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Neuroprotective effects of *Abrus precatorius* Linn. aerial extract on hypoxic neurotoxicity induced rats

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ABSRACT

The present study was carried out to evaluate the neuroprotective effects of petroleum ether extract of aerial parts of *Abrus precatorius* Linn. (100 mg/kg and 200 mg/kg) in hypoxic neurotoxicity induced rats. This was based on the use of *Abrus precatorius* for epilepsy in traditional oriental medicine. Hypoxia was induced by providing sodium nitrite drinking water to rats for 14 days. The extract at tested doses promoted spatial behavior significantly, which was impaired in hypoxic rats. The extract restored the decreased levels of enzymes such as glutamate, dopamine and acetylcholinesterases, which were reduced due to hypoxia. Thus, this study suggests that petroleum ether extract of *Abrus precatorius* posses neuroprotective effects in hypoxia induced neurotoxicity in rats.

Keywords: Neuroprotective, Hypoxia, *Abrus precatorius*, Neurotoxicity, Rats.

1. INTRODUCTION

Oxidative stress is implicated as one of the primary factors that contribute to the development of neurodegenerative diseases i.e. alzheimer, parkinsonism and neurological conditions like epileptic seizures, stroke, brain damage, neurotrauma, hypoxia etc. The highest degree of oxidative damage occurs in brain, heart and skeletal muscles as these organs are composed primarily of post mitotic cells. The central nervous system shows high susceptibility to oxidative stress because of its high oxygen consumption. Lack in oxygen leads to the generation of free radicals.[1] All the cells in our body are equipped with anti oxidative enzymes such as super oxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GRD) and reduced glutathione (GSH). They are generated for protecting the oxidative attack.[2-4]

Abrus precatorius Linn. of leguminosae family with local name Rati. It is a wild plant that grows best in fairly dry regions at low elevations and in tropical, subtropical climate with high climbing, twining woody vine with slender herbaceous branches. The leaves are glabrous with long internodes, alternate compound paripinnate with stipules. The flowers are small and pale violet in color. Fruits are scarlet in color with glossy texture.[5]

2. MATERIALS AND METHODS

2.1. Plant Material

The *Abrus precatorius* plant was collected from Dharmapuri, Tamil Nadu. The plant was identified by a botanist (Dr. V. S. Ramachandran), and voucher specimen was deposited in the Department of Botany, Bharathiyar University, Coimbatore. After authentication, aerial parts were cleaned, shade dried and milled into coarse powder by a mechanical grinder.

2.2. Preparation of aerial extract

The powdered aerial parts of *Abrus precatorius* (2 kg) were extracted with petroleum ether using a maceration process. The petroleum ether extract was filtered and concentrated by distillation process. A greenish colored residue was obtained (yield 6.79 % w/w), which was kept in a dessicator. This petroleum ether extract of APAE (*Abrus precatorius* aerial extract) was used for further experiments.

2.3. Experimental animals

Inbreed strains of *Wistar* rats of either sex weighing 150-200 g were taken from the animal house of Nandha College of Pharmacy and Research Institute, Erode, Tamil Nadu for the study. The animals were maintained in polypropylene cages of standard dimensions at room temperature 28±1^{o}C and standard 12 h day/night cycle. The animals were fed with standard rodent pellet diet (Hindustan lever Ltd.) and water *ad libitum*. Prior to the experiment, the animals were acclimatized to the laboratory conditions. All the experimental procedures and protocol used in this study were reviewed by Institutional Animal Ethics Committee (NCP/IAEC/PG-28/2009) and were in accordance with the CPCSEA guidelines respectively.

2.4. Preliminary phytochemical study

The preliminary phytochemical screening was performed in accordance with the procedures of Kokate *et al.* (2004).[6-7]

2.5. Acute toxicity studies

Acute toxicity study was performed according to organization for economic co-operation and development (OECD) guidelines. Male Swiss mice were selected by random sampling technique. The animals were fasted for 4 h with free access of water. *Abrus precatorius* aerial extract was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then only higher (50, 300, 2000 mg/kg) dose of *Abrus precatorius* extract were employed for further toxicity studies. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours. Based on the results of acute toxicity studies the doses were fixed as 100 mg/kg and 200mg/kg for further studies.[8-9]

2.6. Induction of hypoxia

Hypoxia was induced by administration of sodium nitrite drinking water (sodium nitrite 30 mg/kg dissolved in normal water) by gavages (5 ml/kg dosing volume) for 14 days except the control group, which was provided with normal water.[10-11]

2.7. Experimental design

The animals were divided into 4 groups of 6 rats in each as follows: Group 1 served as control and received normal saline. Group 2 served as hypoxic rats and received sodium nitrite water for 14 days. Group 3 animals received extract (100 mg/kg, p.o) suspended in 0.5% CMC and sodium nitrite water for 14 days. Group 4 animals received extract (200 mg/kg, p.o) suspended in 0.5% CMC and sodium nitrite water for 14 days.

