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PROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF *GLORIOSA SUPERBA LINN.* IN LEAD INDUCED NEUROTOXICITY IN RATS

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ABSTRACT

In the present study, tubers of *Gloriosa superba Linn* (Liliaceae) was selected for evaluating the effect of hydroalcoholic extract of *Gloriosa superba linn* in lead induced neurotoxicity in rats. Thirty six wistar rats (150-200g) were selected and divided into six groups of six in each. Group I (normal control) received distilled water, group II-lead nitrate (10 mg/kg, p.o.), group III-lead nitrate + vitamin E (10 mg/kg, p.o. + 100 mg/kg, p.o.), group IV-lead nitrate + HEGS (10 mg/kg, p.o. + 50 mg/kg, p.o.), group V-lead nitrate + HEGS (10 mg/kg, p.o. + 100 mg/kg, p.o.), group VI-lead nitrate + HEGS (10 mg/kg, p.o. + 200 mg/kg, p.o.) experiment was carried out for 21 days. At end of the experiment various behavioral, biochemical and histopathological assessments were carried out. The animals showed increase in transfer latencies indicating learning and memory implairment in lead control group, but administration of hydroalcoholic extract of *Gloriosa superba Linn* decreased the transfer latencies, strengthened its memory improvement action in drug treated animals.Hence showed decrease in muscle strength measured by rota-rod test whereas, in hydroalcoholic extract of *Gloriosa superba linn* treated group there was improvement in muscle strength. The locomotor activity assessed by actophotometer and open field test was decreased in lead nitrate group compared with hydroalcoholic extract of *Gloriosa superba Linn* treated group. Biochemical analysis of brain revealed that the chronic administration of lead nitrate significantly increased lipid peroxidation and decreased levels of catalase (CAT), reduced glutathione (GSH) and glutathione reductase (GR), an index of oxidative stress process. Administration of hydroalcoholic extract of *Gloriosa superba Linn* attenuated the lipid peroxidation and reversed the decreased brain CAT and GSH levels. Lead exposed rats showed increased levels of various serum parameters like glucose, ALT, ALP, TG and TC. Lead toxicity also leads to alteration in acetylcholinesterase levels, might have caused neurobehavioral changes which was measured by the change in acetylcholinesterase activity but prior administration of hydroalcoholic extract of *Gloriosa superba Linn* ahead of lead nitrate ameliorated the change. There was marked changes at the subcellular level which were observed by histopathology studies in lead treated group and better improvement in these changes was observed in hydroalcoholic extract of *Gloriosa superba Linn* treated group. Therefore hydroalcoholic extract of *Gloriosa superba Linn* helps to combat the oxidative stress produced by the accumulation of lead in the body.

Key words: *Gloriosa superba Linn*, Lead, Muscle strength, Locomotor activity, Biochemical analysis.

Abbreviations: HEGS - Hydroalcoholic extract of *Gloriosa superba Linn*, MDA- Malondialdehyde, CAT- Catalase, GSH- Reduced glutathione, GR- Glutathione reductase, ALP -Alkaline Phosphatase Level, ALT- Alanine Aminotransferase, TG- Triglycerides, TC- Total cholesterol.

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.

Lead, although one of the most useful metals, is also one of the most toxic environmental pollutant which is detected in almost all phases of biological systems. Lead is known to induce a broad range of physiological, biochemical, and behavioural dysfunctions in laboratory animals and humans[1] (Flora et al., 2006), including central and peripheral nervous systems (Bressler et al., 1999)[2], haemopoietic system (Lanphear et al., 2000)[3], cardiovascular system (Khalil- Manesh et al., 1993)[4], kidneys (Poprawa and Kapusta, 2004)[5], hepatic (Patra et al., 2001)[6] and male (Lancranjan et al., 1975)[7]and female reproductive systems (Ronis et al., 1998)[8]. Several researches have demonstrated that lead can cause neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological disorders. Considering that lead toxicity is currently one of the serious problem worldwide, there is still no specific, reliable and safe treatment. Several metal chelators ($CaNa_2EDTA$ and DMSA) have been used to manage lead toxicity in the event of exposure but none are suitable in reducing lead body burden (Osweiler, 1999)[9]. Moreover these chelators in turn are potentially toxic (Gilman, 1991)[10] and often fail to remove Pb burden from all body tissues (Cory-Slechta et al., 1987)[11].

