100 mmol). The yellow color developed was read immediately at 412 nm on a SmartSpec™ plus Spectrophotometer. It was expressed as μmol GSH/g tissue.

### Glutathione reductase (GR)

The 10,000 g supernatant of 5% tissue homogenate was used for estimation of GR activity by the method of Carlberg and Mannervik, 1985. The assay system consisted of phosphate buffer (0.2M, pH 7.0, containing 2mM EDTA), 20 mM GSSG, 2 mM NADPH and supernatant. The enzyme activity was quantitated at 25°C by measuring the disappearance of NADPH at 340 nm. The enzyme activity was expressed as nmole of NADPH oxidized per minute per mg of protein.

### DNA fragmentation assay

DNA fragmentation assay was conducted using the procedure of Wu et al. 2005, with some modifications. The tissue (50 mg) was homogenized in 10 volumes of a TE solution pH 8.0 (5 mmol Tris-HCl, 20 mmol EDTA) and 0.2% triton X-100. 1.0 ml aliquot of each sample was centrifuged at 27,000 × g for 20 min to separate the intact chromatin (pellet, B) from the fragmented DNA (supernatant, T). The pellet and supernatant fractions were assayed for DNA content using a freshly prepared DPA (Diphenylamine) solution for reaction. Optical density was read at 620 nm with (SmartSpec™ Plus Spectrophotometer catalog # 170-2525)

spectrophotometer. The results were expressed as amount of % fragmented DNA by the following formula;

$$\% \text{ Fragmented DNA} = T \times 100 / (T + B)$$

### Histopathological Examination

The heart of rats of all groups was removed immediately and fixed in 10% formalin. The tissue was processed and sections were cut. The slides were prepared and stained with haematoxylin and eosin and examined under high power microscope (100 X) and photomicrographs were taken.

### Statistical analysis

The values were expressed as the mean  $\pm$  SEM for the 06 rats in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) software package for Windows (version 13.0). Post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test. A value corresponding to  $P < 0.05$  was deemed to be statistically significant.

## RESULTS

### Effect of hydroalcoholic extract of *Pueraria tuberosa* on serum parameters in arsenic induced cardiotoxicity study (Table 1)

Data presented in Table 1 show the effect of sodium arsenate, *Pueraria tuberosa* extract, Vit. E and their combination on the serum heart function indices. Exposure to sodium arsenate resulted in toxicity of heart indicated by significant increase in the activity of Triglycerides, Total cholesterol, LDL, LDH and decrease in HDL levels compared to the control group. Administration of Vit. E, HEPT 50mg/kg, HEPT 100mg/kg and HEPT 200mg/kg protected the heart by decreasing the level of Triglycerides, Total cholesterol, LDL, LDH and increase in HDL levels compared to the sodium arsenate group.

### Effect of sodium arsenate and HEPT on % body weight and heart weight

It is depicted from figure 1 and 2 that the % body weight and organ weight is gradually decreased in sodium arsenate treated group compared to control group, while in Vit E, HEPT 50mg/kg, HEPT 100mg/kg and HEPT 200mg/kg treated groups, there was

significant increase in the body weight and heart weight as compared to sodium arsenate induced group.

### Effect of sodium arsenate and HEPT on haematological parameters

Haematological parameters of normal control group and other different experimental groups are presented in Table 2. In sodium arsenate induced group Hb, RBC, MCH, MCV, MCHC levels are significantly decreased and ESR, WBC are increased when compared to control group. Administration of Vit. E, HEPT 50 mg/kg, HEPT100 mg/kg and HEPT 200 mg/kg protected the heart against sodium arsenate toxicity by significant increase in Hb, RBC, MCH, MCV, MCHC levels and decrease in ESR, WBC compared to sodium arsenate induced group.

### Effect of sodium arsenate and HEPT on DNA fragmentation of the heart

Data presented in figure 3 show the effect of sodium arsenate and HEPT on DNA fragmentation. This result demonstrated that sodium arsenate increases the DNA fragmentation when compared to normal control group. Administration of VitE, HEPT50mg/kg, HEPT100mg/kg and HEPT200mg/kg completely ameliorated fragmentation when compared to sodium arsenate treated group.