2.8.1. Open-field test in rats

The apparatus consist of dimly lit green area, 96 × 96 cm, divided into 16 squares. Extract treated or vehicle treated rats were placed individually at one corner of the apparatus. The whole apparatus was elevated 25 cm above the floor in dimly illuminated room. Rats pretreated with extract or vehicle were placed individually in the centre of the maze facing the closed arm, and thereafter the number of entries and time spent in closed and open arms were recorded during the next 3 min. An

arm entry was defined as all four feet in the respective arm.[12-13]

2.9. Estimation of dopamine and metabolic enzymes

On the day of experiment the rats were sacrificed, the whole brain was dissected out and separated. The weighed quantity of tissue was homogenized in 0.1 mL hydrochloric acid- butanol, (0.85 mL of 37% hydrochloric acid in one litre of n-butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2000 rpm. Measured volume of 0.08 mL of supernatant phase was removed and eppendroff reagent tube containing 0.2 mL heptanes (for spectroscopy) and 0.025 mL 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions to separate the two phases. Upper organic phase was discarded and the aqueous phase (0.02 mL) was used for dopamine estimation.[14-15]

2.9.1. Estimation of dopamine

To the above aqueous phase 0.005 mL of 0.4 M hydrochloric acid and 0.01 mL EDTA/ sodium acetate buffer (pH 6.9) were added, followed by 0.01 ml iodine solution (0.1 m in ethanol). The reaction was stopped after 2 min by the addition of 0.01 mL Na_2SO_3 in 5 M NaOH (0.5 g Na_2SO_3 in 2 ml H_2O + 18 mL 5 M NaOH). Acetic acid (0.01 mL, 10M) was added 1.5 min later. The solution was then heated to 100^oC for 6 min. When the sample again reached room temperature, excitation and emission spectra were read in the microcuvette. The readings were taken at 330-375nm.[14-15]

2.9.2. Estimation of acetylcholinesterase (AChE)

About 20 mg of brain tissue per mL of phosphate buffer (0.1 M; pH 8) was homogenized in a potter elvehjem homogenizer. A volume of 0.4 mL aliquot of brain homogenate was added to the cuvette containing 2.6 mL of 0.1 M phosphate buffer. Exactly 100 µL of dithio-bis-nitrobenzoic acid reagent was added to the cuvette and the absorbance was measured at 412 nm and 20 µL of acetylcholine chloride was added. A change in the absorbance/min was calculated. It was then expressed in µmol/min/g tissue.[15]

2.10. Brain glutamate estimation

Weighed quantity of the brain portion was homogenized with two parts by weight of perchloric acid and centrifuged for 10 min at 3000 rpm. Supernatant liquid (3 mL) was adjusted to pH 9.0 with 1 mL phosphate solution. It was allowed to stand in an ice bath and filtered through a fluted filter paper. Absorbance was measured at 340 nm. Similarly a blank reading was measured at 340 nm. The level of glutamate was expressed as µmol/g tissue.[14-15]

3. RESULTS AND DISCUSSION

The preliminary phytochemical screening carried out on pettoleum ether aerial extract of *Abrus precatorius* revealed the presence of phytoconstituents such as alkaloids, carbohydrates, saponins, triterpenoids and flavonoids.

The extract did not produce any toxic effects or mortality up to the dose level of 2000 mg/kg body weight in rats. Hence the extract was considered as safe for pharmacological screening.

There was a decrease in mobility in negative control group in open-field test when compared with the control group ($p<0.001$). The groups treated with 100 mg/kg and 200 mg/kg of extract showed the significant increase in mobility ($p<0.001$). The hypoxia induced group indicated increase in time spent in closed arms and decrease in number of entries compared with the control group ($p<0.001$). The extract treated group 100 mg/kg and 200 mg/kg exhibited significant ($p<0.01$) increase in time spent in open arms and increase in number of entries in open arms. Induction of hypoxia significantly ($p<0.001$) increased the AChE activity and brain glutamate level when compared with the

control group. In the petroleum ether extract treated groups 100 mg/kg and 200 mg/kg, there was significant reduction in enzyme levels when compared with the hypoxia group. The dopamine levels in negative control group was significantly decreased ($p<0.001$) when compared with the control group. The levels of dopamine in 100 mg/kg and 200 mg/kg extract treatment significantly increased the reduced dopamine level ($p<0.01$)

The present study revealed the neuroprotective effects of petroleum ether extract of *Abrus precatorius* on sodium nitrite induced hypoxia deficits in rats. *Abrus precatorius* is a medicinal plant showing antiepileptic activity which serves as the basis for this study. During hypoxia, changes will occur in the diffusion parameters of the extracellular concentration of energy-related metabolites and glutamate in rat cortex. Hypoxia in rat cortex has shown decrease in space volume and in toxicity within few minutes followed by cardiac arrest. Glucose levels were critical in regulating redox state during the hypoxia; the cellular redox state was determined by increasing the reactive oxygen species (ROS). Supply of glucose increases cellular redox state and lowers the reactive oxygen and cell death.[16]

Table 1: Effect of *Abrus precatorius* on open-field test in rats

Groups	Number of squares crossed	Immobility	Rearing
Control	118±2.40	53.4±2.51	26.8 ±2.58
Toxic control	45.8±3.27[b]	92.6 ±7.05[b]	16.8± 1.30[b]
APAE 100mg/kg	66.0±3.03[a]	64.4 ±4.78[a]	21.2 ±1.64[a]
APAE 200mg/kg	97.8 ± 4.30[a]	55.2 ±3.35[a]	25.00 ±1.87[a]

Results are expressed in Mean ± SEM (n=6), P values are, a= p< 0.001, b= p< 0.01, c=p < 0.05.