The mechanisms of lead induced neurotoxicity are complex. Oxidative stress, membrane biophysics alterations, deregulation of cell signalling, and the impairment of neurotransmission are considered as key aspects involved in lead neurotoxicity. The mechanism involves toxicity by oxidative stress[12] and free radical damage via two separate, albeit related, pathways: (a) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide, and (b) the direct depletion of antioxidant reserves[13].

Vitamin E is a fat soluble vitamin with numerous biological functions (Flora, 2002)[14]. It possesses powerful anti-oxidative properties, operative in the membrane to prevent lipid peroxidation by obstructing the free radical chain reaction. Sajitha et al., (2010)[15] reported that vitamin E administered to rats counteracted the deleterious effect of lead by scavenging free radicals and thus preventing oxidative stress. Lead induced ALAD inhibition in the erythrocytes was found to be reversed by the treatment with vitamin E (Rendon-Ramirez et al., 2007)[16]. Vitamin E was also found to be helpful in restoring thyroid dysfunction by maintaining the hepatic cell membrane architecture disrupted indirectly by lead induced lipid peroxidation. Effect of vitamin E in combination with other antioxidants has been found to be more pronounced than its individual administration. Flora et al (2003)[17] reported that co-administration of vitamin E with monoisoamyl derivative (MiADMSA), which is a thiol chelator, exerts an elevated recovery from lead burden in rats. Interestingly, α-tocopherol is capable of reducing ferric iron to ferrous iron (i.e. to act as a pro-oxidant). Moreover, the ability of α-tocopherol to act as a pro-oxidant (reducing agent) or antioxidant depends on whether all of the α-tocopherol becomes consumed in the conversion from ferric to ferrous iron or whether, following this interaction, residual α-tocopherol is available to scavenge the resultant ROS (Yamamoto & Nike, 1988).

Gloriosa superba lilies valued much for their distinctive, showy and vividly colored blooms. While it's unusual climbing habits makes *Gloriosa superba* an eye catching addition to any home garden, its extreme toxicity requires the most cautious of handling. *Gloriosa superpa* is one of the medicinal plant grown as a commercial crop and will give good returns. Among the medicinal crops it gives more returns like cash crops. The generic name *Gloriosa* means 'full of glory' and superb means 'superb', alluding to the striking red and yellow flowers. *Gloriosa* is a genus of ten species in the plant family Colchicaceae[18] and include the formerly recognised genus Littonia. The aimed at investigating the effect of *Gloriosa superba Linn.* Hydroalcoholic extract on lead induced neurotoxicity in rats by the

determination of various behaviour parameters ,biochemical parameters,antioxidant parameters and histological examination.

MATERIALS AND METHODS

Animals : Healthy male Sprague Dawley rats (5–7 week old) weighing 250-300g, obtained from Teena labs, Bachupally, Hyderabad, were used for the present study. The animals were housed in standard polypropylene cagesand maintained at an ambient temperature with natural day-and-night cycles (12:12 h light and dark cycles). Standard pelletized feed and tap water were provided *ad libitum*. 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/2011-12/MPCOL/30).
Animals were grouped, one to five per cage and were allowed a one-week habituation period to the animal room before testing.

Experimental methodology: The rats were randomly assigned into six different groups (n = 6). Group I (normal control) received distilled water, group II-lead nitrate (10 mg/kg, p.o.), group III-lead nitrate + vitamin E (10 mg/kg, p.o. + 100 mg/kg, p.o.), group IV-lead nitrate + HEGS (10 mg/kg, p.o. + 50 mg/kg, p.o.), group V-lead nitrate + HEGS (10 mg/kg, p.o. + 100 mg/kg, p.o.), group VI-lead nitrate + HEGS (10 mg/kg, p.o. + 200 mg/kg, p.o.) experiment was carried out for 21 days.

Behavioral studies:

Rota-rod test: Rats were subjected to rota-rod test over the course of experiment to measure changes in their balancing behavior. Finally, at the end of the study number of falls in 5min interval was measured to assess their balancing behavior.

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: Rats were adapted to the open-field test before the test, and monitored for behavioural changes at the end of the study. Parameters like total time immobile, total immobile episodes were estimated.