### Effect of sodium arsenate and HEPT on antioxidant parameters

The lipid peroxidation product was found to be significantly increased while Catalase, GSH and GR levels are decreased when compared to control group. In Vit. E, HEPT50mg/kg, HEPT100mg/kg and HEPT 200 mg/kg treated rats MDA level is decreased while Catalase, GSH and GR are increased when compared to sodium arsenate group (Table 3).

### Effect of HEPT on histological changes in heart

The histological changes were graded and summarized in Figure 4. The sections of control group showed normal histology including normal myocardium. Marked histological changes were observed in sodium arsenate treated rats. The cross section showed myocardial fibrosis and inflammation. Treatment with HEPT and Vit E in sodium arsenate intoxicated rats markedly recovered the toxic changes near to the control rat (Figure 4).

**Table 1: Effect of HEPT on serum parameters in sodium arsenate induced cardiotoxicity in rats.**

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	LDH (U/L)
Normal Control	66.13 $\pm$ 3.0	46.91 $\pm$ 3.0	38.48 $\pm$ 0.87	18.27 $\pm$ 2.8	3200.2 $\pm$ 2.01
Sodium arsenate (1 mg/kg)	98.26 $\pm$ 0.3**	75.54 $\pm$ 0.3**	19.91 $\pm$ 0.3**	53.47 $\pm$ 0.2**	5630.0 $\pm$ 13.5**
Sodium arsenate (1 mg/kg) + Vitamin E (100 mg/kg)	38.7 $\pm$ 0.5***	43.79 $\pm$ 0.6***	34.06 $\pm$ 0.4***	17.4 $\pm$ 0.3***	3031.6 $\pm$ 32.1***
Sodium arsenate (1 mg/kg) + <i>Pueraria tuberosa</i> (50 mg/kg)	39.61 $\pm$ 0.2***	47.28 $\pm$ 0.3***	32.6 $\pm$ 0.5***	24.6 $\pm$ 0.06***	3490.0 $\pm$ 1.84***

Sodium arsenate (1mg/kg) + <i>Pueraria tuberosa</i> (100 mg/kg)	53.15±0.4***	39.0±0.3***	36.6±0.5***	19.8±0.54***	2943.7±49.0***
Sodium arsenate (1 mg/kg) + <i>Pueraria tuberosa</i> (200 mg/kg)	59.18±0.4***	49.0±0.3***	39.6±0.5***	21.8±0.54***	2949.6±49.0***

Values are expressed as mean ± SEM n=6, \*\* p<0.01; when compared to normal control group and \*\*\*p<0.001 compared to sodium arsenate treated group. The intergroup variation between various groups was conducted by Prism 6.0 software using one way ANOVA followed by Dunnett’s t-test.

**Table 2: Effect of HEPT on hematological parameters in sodium arsenate induced cardiotoxicity in rats.**

Groups	HB (g/dL)	DNA fragmentation test (U/ml)	RBC (millions cells/Cm m)	WBC (L)	ESR (mm/hr)	MCV (cubic/micron)	MCH (pg)	MCHC (%)
Normal Control	18.32±0.18	15.6	9.82±0.04	68.50 ± 28.87	10.39 ± 0.17	92.00 ± 0.40	30.00±0.40	32.00±0.40
Sodium arsenate (1mg/kg)	5.30±0.2*	16.7*	3.56±0.08*	162.50 ± 28.8*	18.02 ± 0.27*	68.76 ± 0.13*	18.43 ± 0.23*	23.57 ± 0.04*
Sodium arsenate (1mg/kg) + Vitamin E (100 mg/kg)	16.67±0.17**	17.90**	7.46±0.12**	66.33 ± 33.33**	9.00 ± 0.58**	87.33 ± 0.23**	23.80±0.05**	29.40 ± 0.05**
Sodium arsenate (1mg/kg) + <i>Pueraria tuberosa</i> (50 mg/kg)	14.23±0.28*	18.2*	5.53±0.14*	76.66 ± 33.33*	7.33 ± 0.33*	76.56 ± 0.14*	20.76±0.08*	27.23 ± 0.20*
Sodium arsenate (1 mg/kg) + <i>Pueraria tuberosa</i> (100 mg/kg)	17.33±0.23*	18.9*	8.46 ± 0.08*	70.66 ± 33.33*	10.00 ± 0.58*	79.33 ± 0.12*	26.60 ± 0.20*	31.53±0.21*
Sodium arsenate (1mg/kg) + <i>Pueraria tuberosa</i> (200 mg/kg)	19.33±0.28*	19.6*	9.47±0.08*	72.66± 32.38*	13.00 ± 0.59*	79.58± 0.16*	29.08± 0.23*	33.43 ± 0.26*

