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Antialzheimer's Potential of *Abrus pectoris* hydro alcoholic root extract

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Abstract

Aim-The present study aims at the extraction of the flavonoid content from the dried root of *Abrus pectoris* using hydroalcoholic mixture and determining the anti-alzheimer's activity and antioxidant potential of the *Abrus pectoris* root extract (APRE).

Methods-Evaluation of anti-alzheimer activity of *Abrus precatorius root* extract using *D-Galactose* model.
Determination of Behavioural pattern of experimental animals during the study period.
Estimation of Acetyl cholinesterase (AChE) levels and Acetylcholine (Ach) levels.
Estimation of antioxidant parameters like Lipid peroxidation, Superoxide Dismutase, Catalase, and Glutathione reductase.

Results-Simultaneous treatment of D-Galactose and *APRE*, in the protective groups III & IV had shown a significant increase in total body weight. Decreased transfer latency in the elevated plus maze, morris water maze by the APRE treatment groups may be because of memory enhancement. A significant increase in ACh levels, decrease in the AchE level was observed in protective groups III & IV. After APRE supplementation for 90 days a significant decrease ($0.46 \pm 0.018^{***}$) in LPO level as well as a significant increase in SOD, CAT and GSH levels has been observed which indicate the antioxidant status of APRE. So, based on the above results, APRE can be used in the treatment of alzheimer's disease.

Key words: treatment, transfer latency, memory, antioxidant, alzheimer's

1. INTRODUCTION

Alzheimer disease (AD) is characterized by a progressive decline in cognitive function. AD shows more impact on the people aged 65 years or more, with a progressive decline in memory, thinking, language and learning capacity.

Alzheimer's disease, a progressive neurodegenerative disorder majorly characterised by memory impairment, cognitive dysfunction, behavior disturbances and deficits in activities of daily living. As stated by the Alzheimer's Association, in India, the prevalence of Alzheimer's disease is about 4 millions, and all over the world it is 44 millions. Generally, the drugs used in the Alzheimer's like donepazil will alter the hemoprofile, and also affects the rhythm of the heart. Usually, the elder persons will have decreased age based functioning of the heart. So, the prolonged use of this antialzheimer medications in turn increases the cardiovascular complications which in turn may worse the cardiovascular functions in the body[1,2]. To prevent the unwanted effects of the antialzheimer's drugs, as the medicinal plants have been traditionally used in the treatment of several human disease, plants with the antioxidants can be a great source to treat various degenerative diseases apart from the plant cholines also favour the synthsesis of Ach in brain thereby improves the cognitive and memory functions of the brain[4,5].

Abrus Precatorius commonly known as Rosary Pea, Gunja and Jequirity peas,consists of choline and other biological constituents having therapeutic potential. Various parts of *A. precatorius* are having different pharmacological activities like anti-diabetic, neuroprotective, anti-viral, neuromuscular, and anti-convulsant activities etc[6,7].

In view of that, from the *Abrus precatorius* plant having many pharmacological actions, the root part was selected, planned and aimed to evaluate the anti-alzheimer activity, using the *in-vivo* experimental methods.

2. MATERIALS AND METHODS:

2.1 Extraction methodology

The dried root powder of *Abrus Pectoris* was collected from government Ayurveda college, Vijayawada. *Abrus precatorius* root powder was extracted with hydroalcohol (30:70) at a temperature not exceeding 60° C. The obtained *Abrus precatorius* root extract (APRE) was concentrated with the help of an evaporator to yield a crude semi-solid mass. The resultant semi-solid extract was dried, weighed, labelled and stored in a dessicator.

2.2 Phytochemical analysis

2.2.1 Qualitative phytochemical analysis

Qualitative chemical tests were carried out for hydroalcoholic extract of *abrus pectoris* root (APRE), to identify different phyto-constituents. Tests for alkaloids, flavonoids, saponins, steroids, tannins and triterpenes are performed.

2.3 Evaluation of antialzheimer's activity

2.3.1 Drugs and chemicals

Drugs and Chemicals used in this study were of analytical grade and of highest purity. It include D-Galactose, Acetylcholine iodide, Di Thiobisnitrobezoic Acid (DTNB), hydroxylamine hydrochloride, Ferric chloride, EDTA

2.3.2 Animals

Healthy adult albino wistar rats weighing 200-250grams of either sex were selected for the study. Animals were housed in individual cages under standard laboratory conditions and fed with standard pellet diet and water ad libitum. They were fasted overnight before the day of experiment. Animals were housed within the departmental animal house and the room temperature was maintained at 27°C.

