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Research Article

PROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF *PUERARIA TUBEROSA AGAINST* ARSENIC INDUCED NEUROTOXICITY IN RATS

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ABSTRACT

Administration of arsenic induces neurotoxicity through excessive formation of reactive oxygen species (ROS) causing oxidative stress and neural damage along with significant decline in the memory and learning capacity. In the present study, the protective effect of hydroalcoholic extract of tubers of *Puerariatuberosa*against arsenic induced neurotoxicity was studied using various behavioural and biochemical parameters in rats. Neurotoxicity was induced by sodium arsenate 1mg/kg by oral route. 36 adult male and female albino rats were used in this study. The animals were divided into six groups of 6 animals each Group 1: Control group (received distilled water 1ml), Group 2: Sodium arsenate (1 mg/kg, p.o.), Group 3: Sodium arsenate + vitamin E (1 mg/kg, p.o. + 100 mg/kg, p.o.), Group 4: Sodium arsenate + hydroalcoholic extract (1 mg/kg, p.o. + 50 mg/kg, p.o.), Group 5: Sodium arsenate + hydroalcoholic extract(1 mg/kg, p.o. + 100 mg/kg, p.o.),Group 6: Sodium arsenate + hydroalcoholic extract (1 mg/kg, p.o. + 200 mg/kg, p.o.). All the groups were At the end of the experimental study the rats were sacrificed by ether anaesthesia and blood was collected by carotid bleeding. The blood was centrifuged for the collection of serum and was analyzed for estimation of serum parameters viz Triglycerides, Total cholesterol, Glucose, ALP, ALT. Anti-oxidant parameters viz Catalase, Reduced glutathione, Glutathione reductase, Melondiadehyde was also estimated. The increased levels of lipid peroxidation (MDA) and decrease in level of enzyme like catalase (CAT), glutathione reductase (GR), and reduced glutathione (GSH) .Significant decline in the spontaneous behaviour and exploratory response in the behavioural tests was observed in the arsenic treated rats. The animals showed decrease in learning and memory in arsenic treated rats. Administration of hydro alcoholic extract strengthens its memory. Muscle strength was measured by rota rod test, the locomotor activity by actphotometer and open field test. Muscle strength and locomotor activity was decreased in arsenic treated rats. These changes were significantly ameliorated by treatment with the extract. Biochemical changes were supported by the histopathological observations, which also revealed chronic exposure to sodium arsenate causes damage to the brain. Thus, the results suggested that the extract possesses neurotoxicity activity.

Keywords: Arsenic, *Puerariatuberosa,* Neurotoxicity, Muscle strength,Locomotor activity.

INTRODUCTION

The root name *neuro* comes from the Greek word meaning "nerve." *Toxicity* means "the action of poisonous properties or materials." Neurotoxicity is defined as the damage to the nervous tissue from toxic substances. It can affect the central nervous system or the peripheral nervous system. Neurotoxicity occurs when the exposure to natural or artificial toxic substances, which are called neurotoxins, alters the normal activity of the nervous system in such a way as to cause damage to nervous tissue. This can eventually disrupt or even kill neurons, key cells that transmit and process signals in the brain and other parts of the nervous system. The agent that causes neurotoxicity is called a neurotoxin or sometimes a neurolysin. A neurotoxin is a substance that has the property of destroying the nerve cells called ganglion and cortical cells. A ganglion is a group of nerve cells that serves as a central point from which transmission of nerve impulses originate. Cortical cells are cells in the cerebral cortex of the brain. Neurotoxins may be natural substances that impair how nerves functions by blocking their electrical activities.