Values are expressed as mean ±SEM n=6, \*\*p<0.01; when compared to control group, \*p<0.001 compared to sodium arsenate treated group. The intergroup variation between various groups was conducted by Prism 6.0 software using one way ANOVA followed by Dunnett’s t-test.

**Table 3: Effect of HEPT on antioxidant parameters in sodium arsenate induced cardiotoxicity in rats**

Groups	MDA tissue (nm/g)	Catalase (K/min)	GSH (µg/ml)	GR (units/ml)
Normal Control	99.75 ±0.85	7.014 ±0.02	54.39±0.67	34.67±0.56
Sodium arsenate(1mg/kg)	145.00 ±0.58***	2.020±0.01***	23.62±0.34***	12.36 ±0.12***
Sodium arsenate(1mg/kg)+VitaminE(100mg/kg)	101.00 ±0.58**	4.66±0.33**	46.65±0.55**	28.56±0.45**
Sodium arsenate (1mg/kg)+ <i>Pueraria tuberosa</i> (50 mg/kg)	110.33±0.88#	2.90±0.15#	38.93±0.49#	21.34±0.34#
Sodium arsenate (1mg/kg)+ <i>Pueraria tuberosa</i> (100 mg/kg)	135.33 ±0.88#	3.33 ±0.23#	33.64 ±0.042#	16.58±0.25#
Sodium arsenate (1mg/kg)+ <i>Pueraria tuberosa</i> (200 mg/kg)	137.35±0.88#	3.55 ±0.26#	35.24 ±0.032#	17.38±0.24#

Values are expressed as mean±SEM, n=6, \*\*\* p<0.001 when compared to control group, \*\*p<0.01 when compared to sodium arsenate treated group, #p<0.05 when compared to sodium arsenate treated group. The intergroup variation between various groups was conducted by Prism 6.0 software using one way ANOVA followed by Dunnett’s t-test.

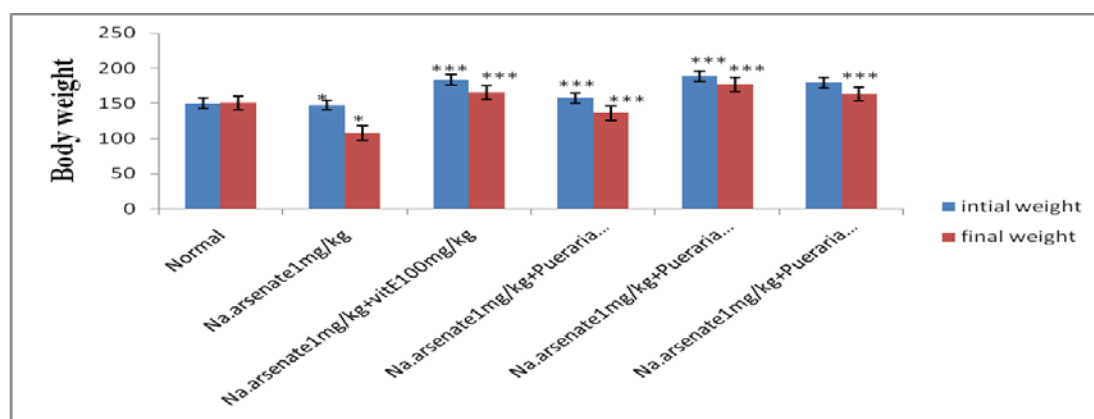


Figure 1: % Difference in Body weight in control, Na arsenate, Vit E, HEPT50mg/kg, HEPT 100mg/kg and HEPT 200mg/kg administered rats. Data are expressed as mean  $\pm$  SEM n=6. \*p<0.1 when compared to control group and \*\*\*p<0.001 when compared to sodium arsenate group. The intergroup variation between various groups was conducted by Prism 6.0 software using one way ANOVA followed by Dunnett's t-test.