2.3.3 Treatment Schedules

Group I treated as disease control, receives D-Galactose (120 mg/kg, p.o). Group II treated as positive control, receives Rivastigmine (0.3 mg/Kg, p.o). Group III and IV are treatment groups, receives the *Abrus precatorius* root hydroalcoholic extract (APRE) at 150 and 300 mg/kg, p.o respectively. All groups should receive D-Galactose followed by Rivastigmine and APRE administration for 90 days in respectively.

2.3.4 Biological effect of D-Gal induced Alzheimer's Disease

Old Age can be induced by oral or Intra peritoneal (IP) injection of D-Galactose, a reducing sugar, which reacts readily with the free amines of amino acids in proteins and peptides both *in-vivo* and *in-vitro* to form Advanced Glycation End-products (AGE) through non-enzymatic glycation[9].

The Advanced Glycation End-product activates its receptors, which are coupled to biochemical pathways that stimulate free radical production. D-Galactose ($C_6H_{12}O_6$), is a physiological nutrient, but over supply of D-Galactose will result in abnormality of metabolism. The oxidative metabolism of D-Galactose produces advanced glycation end products[10].

Reactive Oxygen Species (ROS), which surpass the ability of the cells to eliminate them, consequently causing impairment of cellular membrane, structure and gene expression. Prolonged intake of D-Galactose accelerate the ageing process, influencing the age-related cognitive decline in experimental animals, due to generation of ROS and induces mitochondrial dysfunction.

In addition, prolonged supplementation of D-Galactose into experimental animals leads to the reduction of nerve growth factors expression and its related proteins which is associated with the degeneration of nerve cells and finally reduced the levels of acetylcholine in brain regions.

Thus, long-term oral or intraperitoneal injection of D-Galactose induces AD in normal rat. In the present study the experimental duration selected was 90 days. D-Gal was given for first 14 days period to observe AD symptoms with the assessment of cognitive skills in rats (AD group). Further AD induced rats were again treated with D-Gal as well as APRE simultaneously through the study period of 90 days. To observe the cognitive skills, the rats were subjected to behavioural studies on selected days.[11,12]

2.3.5 Behavioural Aspects [14]

2.3.5.1 Elevated plus-maze

Elevated plus-maze served as the behavioral model for external stimulation related memory evaluation in mice. The procedure, technique and endpoint for testing memory were followed as per the parameters described by the investigators working in the area of psychopharmacology. The elevated plusmaze for mice consisted of two open arms (16cm×5cm) and two covered arms (16cm×5cm ×12cm) extend from a central platform (5cm×5cm), and the maze was elevated to a height of 25cm from the floor. On the study day, the experimental animals were placed on the open arm, away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arm with all its four legs. TL was recorded on the first day (training session) for each animal. The animals were allowed to move through out the maze and then returned to its home cage. Retention of this learned task (memory) was examined 24 hours after the first day trial and on the selected days to check the improvement in cognitive function. Noticeble decline in the TL value of retention indicated improvement in memory.

2.3.5.2 Morris water maze test

Behavioural experiments were performed by using the water maze which was originally designed to test the learning and memory ability in rodents. The tank was filled with water (21-26°C) up to a height of 30 cm and the transparent escape platform made measuring 10 cm in diameter and 29 cm in height was hidden 1.5 cm below the surface of water in a fixed location. Water was made opaque by mixing with powdered non-fat milk. The platform was not visible from just above the water level and transfer trials have indicated that escape on to the platform was not achieved by visual or other proximal cues. The time spent by the animal to reach the hidden platform was called as the Escape Latency and used as the index of memory[15].

2.3.5.3 Y-maze test

Y-maze test was used to determine the short-term memory of mice by recording spontaneous alternation in a single session. The maze used in this study was a Y-maze made of polywood with three identical arms (35 cm length × 8 cm height × 15 cm width) mounted at 120 degrees to one another in a single piece. After the corresponding treatments, the animals are placed at the end of one arm and were allow to move freely through the maze for 8 min. The movement of entry in each arm was recorded for every mouse. For an entry to be counted, the animal should be place all of its four paws inside the arm path. The movement animal into the arm indicates the general locomotor activity. The arms of the maze were cleaned between sessions with 10% ethanol.