Many heavy metals, including As, are known to induce over production of reactive oxygen species (ROS) and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acid contents of membranes[1]. Also, it has been shown to enhance the production of ROS in a variety of cells resulting oxidative stress[2]. ROS are the byproducts of many degenerative reactions in many tissues, which will affect the regular metabolism by damaging the cellular components[3]. Extensive study on oxidative stress has demonstrated that exposure of cells to adverse environmental conditions can induce the over production of ROS, such as superoxide radical (O^{2-}), H_2O_2 and hydroxyl radical (OH^{-}) in plant cells[4]. In addition, ROS are highly reactive to membrane lipids, protein and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage[5-10]. Acetylcholinesterase (AChE) or acetylhydrolase is a serine protease that hydrolyzes the neurotransmitter racetylcholine to be acetyl CoA and choline. AChE is found at mainly neuromuscular junctions and cholinergic brain synapses, where its activity serves to terminate synaptic transmission. Other studies have shown that the deleterious effect of As exposure on memory could be related to its capacity to induce cholinergic dysfunction in brain[11-12]. Since cholinergic system is responsible for the behavioral manifestations in animals, observation of As induced impairments in AChE system can be attributed to cognitive dysfunction[11].Although chelating therapy is currently an available treatment of As neurotoxicity, it is observed to have many adverse effects such as divalent metal ion imbalance and it is also ineffective in improving previous nerve injury induced by As. Currently ,no efficient drugs are available for treating chronic Arsenic induced cognitive deficits[13-14]. Although various countries have established legislation regulating their concentration, they are still sometimes a danger for consumer health. Arsenic has been associated with various forms of cancer, nephrotoxicity, central nervous system effects and cardiovascular diseases in human[15]. The inhalation of arsenic could permanently lower intelligence quotient (IQ), damage emotional stability and cause hyperactivity, poor school performance and hearing loss.*Puerariatuberosa* is a perennial climber and is used as an ayurvedic medicinal herb to cure diseases in various parts of Africa and Southeast Asia. It also faces a low seed set problem, but due to its industrial demand it is now under cultivation *Puerariatuberosa*has gained the importance in medicine in recent years only and is indicated promising drug for the production of flavonoids on commercial scale. *Puerariatuberosa* popularly used in folklore medicine is reported for its nerve tonic, anti-inflammation brain tonic, galactogauge and as a mind power syrup in ayurvedic formulation. [16, 17]

MATERIALS AND METHODS

Plant: Plant: The plant samples such as tubers of *GloriosaSuperba*were collected from Tamilnadu, India and authenticated from **Department of Botany**, **Osmania university**. The tubers were separated and shade dried. The tubers were then ground to powder form.

Animal selection: Thirty six male Wistar Albino rats weighing 150gms-250gms were obtained from Teena labs, bachupally Hyderabad. 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*. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2013-14/MPCOL/24)

Preparation of Extract

The tubers were separated from *Puerariatuberosa*and were shade dried individually. The dried tubers were ground to powder. This dried powder was used for soxhlet extraction. Extraction was done by using the soxhlet apparatus at a temperature below 60^{o}C for 24 hours. The solvent thus obtained was evaporated under vacuum to get a semi-solid form of the extract. Percentage yield was 70% with respect to dried powder.

Preliminary phytochemical screening[18-19]

Preliminary phytochemical screening was done for the presence of alkaloids, carbohydrates, proteins, saponins, flavonoids,iso- flavonoids, tannins, tri-terpinoids and phenolic compounds according to the procedures described in "Text book of Practical Pharmacognosy" by C.K. Kokate.

DETERMINATION OF LD_{50} OF HYDROALCOHOLIC EXTRACT OF*PUERARIA TUBEROSA*

Acute toxicity study of hydroalcoholic extract was carried out for determination of LD_{50} by adopting fixed dose method of CPCSEA, OECD guideline no.423.A group of albino mice was used for this study.No signs of toxicity were found upto the dose of 1000 mg/kg body weight.

EXPERIMENTAL DESIGN

Thirty six Wistar Albino male rats of weight 150g-200g were selected for this study. Animals were divided into six groups of six animals each. Group 1: Control group (received distilled water 1ml), Group 2: Sodium arsenate (1 mg/kg, p.o.), Group 3:Sodium arsenate + vitamin E (1 mg/kg, p.o. + 100 mg/kg, p.o.), Group 4: Sodium arsenate + HEPT (1 mg/kg, p.o. + 50 mg/kg, p.o.), Group 5: Sodium arsenate + HEPT(1 mg/kg, p.o. + 100 mg/kg, p.o.),Group 6: Sodium arsenate + HEPT(1 mg/kg, p.o. + 200 mg/kg, p.o.).All the groups were treated once daily for a period of 30 days. The animals were weighed and behavioral observations were recorded before and at the end of the experiment. After the administration of last dose, the animals were given rest overnight and then on the next day, they were sacrificed under light ether anesthesia. The organs were removed, cleaned, washed with phosphate buffer saline (pH 7.4) for various studies.