Figure 2: Effect of *Pueraria tuberosa* on organ weight in sodium arsenate induced cardiotoxicity in rats

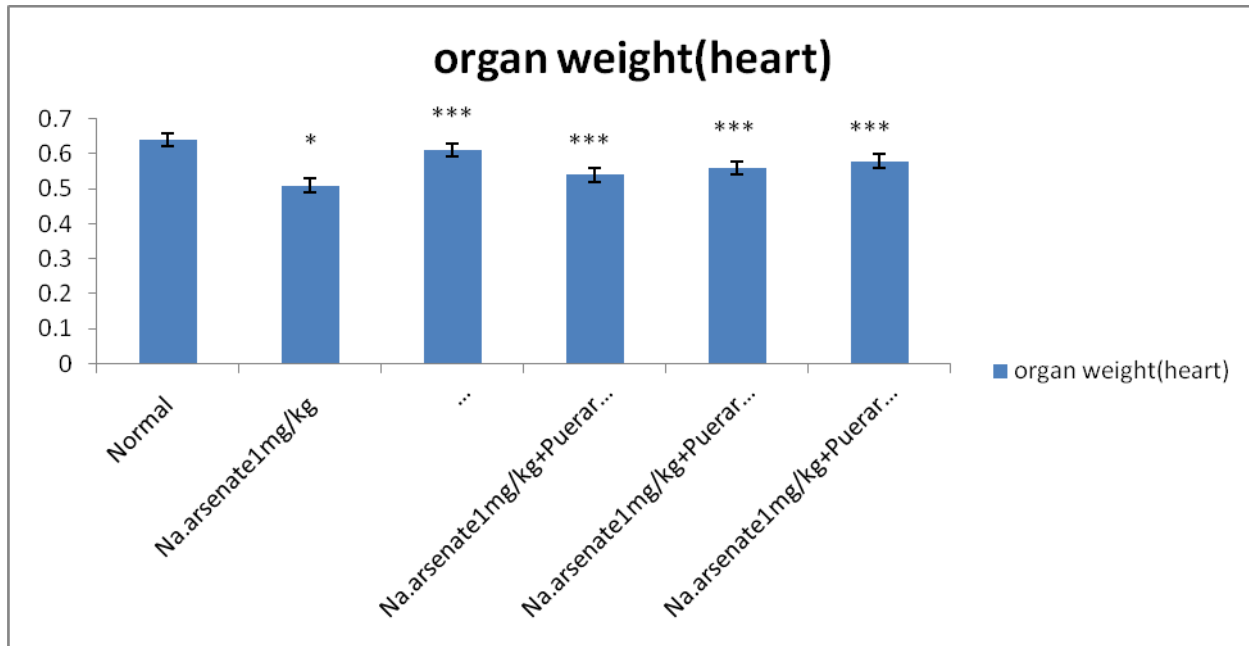
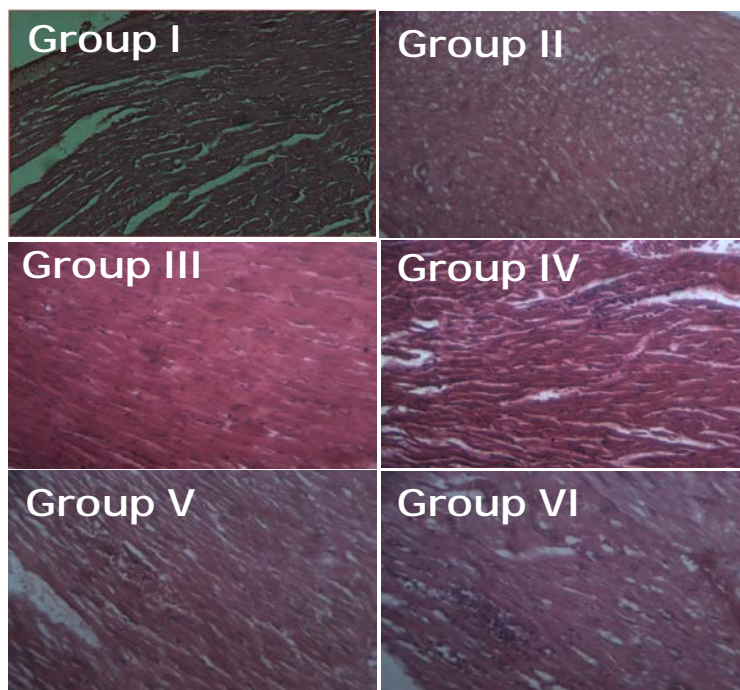