2.3.5.4 Organ weight

The various organs under study were excised from the animal and weighed. The weights of the organs such as liver, heart, lungs, thymus glands, spleen, adrenal glands, kidneys, testes, uterus and ovaries were recorded and studied for any abnormal gain or loss of weight. This gives a preliminary confirmation regarding the adverse effects (if any) of the drug under test.

2.4 Biochemical Estimation of Cholinergic System

2.4.1 Estimation of Acetylcholine

The rat brain regions such as Cerebral Cortex (CC) and Hippocampus (HC) were weighed accurately, transferred to test tubes and placed in a boiling water bath for 5 minutes to terminate the Acetylcholinesterase enzyme activity and also to release the bound ACh. Then the tissues were homogenized in 1ml of distilled water. To the homogenate, 1 ml of alkaline hydroxylamine hydrochloride was added followed by 1 ml of 50 % hydrochloric acid solution. The contents were mixed thoroughly and centrifuged. To the supernatant, 0.5 ml of

0.37 M ferric chloride solution was added and the brown colour developed was read at 540 nm against a reagent blank (1 ml of alkaline hydroxylamine hydrochloride +1 ml of 50 % hydrochloride + 1 ml of distilled water + 0.5 ml of 0.37 M ferric chloride solution) in a spectrophotometer. The Acetylcholine content was expressed as µ moles of ACh/gm wet weight of tissue.

2.4.2 Estimation of Acetyl cholinesterase

Acetyl cholinesterase activity as estimated as below. 10% homogenates of different regions of rat brain were prepared. The reaction was started with the addition of 100 µ liters of homogenate to the reaction mixture containing 3.0 ml of phosphate buffer (PH 8.0) + 20 µ moles of substrate (0.075M) + 100 µ moles of Dithiobis Trinitrobenzene (DTNB, 0.01M). The contents were incubated at 37°C for 15 minutes. The developed colour was read at 412 nm in a spectrophotometer against a reagent blank containing 3.0 ml of phosphate buffer (pH 8.0) + 20 µ moles of substrate (0.075M) + 100 µ moles of Dithiobis Trinitrobenzene (DTNB,0.01M). The enzyme activity was expressed as µ moles of ACh hydrolysed mg protein/hour.

2.5 Antioxidant System

2.5.1 Estimation of lipid peroxide levels (LPO)

Blood plasma of 0.2 ml was mixed with 1 ml of 20% acetic acid; subsequently 0.2 ml of 8% SDS was added to the above mixture and pH was adjusted to 4. Following that, 1.5 ml of 0.8% TBA and 1.1 ml of distilled water was added. This reaction mixture was incubated in a boiling water bath for 1 hour. After cooling, 3 ml of n-butanol was mixed, and then centrifuged at 10,000 g for 15 minutes. A clear butanol fraction thus obtained was used for measuring the absorbance at 532 nm [16]].

2.5.2 Estimation of superoxide dismutase (SOD)

The supernatant from the above extraction step was divided into two portions, experimental and reference. To the experimental test tube we added 0.3 ml Nitroblue tetrazolium (NBT), 0.2 ml Phenazine Metho Sulphate (PMS) and 0.2 ml pyrophosphate buffer, 1 ml D.D.W, and 2 ml enzyme for analysis. In the reference test tube, everything was added along with NADH except the enzyme.The reaction was run for 90 seconds at 37°C with constant stirring. The reaction was stopped by adding 1 ml acetic acid. After 10 minutes, the enzyme was added in the reference test tube and optical density (OD) was read at 560 nm. Blank included NBT, PMS Buffer, and Triple Distilled Water [20]

2.5.3 Estimation of reduced glutathione (GSH)

One milliliter of the supernatant after ethanol and chloroform extraction was mixed with 1 ml DTNB reagent just before measuring the absorbance of the sample at 412 nm. GSH solution of known concentration was similarly processed to prepare a standard curve. The amount of GSH in the sample was determined from the standard curve [20, 22]

2.5.4 Estimation of Catalase activity

2000 µl Hydrogen peroxide, 6 ml Working reagent,1000 µl sample were taken and the test tubes were vortexed and incubated at room temperature for 10 min, and absorbance 153 was read at 550 nm[17-22]

2.6 Statistical Analysis

The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant at $F < 0.05$ for ANOVA and $P < 0.05$ for t-test.