ESTIMATION OF BEHAVIORAL PARAMETERS

Rota-rod test

The effect of Arsenic as well as*Puerariatuberoa*extracttreatment on muscle performance was evaluated using Rota-rod (Techno) test. Allthe rats were given two initial training trials of300 s, approximately 10 min apart, tomaintain posture on the Rota-rod (3 cm indiameter and rotating at a constant 20rev/min). After the initial training trials, a baseline trial of 120 s was conducted.

The time each animal remained on the rota-rod was recorded. The animals that did not fall off the Rota-rod were given a maximum score of 120 seconds.

Locomotor activity

The spontaneous locomotor activity of each rat was recorded individually for 10 min using actophotometer. The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photo cell is cut off by the animal, a count is recorded.

Open field test

The open field test, which provides simultaneous measures of locomotion, exploration and anxiety, was used for this study. The open field is a 400×400×300 mm area with thin black stripes painted across the floor, dividing into 16 quadratic blocks. Animal was placed in the center of arena and an observer quantified the spontaneous ambulatory locomotion of each animal for 5min. during this period, the number of squares crossed and number of rearings was measured.

ESTIMATION OF BIOCHEMICAL PARAMETERS

Serum sample preparation

At the end of experimental period 30 days, animals were sacrificed using light ether anaesthesia and the blood samples were collected into blood collecting tubes by carotid bleeding from all animals. The blood samples were allowed to clot for 60 min at room temperature. Serum was separated by centrifugation using cool centrifuge (REMI) at 4000 rpm for 15 min and stored at -40ºC. Which was later used for estimation of various serum parameters like TG, TC, ALP, ALT, glucose.

Tissue sample preparation

Animals were sacrificed by cervical dislocation with light ether anesthesia and the brain of each rat was removed and washed well with ice cold saline to remove blood and stored at -40ºC. later the brain was taken and minced into small pieces and a total of 10% homogenate was prepared using phosphate buffer (0.1M, pH 7.4) containing 1mmol ethylene diamine-tetra-acetic acid (EDTA), 0.25M sucrose, 10mM potassium chloride (KCL) and 1mM phenyl methyl sulfonyl fluoride (PMSF) with a homogenizer (REMI) fitted with a Teflon plunger, which was centrifuged at 8000 rpm for 30min at 4^{o}c to yield the supernatant. Later the supernatant was used for the estimation of acetyl cholinesterase (AChE) and antioxidant parameters (MDA, GSH, CAT, and glutathione reductase). (Followed according to stranded procedures)

RESULTS AND DISCUSSION

PERCENTAGE YIELD OF EXTRACT

The percentage yield of hydroalcoholic extract of *Puerariatuberosa*with respect to the dried powder was found to be 70%.

PRELIMINARY PHYTOCHEMICAL SCREENING OF HYDROALCOHOLIC EXTRACT OF *PUERARIA TUBEROSA*

The main chemical constituents that are found in the hydroalcoholic extract of *Puerariatuberosa*flovonoids are abundantly found. Carbohydrates, Tannins, alkaloid,carbohydrates,saponins,phenolic compounds,gums and mucilage.

NEUROPROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF *PUERARIA TUBEROSA*IN ARSENIC INDUCED NEUROTOXICITY

Effect of Hydroalcoholic extract of *puerariatuberosa*on Serum Parameters in Arsenic induced Neurotoxicity Study(Table 1)

Arsenic exposure produced significant increase P<0.0001 in glucose (140.46±0.118), ALP (135.59± 0.78), ALT (116.87±0.2), TG (125.03±0.62) and in TC (135.6±3.92) when compared to normal control group.Hydroalcoholic extract of *puerariatuberosa* pretreatment before Arsenic exposure showed significant decrease P<0.0001 in glucose (96.6±0.87), ALP (85.46±1.43), ALT (82.5±0.31) , TG (73.45±1.35) and TC (79.5±0.6) in Arsenic+ extract 3 group when compared to Arsenic treated rats. (Table 3)