Serum sample preparation: At the end of experimental period 21 days, animals were sacrificed using light ether anaesthesia and the blood samples were collected into blood collecting tubes by cardiac puncture 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 -20^0 C 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 -80ºC. 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 4ºc 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).

Tissue Biochemical studies

Lipid Peroxidation assay: The method of Ohkawa et al., (1979) was used for the estimation[19].The principle is based on polyunsaturated fatty acid from the lipid of cell membrane on lipid peroxidation, caused by reactive oxygen species converted to TBARS like MDA. In case of oxidative stress TBARS increase in plasma and tissue. The supernatant (0.2 ml) of the homogenate was mixed with sodium laurylsulphate (8.1%; 0.2 ml), acetic acid (30%; pH 3.5; 1.5 ml), thiobarbituric acid (1.5 ml), and the volume was made up to 4 ml with distilled water. It was incubated for 1 h at 95^0C to develop a pink color, and the solution is cooled followed by addition of distilled water (1 ml).and absorbance was measured spectrophotometrically at 532 nm using U.V – Visible spectrophotometer and the MDA content with extinction coefficient of 156 $nM^{-1}cm^{-1}$ and was expressed as nM/mg of protein.

Reduced Glutathione Assay: GSH concentration was measured by method described by Ellman et al., (1959)[20].To 2ml of 0.1M potassium phosphate pH 8.4, 0.1ml of standard or experimental sample, 0.5ml of DTNB were added and made the volume up to 3 ml with DDW. Then the mixture was incubated for 10 min at room temperature and measured the

absorbance at 412 in U.V – Visible spectrophotometer and calculated the GSH content from standard graph.Reduced glutathione is taken as a reference standard for preparation of standard graph.

Catalase Assay: Catalase activity in tissue was determined by measuring the rate of decomposition of hydrogen peroxide at 240 nm, according to the method given by Aebi et al (1974)[21]. The CAT activity was estimated using method of Aebi (1974). The supernatant (50 ml) was added to a cuvette that contained phosphate buffer (pH 7.0; 50 mM; 1.95 ml). Hydrogen peroxide (H_2O_2; 30 mM; 1.0 ml) was added in it and changes in absorbance were followed at 15-s intervals for 30 s at 240 nm. The catalase activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.071 mmol $cm^{-}1$) and the activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.

Acetyl Cholinesterase (Ache) Activity: An aliquot of the brain homogenate (0.4 ml) was added to a cuvette containing 2.6 ml of phosphate buffer (pH 8.0, 0.1M), 100 µl of 2,4dithionitrobenzene (DTNB) reagent was added to the cuvette followed by the addition of 20 µl of acetylthiocholine iodide (AChI).Thiocholine reacted with DTNB reagent to produce a yellow color and the change in absorbance was read spectrophotometrically at 412 nm. (Ellman et al, 1961)[22].

Calculations:

The enzyme activity is calculated using the following formula;

$R = 5.74x\ 10^{-4}x\ A/CO$

Where, R = Rate in moles of substrate hydrolyzed /minute / gm tissue

A = Change in absorbance / min

CO = Original concentration of the tissue (mg / ml).

Histopathological evaluation: Histopathological evaluation of various brain sections was done with hematoxylin and eosin staining, Humanson et al, (1962)[23]. Transverse sections of brain were fixed in formal-calcium and embedded in paraffin wax. The sections of 7– 9 mm of thickness were cut and dewaxed in xylene, hydrated in decreasing percentage of alcohol and stained with hematoxylin. They were then dehydrated in increasing percentage of alcohols, till 70% and stained with 1% alcoholic eosin. They were differentiated in 90% alcohol and cleared in xylene. These stained sections were observed under the microscope (Zeiss Primo star) for histopathological analysis.

Statistical analysis: All the results were expressed as mean ± SEM. The data was analyzed using one-way analysis of variance (ANOVA) followed by Student t-test. P-values <0.0001 were considered as statistically significant for all comparisons.

RESULTS

Preliminary phytochemical screening of hydroalcoholic extract of *gloriosa superba linn.:*
The main chemical constituents that are found in the hydroalcoholic extract of *Gloriosa superba* are alkaloids, carbohydrates are abundantly found. Saponins, phenolic compounds, tannin, gums and mucilage are slightly found.