Figure 2: Heart weight in control, Na arsenate, Vit E, HEPT50mg/kg, HEPT100mg/kg and HEPT200mg/kg and Rutin70mg/kg administered rats. Data are expressed as mean  $\pm$  SEM n=6. \*p<0.1 when compared to control group and \*\*\*p<0.001 when compared to sodium fluoride group. The intergroup variation between various groups was conducted by Prism 6.0 software using one way ANOVA followed by Dunnett's t-test.



Group I: Control group myocardium appeared, Group II: In Sodium arsenate group arrow indicates the Vacuolar degeneration in cardiac muscle fiber normal. No necrosis and inflammation observed Tissue is stained with Haematoxylin and Eosin at in the myocardium. Tissue is stained with magnification (100X). Haematoxylin and Eosin at magnification (100X). Group III: In Arsenic + Vitamin E group arrowGroup IV : In Arsenic + *Pueraria tuberosa* (50mg/kg) indicates the degeneration in cardiac muscle fiber group arrow indicates the Focal myocarditis and Tissue is stained with Haematoxylin and Eosin at infiltration of mononuclear cell. Tissue is stained with magnification (100X). Haematoxylin and Eosin at magnification (100X). Group V: In Arsenic + *Pueraria tuberosa* (100 mg/kg) Group VI: In Arsenic + *Pueraria tuberosa* (200 mg/kg) group arrow indicates the Myocardial hemorrhages group arrow indicates the Focal myocarditis and Tissue is stained with Haematoxylin and Eosin infiltration of mononuclear cell. Tissue is stained with at magnification (100X). Haematoxylin and Eosin at magnification (100X).

## DISCUSSION

Through the twentieth century, sodium arsenate attracted the interest of toxicologists due to its deleterious effects at high concentrations in human populations and experimental models (Barbieret al., 2010). The main objective of this study was to

investigate some of the cardiotoxic effects of sodium arsenate and the possible protective role of *Pueraria tuberosa* extract against it in adult albino rats. In the current study, the serum levels of triglycerides, LDH, total cholesterol, LDL and HDL were significantly elevated in the sodium arsenate treated group as

compared to the control groups. They stated that this may be attributed to a generalized increase in membrane permeability and is particularly useful in the diagnosis of muscular dystrophy. Akhlaghi & Bandy, 2010 reported that the release of LDH to the medium is a commonly used indicator of myocardial necrosis in the isolated heart model and has been used clinically as an indicator of myocardial infarction. Guan et al., 2012 stated that cardiomyocyte damage can be confirmed by assessing LDH, triglycerides, total cholesterol, LDL which are the cardiac-specific markers that reflect the degree of myocardial damage. Administration of *Pueraria tuberosa* extract ameliorated the myocardial damage.