Table 2: Effect of Abrus precatorius on elevated-plus maze test in rats

Groups	Number of entries		Time spent (sec)	
	Open arms	Closed arms	Open arms	Closed arms
Control	6.8±0.84	11.8±0.83	37.8±0.66	192.6±1.77
Toxic control	3.6±0.55[a]	7.8±0.83[a]	21.20±0.58[a]	178.8±0.58[a]
APAE 100mg/kg	8.6±0.54[a]	10.4±0.54[a]	35.00±0.54[a]	165.2±0.37[a]
APAE 200mg/kg	11.2±0.84[a]	13.6±0.52[a]	41.80±0.80[a]	158.2±0.80[a]

Results are expressed in Mean ± SEM (n=6);P values are a= p< 0.001, b= p< 0.01, c=p < 0.05.

Table 3: Effect of *Abrus precatorius* on brain enzymes

Groups	AChE (µmol/min/mg protein sec)	Glutamate(µmol/g tissue)	Dopamine(pg/mg tissue)
Control	16.99 ± 0.05	75.67 ± 0.01	664.35 ± 0.01
Toxic control	20.27 ± 0.03[a]	84.23 ± 0.02[a]	542.50 ± 0.008[a]
APAE 100mg/kg	18.61 ± 0.03[a]	80.66 ± 1.30[a]	580.80 ± 0.01[a]
APAE 200mg/kg	15.08 ± 0.06[a]	76.99 ± 0.01[a]	610.50 ± 0.04[a]

Results are expressed in Mean ± SEM(n=6); P values are a= p< 0.001, b= p< 0.01, c=p < 0.0

There was a decrease in mobility in negative control group in open-field test when compared with the control group ($p<0.001$). The groups treated with 100 mg/kg and 200 mg/kg of extract showed the significant increase in mobility ($p<0.001$). The hypoxia induced group indicated increase in time spent in closed arms and decrease in number of entries compared with the control group ($p<0.001$). The extract treated group 100 mg/kg and 200 mg/kg exhibited significant ($p<0.01$) increase in time spent in open arms and increase in number of entries in open arms. Induction of hypoxia significantly ($p<0.001$) increased the AChE activity and brain glutamate level when compared with the control group. In the petroleum ether extract treated groups 100 mg/kg and 200 mg/kg, there was significant reduction in enzyme levels when compared with the hypoxia group. The dopamine levels in negative control group was significantly decreased ($p<0.001$) when compared with the control group. The levels of dopamine in 100 mg/kg and 200 mg/kg extract treatment significantly increased the reduced dopamine level ($p<0.01$).

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Exposure to hypoxia alters the dendritic carbonization of hippocampal neurons and impairs the spatial behavior viz learning and memory. Hypoxia will affect the dendric morphology of the Cornu Ammonis1 neurons. Exposure to more days result in significant reduction in branching points, intersections and dentric length in most of the segments, significantly elevated levels of calcium and protein synthesis may lead to delayed neuronal death in CA1 region after hypoxia and this CA1 region is more vulnerable to hypoxia.[16-17]

Glutamate is a excitatory neurotransmitter in brain. Down regulation of glutamate transporter expression and activity was observed during hypoxia. GABA levels were correlated with endogenous glutamate levels during hypoxia; it increases GABA levels when glutamate level increases above normal level and vice versa. ATP depletion effects on the release and redistribution of glutamate and aspartate in rat hippocampal slices, glutamate is released during ATP depletion by reversal of co-transporters.[18]

The treatment with petroleum ether aerial extract ameliorated cognitive deficits in sodium nitrite drunken rats. In open-field test consumption of extract increased the mobility almost to the normal levels in dose dependent manner and in elevated plus maze task the consumption of extract increased the cognitive effects which was impaired by sodium nitrite water, thus neuroprotection play a role in favorable effect of *Abrus precatorius* on sodium nitrite induced cognitive effects.

The AChE activity has been shown to be increased in and around the hypoxia brain. The calcium influx followed by oxidative stress is involved the increase in the activity of AChE induced by sodium nitrite, decreasing cell membrane order and ultimately lead to the exposure of more active sites of the enzyme. Increasing in AChE activity and ROS production indicates that it can be possible to

ameliorate cholinergic function by inhibiting sodium nitrite, which induces increase in AChE activity.

The AChE activity in the brain was increased in rats treated with sodium nitrite when compared to normal.

4. CONCLUSION

In conclusion, this study suggests that markedly petroleum ether extract improves antihypoxic effects induced by sodium nitrite and this effect may be mediated by antioxidant mechanism thereby enhancing neuroprotection.

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