Effect of Hydroalcoholic extract of*Puerariatuberosa*on Antioxidant Parameters in Arsenic induced Neurotoxicity Study(Table 2)

Arsenicexposure produced significant decrease P<0.0001 in catalase (12.45±0.1), in GSH (18.23±0.23),in glutathione reductase (10.56±0.2), and significant increase P<0.0001 in MDA (98.06±2.5) in Arsenic control group when compared to normal control group.Hydroalcoholic extract of *Puerariatuberosa*pretreatment before Arsenic exposure showed significant increase P<0.0001 in catalase (25.8±0.3), GSH (23.85±0.8) , glutathione reductase (18.91±0.69) and significant decrease P<0.0001 in MDA (77.93±1.0) in Arsenic extract 3 group when compared to Arsenic treated group (Table 4).

Effect of Hydroalcoholic extract of *Puerariatuberosa*on Behavioural Parameters in Arsenic induced Neurotoxicity Study(Table3)

Arsenic exposure produced significant decrease P<0.0001 in muscular strength (14.92±0.70) in Rota rod test, locomotor function (14.45±0.73) in actophotometertest,P<0.0001 in number of squares crossed in open field test (52.14±3.2) when compared to normal control group.Hydroalcoholic extract of *Puerariatuberosa* pretreatment before Arsenic exposure showed significant increase in P<0.0001 in muscular strength (31.22±0.75) in Rota rod test, locomotor function (23.25±1.15) in actophotometer test and significant increase P<0.0001 in number of squares crossed in open field test (124.14±5.5) in Arsenic+ extract 3 group when compared to Arsenic treated group (Table 5,6).

Effect of Hydroalcoholic extract of*Puerariatuberosa*on Acetyl Cholinesterase (AChE) Activity in Arsenic induced Neurotoxicity Study(Table4)

Arsenic exposure produced significant decrease P<0.0001 in AChE activity (2.76±0.01), when compared to normal control group.Hydroalcoholic extract of *Puerariatuberosa* pretreatment before Arsenic exposure showed significant increase P<0.0001 in AChE activity (3.45±0.03), in Arsenic+ extract 3 group when compared to Arsenic treated group. (Table 7).

HISTOPATHOLOGY OBSERVATIONS

Effect of hydroalcoholic extract of*Puerariatuberosa*on brain histopathology

Transverse section of the brain sample were stained with eosin and haematoxylin to study the neurodegeneration. In the normal control group the section was found to be intact and no neuronal loss was observed. Gross histopathology changes, including neurodegeneration and vacuolated cytoplasm was observed in lead control group whereas these changes were not found in lead plus vitamin E on the other hands, remarkable improvements were in the Arsenic plus Hydroalcoholic extract of*Puerariatuberosa.*

Table 1: Effect of *Puerariatuberosa*on Serum Parameters in Arsenic induced Neurotoxicity

Groups	Glucose (mg/dL)	ALP (IU/L)	ALT (IU/L)	TG (mg/dL)	TC (mg/dL)
Normal	80.56 ±0.74	75.92 ± 1.37	52.56± 0.3	65.89± 0.95	96.72± 1.11
Arsenic(1mg/kg)	140.46±0.118	135.59± 0.78	16.87 ± 0.2	125.03± 0.62	135.6± 3.92
Arsenic(1mg/kg)+VitE(100mg/kg)	125.08± 1.83	120.45± 1.18	102.4± 0.31	18.9± 1.11	123.8± 0.71
Arsenic(1mg/kg) +HEPT(50mg/kg)	116.9± 1.10*	102.89± 2.59*	72.45 ± 0.5*	107.15± 1.56*	102.6± 1.71*
Arsenic(1mg/kg) +HEPT(100mg/kg)	104.2± 1.96**	93.78± 1.90**	65.8± 0.2**	88.84± 0.79**	86.7± 1.21**
Arsenic(1mg/kg)+HEPT(200mg/kg)	96.3± 0.87***	85.46± 1.43***	6.5± 0.31***	73.45± 1.35***	79.5±0.6***

Values are expressed as mean ± SEM,

A significant increase in the level was seen in the Arsenic treated rats whereas treatment of Extract groupsin rats along with reduced the levels. and the treatment group of Extract at the dose of 50mg/kg showed better neuroprotective effect in comparison with the other groups with *P<0.01.