Effect of *Gloriosa superba* on Serum Parameters in lead induced Neurotoxicity Study: Lead exposure produced significant increase $^{\#}P<0.0001$ when compared to normal control group. Hydroalcoholic extract of *Gloriosa superba* treatment before lead exposure showed significant decrease $^{c}P<0.0001$ in Lead nitrate + HEGS group 6 when compared to lead treated rats.

Effect of *Gloriosa superba Linn.* on Antioxidant Parameters in lead induced Neurotoxicity Study: Lead exposure produced significant decrease $^{\#}P<0.0001$ in lead control group when compared to normal control group. Hydroalcoholic extract of *Gloriosa superba* pretreatment before lead exposure showed significant increase ^P<0.0001 in catalase, GSH, GR and significant decrease ^P<0.0001 in MDA in Lead nitrate + HEGS group 6 when compared to lead treated group.

Effect of *Gloriosa superba Linn.* Extract on Behavioural Parameters in lead induced Neurotoxicity Study: Lead exposure produced significant decrease $^{a}P<0.0001$ in muscular strength in Rota rod test, locomotor function in actophotometer test,$^{*}P<0.0001$ in number of squares crossed in open field test when compared to normal control group. Hydroalcoholic extract of *Gloriosa superba* pretreatment before lead exposure showed significant increase in $^{\#}P<0.0001$ in muscular strength in Rota rod test, locomotor function in actophotometer test and significant decrease (^P<0.0001) in number of squares crossed in open field test in Lead nitrate + HEGS group 6 when compared to lead treated group.

Effect of *Gloriosa superba Linn.* Extract on Acetyl Cholinesterase (AChE) Activity in Aluminium induced Neurotoxicity Study: Lead exposure produced significant decrease ^P<0.0001 in AChE

activity when compared to normal control group. Hydroalcoholic extract of *Gloriosa superba* pretreatment before lead exposure showed significant increase ^^P<0.0001 in AChE activity in Lead nitrate + HEGS group 6 when compared to lead treated group.

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 lead plus Hydroalcoholic extract of *Gloriosa superba.*

DISCUSSION

There has been insufficient concern about toxic consequences of lead ingestion because its bioavailability was considered to be poor and the gastrointestinal tract normally represented a barrier to lead absorption under normal circumstances but this barrier can be breached. It has been shown that individuals ingesting large amounts of lead compounds to absorb significant amounts resulting in elevated plasma levels[19].

The present study is to evaluate the potential protective effect of the hydroalcoholic extract of *Gloriosa superba* in lead induced neurotoxicity. It is evident from the results of the present investigation that supplementation of *Gloriosa superba* hydroalcoholic extract with lead protected the animals from toxic effects of lead.

As the *Gloriosa superba* contain alkaloid compounds as active constituents, hence the hydroalcoholic solvent was selected for extraction process[20].

A decrease in muscle strength, locomotor activities, number of squares crossed was observed in lead-exposed rats compared with normal control. On the other hand, significant improvement in muscle strength, locomotor activities, number of squares crossed occurred in *Gloriosa superba* extract -treated rats compared with lead-treated rats. Lead-associated toxicity diminished motor activities and grip strength. Thus, improvement in a neurotransmitter enzyme such as AChE, and nuclear materials in rat blood and brain could be responsible for the improvement in learning, memory, cognition and locomotor functions. The importance of neurobehavioral studies in risk assessment lies in the fact that behavior can be regarded as the net output of the sensory, motor and cognitive functions occurring in the nervous system and can serve as potentially sensitive end-points of chemically-induced neurotoxicity.[21]

Lead-exposed rats showed increased blood glucose level which may be due to decreased pyruate formation that affects the synthesis of acetylcholine[22]. It appears that lead 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 AChE in the cortex and hippocampus of Alzheimer's 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. Lead accumulation in the liver leads to liver damage as a result of increased enzymes levels of ALP, TC, TG and glucose in the serum. Increased enzyme levels may cause cellular degeneration or destruction of hepatic cells. Decreased plasma concentration of ALP, TC, TG and glucose levels in *Gloriosa superba* extract treated animals suggests that the drug exhibits neuro-protective activity.[23]