3. Results And Discussion

3.1 Morphological Features

The observed body weight of all the animals in Alzheimer's inducer Group I was less than that of other treatment groups. There was 12% variation among group I and II, 10% variation among group I and IV for 60 days where there is a 17.5 % variation among group I and II and 15.4% variation among group I and IV for 90 days. When compared with the control group, AD-model rats showed gradual loss of hair, skin elasticity which became stiff, thin and saggy. Simultaneous treatment of AD induced group with *APRE* in the protective groups III & IV had shown a significant increase in total body weight, when compared with the AD-model group.

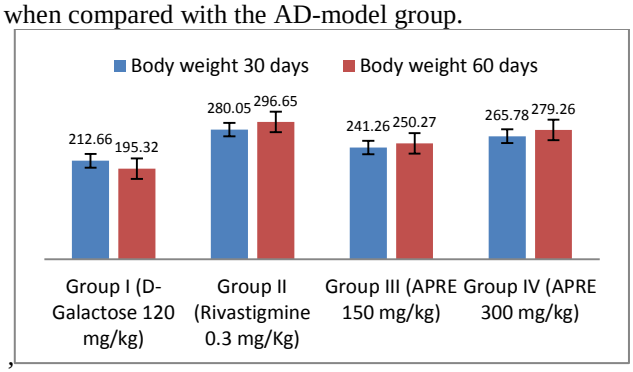

Figure 1: Morpological variation after APRE treatment

3.2 Visceral organ Index:

The organ index of protective groups III and IV, exhibited better gain in organ weight than that of AD-model group. The visceral organ weight was found to be slightly increased in all the treatment groups, there was a slight variation of weights in between 60th day and 90th day of the study.

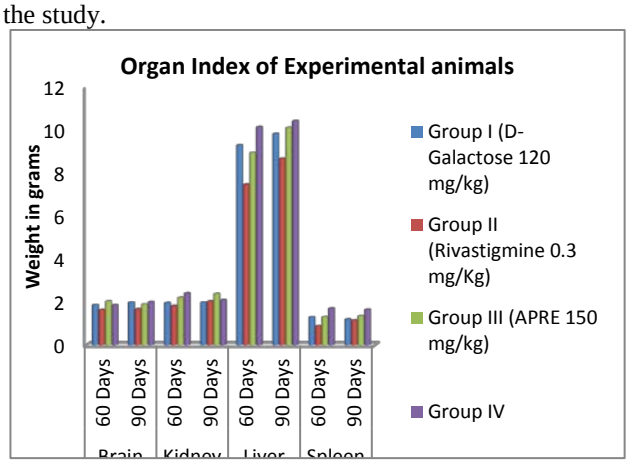

Figure 2: Organ Index

3.3 Behavioural Aspects

3.3.1 Elevated plus maze

Galactose had shown increase in the transfer latency compared with the control group, may be because of increased spontaneous movement. The increased TL is an indication of memory impairment. The decreased TL in the standard and the treatment groups may be because of inhibition of spontaneous activity produced by galactose by the corresponding treaments.

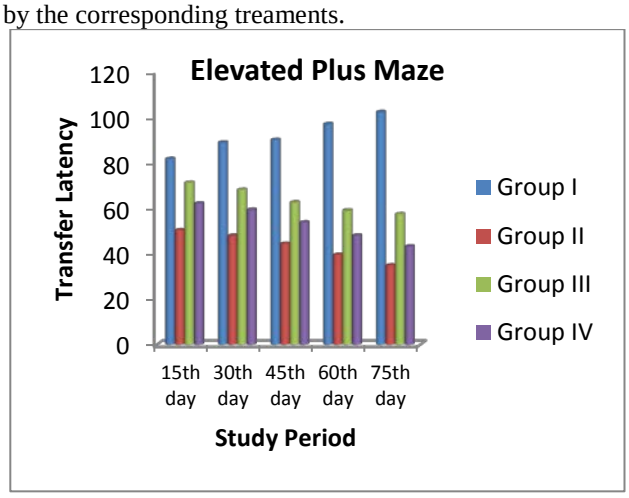

Figure 3: Elevated Plus Maze

3.3.2 Morris Water Maze Test

There is an increase in escape latency and decrease in the time spent on the platform in case of D-Galactose treated negative control group when compared with the positive control group (P<0.01). There is an decrease in escape latency and increase in the time spent on the platform in case of APRE treated animals (P<0.01). The group treated with 150 & 300 mg/kg APRE showed the significance of (P<0.01 andP<0.001) respectively. There was a decrease in escape latency and increase in time spent on the platform during testing on selected days that indicate improvement of memory by APRE.