Table 2: Effect of*Puerariatuberosa*on Antioxidant Parameters in arsenic induced Neurotoxicity study

Groups	MDA	Catalase	GSH	Glutathione Reductase
	(nm/g tissue)	(mM H2O2 Consumed/min/g Wet tissue)	(µg/ml)	(Units/ml)
Normal Control	92.108±1.7	31.2±0.5	28.46±0.7	21.30±1.02
Arsenic(1mg/kg)	98.06±2.5	12.45±0.1	18.23±0.23	10.56±0.2
Arsenic(1mg/kg)+ VitE(100mg/kg)	88.41±1.1	21.05±0.2	22.16±0.5	16.89±0.28
Arsenic(1mg/kg)+ HEPT(50mg/kg)	83.01±1.03*	18.6±0.21*	19.45±0.6*	11.21±0.2*
Arsenic(1mg/kg)+ HEPT(100mg/kg)	80.79±1.0**	22.89±0.31**	21.24±0.3**	14.45±0.51**
Arsenic(1mg/kg) + HEPT(200mg/kg)	77.93±1.00***	25.8±0.3***	23.85±0.8***	18.91±0.69***

Values are expressed as mean ± SEM.

A significant increase in the TBARS level was seen in the Arsenic treated rats whereas treatment of Extract groupsin rats along with reduced the level of TBARS and the treatment group of Extract at the dose of 50mg/kg showed better neuroprotective effect in comparison with the other groups with $P<0.01$.

Catalase, Reduced Glutathione and Glutathione Reductase levels were significantly reduced on treatment with Arsenic in comparison to the control treated group whereas *Extract* reversed the reduction in the levels of catalase, reduced glutathione and glutathione reductase induced by Arsenic and further it was found that the treatment group of *Extract* at the dose of 200mg/kg showed better response with ***$P<0.0001$ in comparison to the treatment group of *Extract* at the dose of 100mg/kg which showed slight improvement in the catalase, glutathione reductase and reduced glutathione levels in the cerebral cortex of rats.

Table 3:Effect of*Puerariatuberosa* Extract on Muscular Strength and Locomotor Function in Arsenic induced Neurotoxicity Study

Groups	Muscular Strength (Seconds)	Locomotor Function (Score in 5 min)
Normal Control	37.32 ± 0.72	26.18± 0.91
Arsenic Control(1mg/kg)	14.92± 0.70	14.45±0.73
Arsenic(1mg/kg)+Vit E(100mg/kg)	31.88 ± 0.90	19.87±0.61
Arsenic(1mg/kg)+ HEPT(50mg/kg)	23.54 ± 1.17*	21.09±0.62*
Arsenic (1mg/kg)+HEPT(100mg/kg)	28.81±1.27**	23.07±0.89**
Arsenic (1mg/kg)+HEPT(200mg/kg)	31.22± 0.75***	23.25±1.15***

Values are expressed as mean ± SEM, n=6.

In the Rota Rod test, it was observed that in comparison to the control rats, the "Fall of Time" of the Arsenic treated rats were significantly decreased whereas the *Extract* treated groups the rats showed improvement in motor activity and balance with* $P<0.01$ at the dose of 50mg/kg Extract ,**$P<0.001$ at the dose of 100mg/kg of Extract,*** $P<0.0001$ at the dose of 200mg/kg of Extract, thereby showing marked increase in the "Fall of Time" in comparison to the Arsenic treated rats.

In case of the Locomotor activity test, the ambulatory behavior of the Arsenic treated rats were significantly decrease in comparison to the control group. Futher the treatment of Extract showed enhancement in ambulatory behavior and increase in the locomotor activity with *$P<0.01$ at the dose of 50mg/kg ofExtract, **$p<0.001$ at the dose of 100mg/kg ofExtract and***$p<0.001$ at the dose of 200mg/kg of Extract.