Increased levels of MDA reflect the stimulatory effects of lead on lipid peroxidation. The depletion of CAT and GSH enzymes were observed which is taken as marker of oxidative stress. The decreased activity might have resulted from the oxidative modification of genes that control these enzymes. In lead nitrate treated rats, the lower activity of total antioxidants resulted in an increase of oxidants such as TBARS. The decrease of the antioxidant versus oxidant ratio plays a crucial role in generating a condition of oxidative stress. All of these records are in agreement with a decrease in the total antioxidant capacity. Several antioxidants have been studied for the reduction of oxidative stress occurring in neurodegenerative disorders. Co-administration of *Gloriosa superba* extract (200 mg/ kg) to lead exposed groups, prevented oxidative stress revealing its protective role. In the present study, *Gloriosa superba* extract has shown significant decrease in the MDA levels, increased the antioxidant defensive enzymes such as CAT and GSH, indicating the antioxidant activity of the extract.

These results could confirm that the hydroalcoholic extract of *Gloriosa superba* is having the protective effect against the lead induced neurotoxicity.

Table 1: Effect of *Gloriosa superba* Extract on Muscular Strength in lead induced Neurotoxicity Study

Groups	Muscular Strength (Seconds)
Normal Control	124.35 ± 0.15
Lead Control (10 mg/kg)	44.30 ±0.11[a]
Lead(10mg/kg)+ Vit E(10mg/kg)	107.10±0.05[#]
Lead(10 mg/kg)+ HEGS (50mg/kg)	80.10±0.05[##]
Lead (10 mg/kg) + HEGS (100mg/kg)	98.10 ±0.05*
Lead (10 mg/kg) + HEGS(200mg/kg)	102.20 ±0.11**

Values are expressed as mean ± SEM, *P< 0.01; significantly different from control.

Table 2: Effect of *Gloriosa superba* Extract on Locomotor Function in lead induced Neurotoxicity Study

Groups	Locomotor Function (Score in 5 min)
Normal Control	20.05 ±0.05
Lead Control(10mg/kg)	8.10±0.05[a]
Lead(10mg/kg)+ Vit E(10mg/kg)	14.10 ±0.05[#]
Lead(10mg/kg)+ HEGS(50mg/kg)	16.10 ±0.05[##]
Lead(10mg/kg)+ HEGS (100mg/kg)	18.36 ±0.12*
Lead(10mg/kg)+ HEGS(200mg/kg)	20.06 ±0.12**

Values are expressed as mean ± SEM, *P< 0.01; significantly different from control.

Table 3: Effect of *Gloriosa superba* Extract on Open Field Test in lead induced Neurotoxicity Study

Groups	Number of Squares Crossed			Time Spent in Central Squares	Number of Rears
	Peripheral	Central	Total		
Normal Control	24.15 ±0.05	4.75 ±0.05	29.10 ±0.10	29.250 ±0.05	10.05 ±0.05
Lead Control (10mg/kg)	13.30 ±0.05*	22.10 ±0.05*	35.16 ±0.12*	16.10 ±0.05*	7.70 ±0.05*
Lead(10mg/kg) Vit E (10mg/kg	25.10 ±0.05**	5.10 ±0.05**	30.13 ±0.08**	17.30 ±0.05**	6.00 ±0.00**
Lead(10mg/kg)+ HEGS (50mg/kg)	26.60 ±0.05[#]	4.10 ±0.05[#]	30.05 ±0.11[#]	22.60 ± 0.05[#]	5.70 ±0.05[#]
Lead(10mg/kg)+ HEGS(100mg/kg)	26.8 ±0.05[# #]	2.70 ±0.05[# #]	31.05 ±0.02[# #]	20.40 ±0.05[# #]	6.10 ±0.00
Lead(10mg/kg)+ HEGS(200mg/kg)	29.10 ±0.05^	2.86 ±0.03^	32.10 ±0.05^	19.83 ±0.08^	6.30 ±0.00^

Values are expressed as mean ± SEM, *P< 0.01; significantly different from control.