Blood parameters like WBC and ESR are increased and Hb, RBC, MCH, MCHC and MCV levels are reduced in sodium arsenate treated group compared to normal control group. In the present study myocardium from sodium arsenate treated rats showed that myocytes were separated from each other by wide intracellular spaces. Some of myocardial fibers attained vacuolated sarcoplasm with localized area of hemorrhage and congested blood capillaries with extravasated red blood cells. These finding observed also by Mahaboob and Sujitha, 2011 who reported that sodium arsenate causes histological changes in the heart like oedema, plasmic vacuolization small, hemorrhage and fibrous necrosis. Ciceket al., 2005 found significant histopathological changes in the myocardial tissue of rats treated sodium arsenate. These were myocardial cell necrosis, extensive cytoplasmic vacuole formation, inflammation and clumped myocardial fibers, fibrillolysis, interstitial oedema, small hemorrhagic areas and hyperaemic vessels. The result of the current study are in agreement also with Stawiarska-Pięta et al., 2012 who reported that long term exposure to sodium arsenate compounds induces morphological changes in many organs, leading to an impairment of their function. They reported the occurrence of pathological changes in the heart. Varolet al., 2010 assumed that myocardial cell damage and cell death could be induced by the generation of reactive oxygen species in arsenate. When reactive oxygen system is out of balance in chronic stage, the free radicals will increase relatively, leading to degradation of cytomembranes, which cause descending fluidity and elevated permeability of the cell membrane, and even collapse of membrane structure. Many investigations have previously demonstrated that arsenate is a cytotoxic agent inducing apoptosis, and disrupting cell cycle progression in many types of cells. It is well known that collagen is a target of excessive sodium arsenate exposure (Yan et al., 2011). In most cases, sodium arsenate inhibits enzymatic activity, but in other cases, ions actually stimulate enzymatic activity. Sodium arsenate exposure has been linked to increase in the risk of coronary artery disease. They have thought that arsenic had an effect on all

cardiovascular system including heart and major vessels arising from the heart. Hence structural changes observed in myocardium of arsenic treated group may be due to occurrence of minute segmental infarction. Also they found that MDA, catalase, glutathione reductase and reduced glutathione contents decreased within myocardium and all these antioxidant agents located mainly in mitochondria which help to protect the biological tissue. Moreover, Ingrida et al. (2009) stated that increase the concentration of sodium arsenate resulted in reproducible decrease in oxidation rate of rat cardiac mitochondria. In the present study there was disorganization of the intercalated discs in the myocardium treated with arsenic. Hu et al., 2005 stated that deformed intercalated discs lead to loss of effective contraction force in most of the affected area.

Glutathione provides a first line of defence and scavenges free radical oxygen species (ROS). The decreased concentration of GSH in heart may be due to NADPH reduction or GSH utilization in the exclusion of peroxides (Yadav et al., 1997). GSH-dependent enzymes offer a second line of protection as they primarily detoxify noxious by-products generated by ROS and help to avert dissemination of free radicals (Gumieniczek, 2005). GSH-Px detoxifies peroxides by reacting with GSH and converting it into GSSG, which is reduced to GSH by GSR (Maritim et al., 2003). Our study revealed that sodium arsenate treatment in rats markedly changed the activity of antioxidant enzymes, which was reverted by the co-administration of HEPT. Thiobarbituric acid reactive substances (TBARS), the final metabolites of peroxidized polyunsaturated fatty acids, are considered as a late biomarker of oxidative stress (Cheeseman, 1993). In our experiment, major decrease in lipid peroxidation and consequent reduction in TBARS were obtained by treatment with HEPT. The increment in lipid peroxidation, as assessed by the elevated levels of TBARS following sodium arsenate administration, has been well documented. Data of the present study indicated that lipid peroxidation induced by oxidative stress caused DNA damage. Administration of HEPT markedly reduced the DNA damage through its antioxidant mechanisms.

All the above mentioned biochemical & histological changes support the hypothesis that arsenic affects the heart muscle fibers. Since the heart has low cell proliferation capacity, attempts directed to prevent death of myocardial cells are of therapeutic value. Therefore, the present study aimed to avoid cardiac damage resulting from arsenic exposure through HEPT administration. The results of the current study show that supplementation of dietary HEPT has been shown to reduce the bioavailability of arsenic in rats, reduced clinical sign of arsenic poisoning and reduced arsenic accumulation and prevented the damage through its free radical scavenging activity.

## CONCLUSION

Data from this study concluded that HEPT protected against sodium arsenate induced cardiotoxicity in rats. Sodium arsenate altered heart by inducing oxidative damage, however, HEPT administration was able to avert the damage. HEPT had the abilities to safeguard the activity of antioxidant enzymes, serum levels, hematological, DNA fragmentation and protect the heart from sodium arsenate induced damage.

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## CONFLICT OF INTEREST

None declared.

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