Effect of*Puerariatuberosa* Extract on Open Field Test in Arsenic induced Neurotoxicity Study

Groups	Number of Squares Crossed		Time Spent	Number	Groom
	in Central	Peripheral	Central Squares	Rears	ing
Normal Control	79.3±2.55	9.23±2.6	23.89±1.55	21.65±1.2	10.6±1.5
Arsenic (1mg/kg)	32.02±1.15	20.12±2.05	7.66±0.6	6.3±0.1	3.2±0.5
Arsenic (1mg/kg)+ Vit E (100mg/kg)	53.25±1.3	33.02±2.01	13±1.15	12.4± 0.5	5.6±1.3
Arsenic(1mg/kg)+ HEPT(50mg/kg)	55.65±1.25*	43.1 ±2.9*	16.12±1.02*	15.2±0.3*	6.56±1.5*
Arsenic(1mg/kg)+ HEPT(100mg/kg)	68.5 ±1.6**	47.8±3.01**	18.5 ±1.12**	16.8 ±0.16**	8.6±1.4**
Arsenic(1mg/kg)+ (HEPT(200mg/kg)	72.14±2.1***	52.5±3.4***	20.4±1.5***	19.45±0.7***	9.1±1.5***

Values are expressed as mean ± SEM, n=6.

In the Open field test, the Arsenic treated rats showed significant decrease in the number of peripheral and central squares crossing along with the number of rears and grooming whereas in the Extracttreated animals there was marked increase in the number of lines crossed, enhanced exploratory activity and increase in the number of rears and grooming frequency with ***P<0.0001 at the dose 200mg/kg of Extract ,** P<0.001 at the dose of 100mg/kg of Extract and *P<0.01 at the dose 50 mg/kg of Extract.

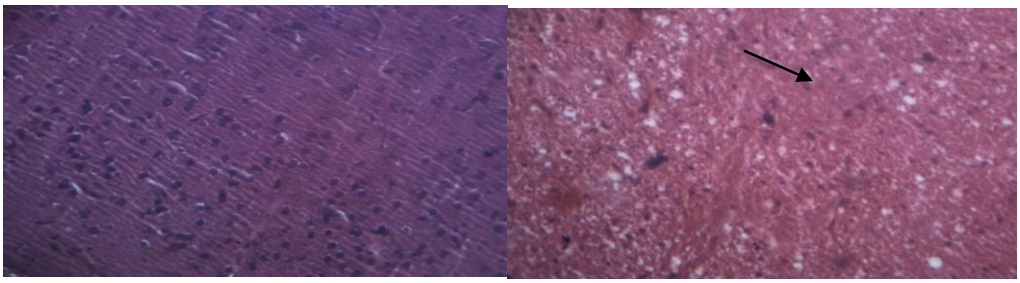
Table 4:Effect of*Puerariatuberosa* Extract on Acetyl Cholinesterase (AChE) Activity in Arsenic induced Neurotoxicity Study

Groups	AChE activity (µmoles/mg protein)
Normal control	6.06 ± 0.34
Arseniccontrol(1mg/kg)	2.76 ± 0.01
Arsenic(1mg/kg)+ vit E(100mg/kg)	5.19 ± 0.2
Arsenic (1mg/kg)+HEPT(50mg/kg)	2.43 ± 0.02*
Arsenic(1mg/kg) +HEPT(100m/kg)	2.78 ± 0.024**
Arsenic(1mg/kg)+ HEPT(200mg/kg)	3.45 ±0..03***

Values are expressed as mean ± SEM.

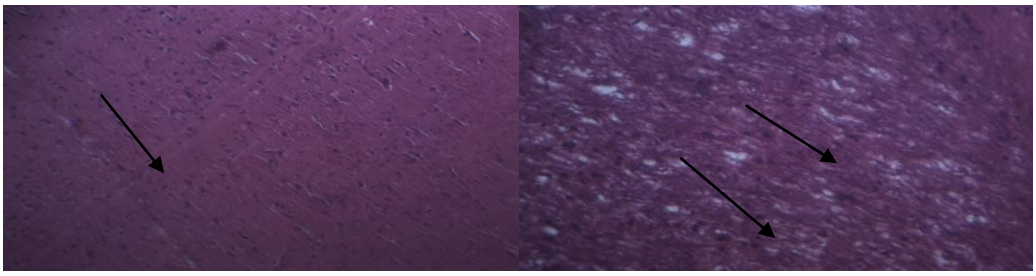
The levels of Arsenic treated rats were significantly decrease in comparison with the control whereas in the Extract treated groups there was marked increase in the levels of Ach and it was also observed that the treatment group of Extract at the dose of 200mg/kg showed better neuroprotective effect with ***P<0.0001.