Table 4: Effect of *Gloriosa superba* on Serum Parameters in lead induced Neurotoxicity

Groups	Glucose (mg/dL)	ALP (IU/L)	ALT (IU/L)	TG (mg/dL)	TC (mg/dL)
Normal	142.42 ± 0.42	107.39 ± 2.81	32.50 ± 0.5	56.67 ± 0.52	71.06 ± 4.46
Lead Control (10mg/kg)	185.57 ±0.29#	162.85 ± 0.86#	64 ± 0.58#	106.67 ± 16.67#	103.3 ± 61.60#
Lead(10mg/kg)+ Vit E (10mg/kg)	157.38 ± 0.31**	55.09 ± 0.16**	36.20 ± 0.61**	95 ± 28.87**	80 ± 5.77**
Lead(10mg/kg) + HEGS(50mg/kg)	128.19 ± .60 a	28.20 ± 0.41a	26.13 ± 0.08 a	89.03 ± 0.33 a	85.03 ± 0.03 a
Lead(10mg/kg) + HEGS (100mg/kg)	114.09 ± 0.58b	32.30 ± 0.11b	29.13 ± 0.08b	81.00 ± 0. 57b	81.23 ± 0.06b
Lead(10mg/kg) + HEGS(200mg/kg)	128.19 ±0.60c	81.30 ± 1.18c	31.64 ± 0.58c	79.46 ± 0.06c	79.20 ± 0.05c

Values are expressed as mean ± SEM, *P< 0.01; significantly different from control.

Table 5: Effect of *Gloriosa superba* on Antioxidant Parameters in lead induced Neurotoxicity study

Groups	MDA (nm/g tissue)	Catalase (mM H_2O_2 Consumed/min/g Wet tissue)	GSH (µg/ml)	Glutathione Reductase (Units/ml)
Normal Control	201.14 ± 2.54	18.2 ± 2.2	27.15 ± 0.05	22.57 ± 0.08
Lead Control (10mg/kg)	355.13 ± 7.83#	7.5 ± 0.85#	19.03 ± 0.08#	11.21 ± 0.10#
Lead(10mg/kg)+ Vit E(10mg/kg)	191.88 ± 29.26##	18.5 ± 2.6##	35 ± 0.05##	24.35 ± 0.16##
Lead(10mg/kg)+ HEGS(50mg/kg)	231.40 ± 1.30*	11.5 ± 2.2*	37.33 ± 0.24*	26.39 ±0.16*
Lead(10mg/kg)+ HEGS(100mg/kg)	218.33 ± 0.28**	16.2 ± 2.6**	38.30 ± 0.153**	28.01 ±0.06**
Lead(10mg/kg) + HEGS(200mg/kg)	207.16 ± 0.06^	17.37 ± 1.17^	39.03 ± 0.08^	30.56 ±0.19^

Values are expressed as mean ± SEM, *P< 0.01; significantly different from control.

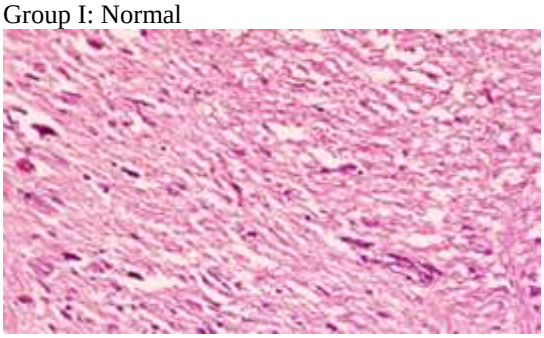
Figure 6: Effect of *Gloriosa superba* Extract on Acetyl Cholinesterase (AChE) Activity in lead induced Neurotoxicity Study

Groups	AChE activity (µmoles/mg protein)
Normal control	8.85 ± 0.08
Lead (10mg/kg)	3.54 ± 0.3^
Lead(10mg/kg) + vit E(10mg/kg)	6.1 ± 0.32*
Lead(10mg/kg) + HEGS(50mg/kg)	6.33 ± 0.03#
Lead(10mg/kg) + HEGS(100mg/kg)	6.79 ± 0.03##
Lead(10mg/kg) + HEGS(200mg/kg)	7.13 ± 0.03^^

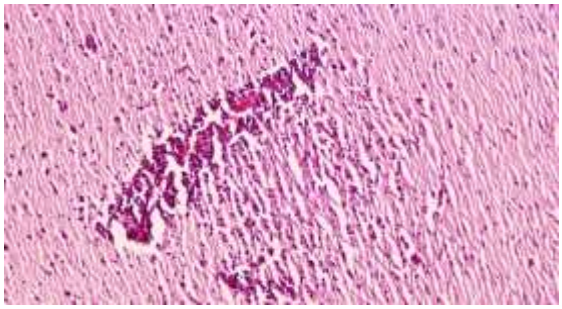
Values are expressed as mean ± SEM, *P< 0.01; significantly different from control.