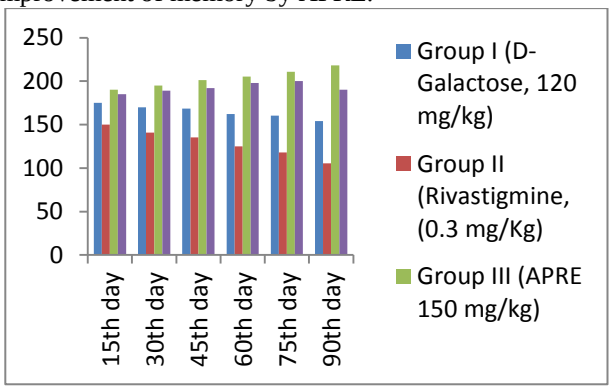

Figure 4: Morris Water Maze Test

3.3.3 Effect of *Abrus pectoris* on Y Maze

The total number of arm entries reflects general locomotor activity. The amnesia induced group (negative control/AD group) indicates increase in total number of arm entries (P<0.01). The treatment groups had shown significancant results by (P<0.01) decrease in the alternation of behaviour in group III, 150 mg/kg of APRE and Group IV 300 mg/kg of APRE when compared with that of the negative control group.

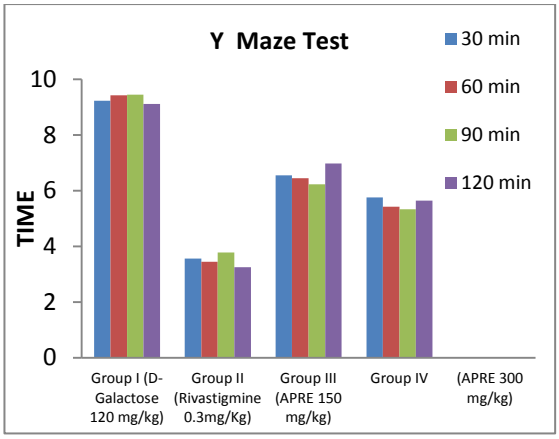

Figure 5: Y Maze Test

3.4 Biochemical Estimation of Cholinergic System

3.4.1 Estimation of Acetylcholine

As compared with control group rats, the ACh content significantly declined in the AD-model group rat. A significant increase in ACh levels was observed in protective groups III & IV when observed at the end of the study. Treatment with *APRE* had shown a significant elevation of ACh in both cerebral cortex and hippocampus.

3.4.2 Estimation of Acetylcholinesterase activity

When treated with APRE, there was an increase in Acetylcholinesterases in the inducer group (167 ± 0.45), which inturn decreases Ach levels in the brain. In the positive control (84 ± 0.09) and treatment groups, there was decrease in AchE, which further decreases degradation of Ach and increases actions of Ach like enhancement of memory. Less the AchE, more will be the cholinergic activity and increase in learning and memory.

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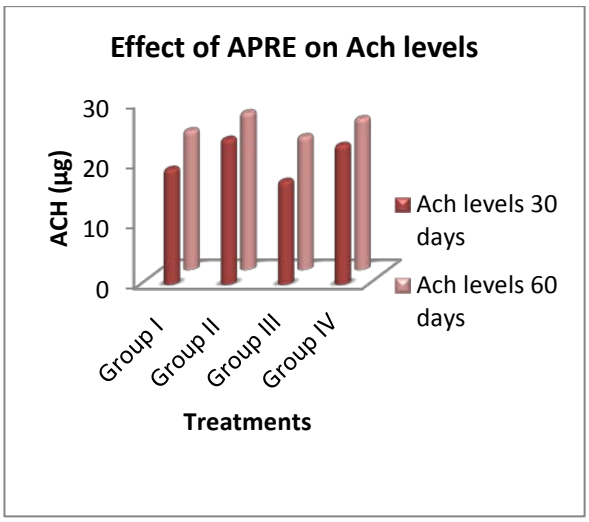