Effect of Hydroalcoholic Extract of *Puerariatuberosa*on brain histopathology in Arsenic induced neurotoxicity in rats



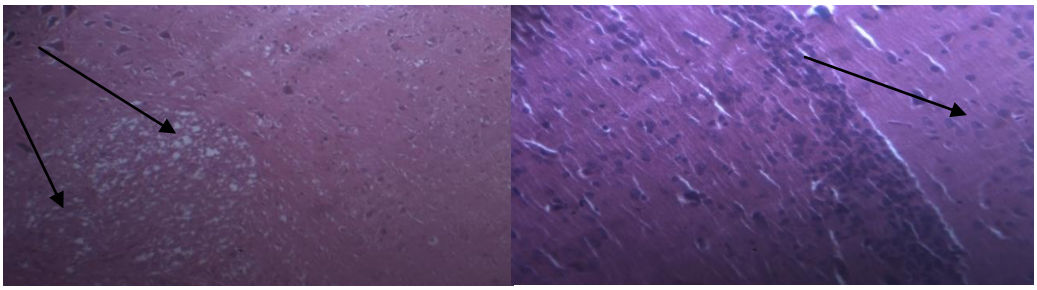
Group 1: ControlGroup Showing normal architecture of brain. No abnormality wasshowing vacuolation in brain detected

Group2: Arsenic TreatedArrow



Group 3: Arsenic+100mg/kg of Vit-E TreatedArrow showing Showing normal architecture of brain. No abnormalitydetected

Group 4: Arsenic+50mg/kg of Extract Treated Arrow showing vacuolation in brain.



Group 5: Arsenic + 100mg/kg of Extract Treated Arrow showing vacuolation in brain (20X)

Group 6: Arsenic + 200mg/kg of Extract Treated Arrow showing inflammatory cell infiltration in brain.(20X)

DISCUSSION

Stress is one of the primary reasons for referrals to psychiatrists and root cause of a number of brain disorders. Modern day diseases are largely due to this hideous stress. The brain is particularly vulnerable to oxidative stress because of its high metabolic rate and low antioxidant defences.[43] Oxidative stress and ROS are said to be the major contributors to the aging process and many age linked neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.[44]

It is known that Arsenic has neurotoxic efficacy and injection of Arsenic can induce neurological and behavioural impairments in rodents due to some biochemical changes in the brain including increased production of ROS and reduced antioxidant enzyme activity. So Arsenic treated rat serves as an induced neurotoxicity model due to excessive formation of ROS followed by neuronal damage and has been used for neurotoxicity investigation and drug testing. The oxygenic metabolism of Arsenic produced many ROS which may result in direct or indirect impairment of learning and memory[18]. Arsenic causes disturbances in cholinergic neurotransmission and disrupts the cognitive behaviour of animals by increasing the stress levels. Studies have shown that Arsenic exposure results in a decrease in hexokinase

activity , which in turn decreases the pyruvate formation and hence affect the synthesis of acetylcholine and subsequently the enzyme activity of AChE. Found that Arsenic is a pro-oxidant and indirectly results in the production of free radicals leading to oxidative damage and reduced levels of reactive oxygen species (ROS) which indirectly affects acetyl cholinesterase enzyme activity. The present study was focused to evaluate that the *Puerariatuberosa*protects Brain Cells against Arsenic induced damage via the scavenging of Reactive Oxygen Species. The results of the present study revealed that arsenic intoxication causes significant increase in catalase, glutathione, and reduced glutathione levels. and significant decrease in glucose,total cholesterol, alanine transaminase,triglycerides, alkaline phosphatase, and lipid peroxidation level. There was significant increase in serum acid phosphatase activity after arsenicexposure. Similar reports have also been reported by Mehra and Kanwar (1986)following arsenic administration. Acid phosphatase activity is localized in cellular lysosomes. An enhanced peroxidation of lysosomal membranes due to arsenic intoxication causes lysis of membrane and oozing out of the enzyme hence results in an increased acid phosphatase activity. In the liver enzyme, alkaline phosphatase is closely connected with lipid membrane in the canalicular zone, so that any interference with the bile flow, whether extra hepatic or intra hepatic leads to decrease in serum alkaline phosphatase activity (Vandenberghe,1995). Arsenic causes cell membrane damage (lipid peroxidation), which leads to the imbalance between synthesis and degradation of enzyme protein, thus lowering the enzyme activity (Hardonk and Koudstaal, 1976)[19]. Present findings are in agreement with the findings of El-Demerdash (2001)and Sharma et al. (2002) who showed that sodium arsenate (1mg/kg b.w.) intoxication significantly decreases the alkaline phosphatase activity in rats.