Histopathological observations:

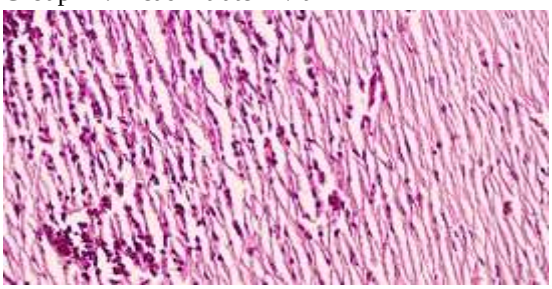
Group I: Normal



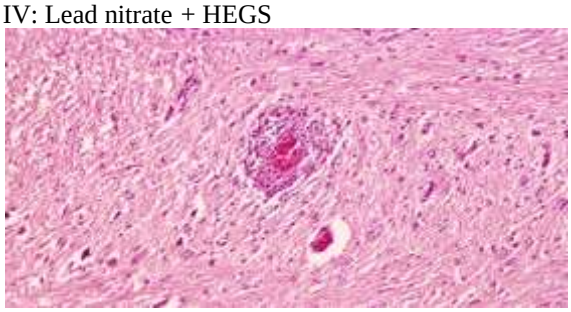
Group II: Lead nitrate



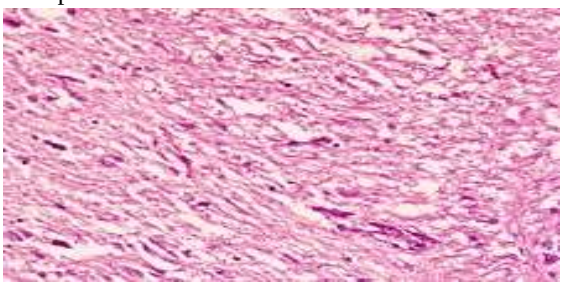
Group III: Lead nitrate + vit E



Group IV: Lead nitrate + HEGS



Group V: Lead nitrate + HEGS



Group VI: Lead nitrate + HEGS

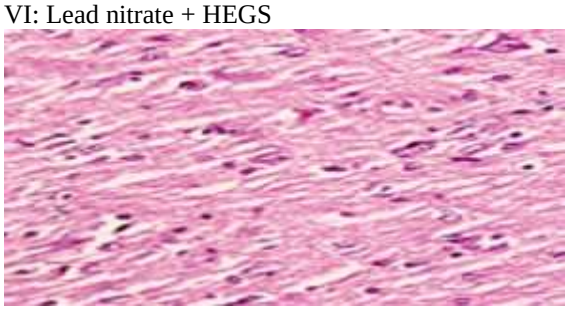


Figure 9: Effect of *Gloriosa superba* Extract on brain histopathology in lead induced Neurotoxicity Study

Group I: (normal control) In the normal control group the transverse section was found to be intact and no neuronal loss was observed. Tissue is stained with Haematoxylin and Eosin at magnification (100X).

Group II: (lead nitrate control) Gross histopathological changes including neurodegeneration and vacuolated cytoplasm was observed in lead group (10mg/kg). Tissue is stained with Haematoxylin and Eosin at magnification (100X).

Group III: (lead nitrate + vitamin E) Where as these changes were not found in hydroalcoholic extract of Lead nitrate + Vitamin E (10mg/kg + 100mg/kg). Tissue is stained with Haematoxylin and Eosin at magnification (100X).

Group IV: (lead nitrate + HEGS) Gross histopathological changes including neurodegeneration and vacuolated cytoplasm was observed in lead nitrate + HEGS (10mg/kg + 50mg/kg). Tissue is stained with Haematoxylin and Eosin at magnification (100X).

Group V: (lead nitrate + HEGS) Gross histopathological changes including neurodegeneration and vacuolated cytoplasm was observed in lead nitrate + HEGS (10mg/kg + 100mg/kg). Tissue is stained with Haematoxylin and Eosin at magnification (100X).

Group VI: (lead nitrate + HEGS) Where as these changes were not found in hydroalcoholic extract of Lead nitrate + HEGS (10mg/kg + 200mg/kg). Tissue is stained with Haematoxylin and Eosin at magnification (100X).

CONCLUSION:

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

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