Figure 6: Ach levels

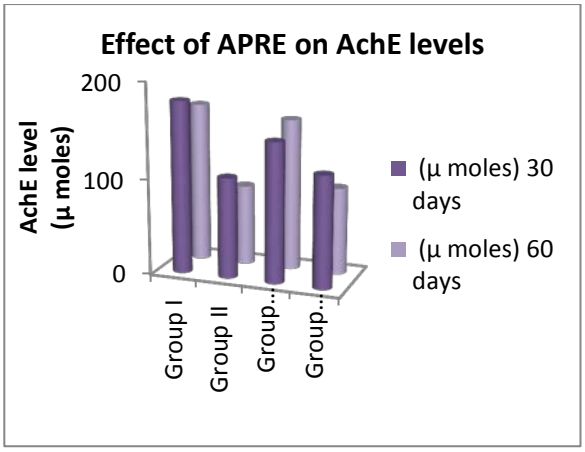
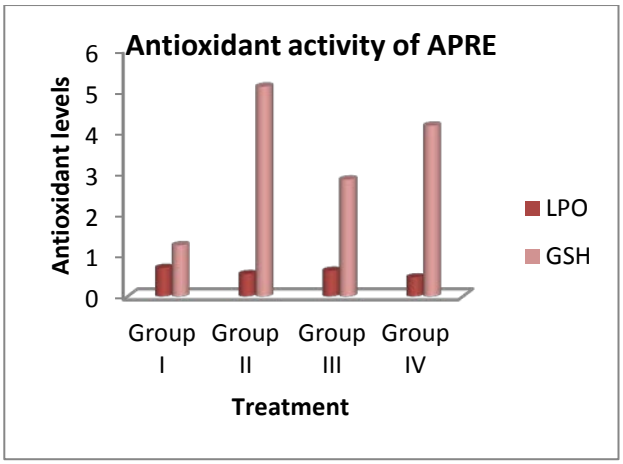


Figure 7: AchE levels

3.5 Antioxidant activity of *Abrus pectoris*

Oxidative stress can simply inactivate anti-oxidant enzymes *i.e.*, SOD, CAT, GSH and induces oxidative stress which in turn causes lipid peroxidation. After APRE supplementation for 90 days a significant decrease ($0.46 \pm 0.018^{***}$) in LPO level as well as a significant increase in SOD, CAT and GSH levels has been observed which indicate the efficacy of plant extract in attenuating antioxidant status and play an important role in prevention of incidence of alzheimer's or nerve degeneration. Values are expressed as Mean ± SEM of 6 rats in each group.



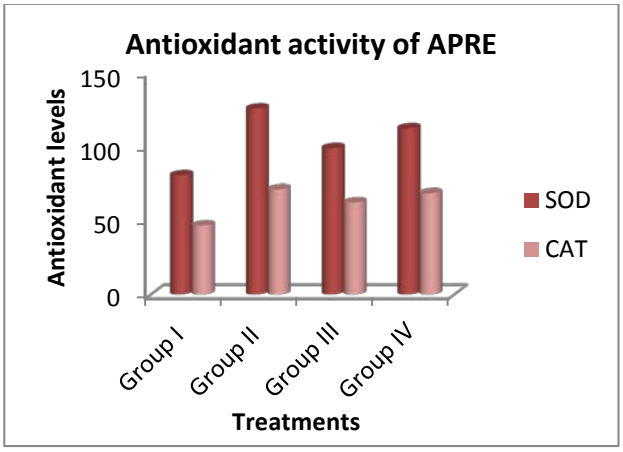


Figure 8: Antioxidant status

4. SUMMARY

The dried root powder of *Abrus Pectoris* was collected from government Ayurveda college, Vijayawada. *Abrus precatorius* root powder was extracted with hydro-alcohol (30:70) at a temperature not exceeding 60° C. Extracts were tested for their anti-Alzheimer's activity in rats. APRE was found to consist the constituents like flavonoids, alkaloids, saponins, phenols and tannins. Morphologically, there was an increase in the body weight and organ index of the treatment groups. Behavioral tests indicate the improvement of memory produced by APRE. Hydroalcoholic extract of APRE (150 and 300 mg/kg) had shown an increase in Cholinergic system that improves the cognition. APRE had improved the antioxidant status, and thereby favours the stress induced AD.

5. CONCLUSION

Abrus pectoris, a rosary pea is having many pharmacological uses. Though leaves, seeds are evaluated before, the pharmacological actions of root was not clearly established. Our present study indicates the presence of alkaloid in the hydroalcoholic root extract, that had the potential to improve the condition of Alzheimer's disease. The future study should be planned to elucidate the specific constituents from the root and to establish the complete therapeutic profile of *Abrus pectoris roots.*

Conflict of Interest

There are no conflicts of interest.

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