In the present investigation, Arsenic-exposed rats showed increased blood glucose level which may be due to decreased pyruvate formation that affects the synthesis of acetylcholine[20]. It appears that Arsenic toxic potency, which leads to alteration in acetylcholine level, might have caused neurobehavioral changes. Davis has observed that acetyl choline affects human memory and cognitive function by dramatically decreasing the activity of acetylcholine tranferase in the cortex and hippocampus of Alzheimer patients. Increase in blood glucose level may have disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by increase in adrenocorticotrophic and glucagon hormones and/or reduced insulin activity. Sallam*etal*reported that Arsenic accumulation in the liver leads to liver damage as a result of increased enzymes levels of ALP, TC, TG, ALT and Glucose in the serum. Increased enzyme levels may cause cellular degeneration or destruction of hepatic cells[21]. Decreased plasma concentration of ALP, TC, TG, ALT and Glucose levels in Extract treated animals suggests that the drug exhibits hepato-protective activity.

In the present investigation, Arsenic-exposed rats showed decrease in muscle strength (rota rod test), locomotor activities, number of squares crossed(open field test), retention time was observed in Arsenic-treated rats compared with normal control. On the other hand, significant improvement in muscle strength, locomotor activities, number of squares crossed, retention time occurred in treated rats compared with Arsenic -treated rats.

Recent studies demonstrated that arsenic compounds during their metabolism generate excessive amount of ROS leading to oxidative stress impairing endogenous antioxidant defense mechanisms and simultaneously damaging the cellular macromolecules such as lipids, proteins and DNA, resulting in disruption of cell structure and functions (Li et al., 2001; Shi et al., 2004; Manna et al., 2008). In the present finding, arsenic administration induced oxidative stress in rat brain as evidenced by perturbations in various antioxidative parameters, which can be suppressed by treatment with *Puerariatuberosa*.

In the present investigation the blood lipid peroxidation level showed a highly significant elevation and GSH level, CAT level showed a highly significant depletion following arsenic exposure. Lipid peroxidation is considered as a molecular mechanism of oxidation of cellular lipid based macromolecules. Overproduction of ROS enhances the lipid peroxidation and subsequently increases the lipid peroxidation products like malondialdehyde (MDA) and other TBARS levels which lead to degradation of cellular macromolecules.

GSH is the major thiol, which binds electrophilic molecular species and free radical intermediates. It plays a central role in the antioxidant defence system, metabolism and

detoxification of exogenous and endogenous substances (Ketterer et al., 1983; Meister and Andersen, 1983).Arsenic high affinity for GSH and causes the irreversible excretion of, up to two GSH tripeptides (Zalups and Lash, 1996, Patrick, 2002). The metal–GSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. However, it depletes the GSH from the cell and thus decreases the antioxidant potential. Hydrogen peroxide subsequently converts into non toxic water and oxygen molecules by the action of catalase (CAT).

Histopathological changes also support the above results. Histological sections of brain in control and*Puerariatuberosa*treated rats showed the normal neuroocytes, and central vein. Arsenic intoxication produced various pathological lesions in the brain such as cytoplasmic vacuolization, and centrilobular necrosis. Concomitant treatment of *Puerariatuberosa*with arsenic showed prominent recovery and normal architecture with mild residual degeneration.

CONCLUSION

The hydroalcoholic extract has shown the ability to maintain the normal functional status of brain.From above preliminary study,we conclude that the hydroalcoholic extract of puerariatuberosa is proved to be one of the herbal remedies for brain functioning.

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