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Research Article

EFFECTS OF HYDROALCOHOLIC EXTRACT OF *PARKINSONIA ACULEATE L.* SEEDS AND *ANANAS COMOSUS* FRUITS ON ROTENONE INDUCED PARKINSON'S DISEASE IN RATS

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ABSTRACT

In present study Hydroalcoholic extracts of Parkinsonia aculeata L. seeds and Ananas comosus L. fruits were examined to assess for effectiveness against the rotenone induced Parkinson symptoms. Hydroalcoholic extracts of both the plants were prepared. Formulation was prepared using suitable suspending agent and suspension was subjected to evaluation of organoleptic property and physicochemical property. Rotenone (1.5 mg/kg, s.c, dissolved in dimethyl sulfoxide and sesame oil) was given in animals on alternate day for 28 days after 30 min of administration of extracts. The treatments were given daily starting from the first day of rotenone injection and continued thereafter for a total period of 28 days. Behavioural scores, catalepsy score and locomotion activity were determined for each animal before the start of experiments and then regularly at the interval of 7 days and last observation were done after the 24 hrs. of last dose. Behavioural score and catalepsy score were increase at each weekly interval in control group, where as administration of Parkinsonia aculeate L. seed extract at the dose 200 mg/kg and Ananas comosus L. at the dose 250 mg/kg were able to reverse the both the score to significant level ($P < 0.05$) while locomotor activity was reduced in control group and that was increased by administration of both the plant extracts. While formulation containing both the plant extracts were able to reverse both the score at $P<0.01$ and locomotor score were increased by administration of formulation. From the present study we can concluded that hydroalcoholic extracts of both plants were effective against the rotenone induced parkinsonism.

Keywords: Rotenone, Parkinson disease, Phytoconstituents, Parkinsonia aculeata L., Ananas comosus L.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease that occurs due to progressive damage to the Dopaminergic neurons in the nigrostriatal tract of the brain. Damage or loss of dopaminergic neurons in this brain region results in the depletion of dopamine from terminals in the striatum involved in coordinating smooth movement. The disease generally affects persons aged 55–64 years, although occasionally much younger individuals are affected. Although the causes for degeneration of dopaminergic neurons in Parkinson's disease are not well understood, emerging data from recent studies show that any sustained adverse interaction between neurotoxins arising from environmental, dietary and lifestyle factors, or from normal metabolism influenced by genetic factors could initiate degeneration in dopaminergic neurons. Epidemiological studies suggest that PD is the second most common neurodegenerative disorder after Alzheimer's disease (AD) affecting ~ 2 – 3 % of the population over the age of 60 years. PD cases are far more unusual in people under the age of 40, and usually these so-called juvenile onset types associate with a clear genetic origin, while non-genetic disease types are principally considered a disease of the elderly. The average age of onset for the sporadic, non-genetic form of PD is approximately 55-60 years, with the rate of PD rising sharply after the fifth decade Among persons over age 65 the prevalence of PD has been

estimated at 1800 per 100,000 (1.8%) individuals, increasing from 600 per 100,000 (0.6%) for persons between the ages of 65 and 69 to 2600 per 100,000 (2.6%) for those 85 to 89 years [1, 2].

Several biochemical abnormalities have been identified in the PD brain. These are due to oxidative stress and damage, mitochondrial dysfunction and evidence of inflammatory change. The major neuropathological change in PD is the loss of the pigmented dopaminergic neurons in the substantia nigra with degeneration of the nigrostriatal tract. This neuronal loss leads to marked decreases in the concentrations of striatal DA, the DA-synthesizing enzymes tyrosine hydroxylase and DOPA decarboxylase, and the DA metabolites homovanillic acid, dihydroxy phenylacetic acid and 3-methoxytyramine[3].

Several groups have demonstrated evidence for increased free radical generation in the PD brain. Such changes include a decrease in reduced glutathione (GSH), an increase in the reduced and oxidised glutathione ratios (GSH/GSSG), increased activity of superoxide dismutase and increased levels of malondialdehyde and lipid hydroperoxides. Increased level of protein carbonyls and of free radical damage to DNA further support free radical damage to proteins in Parkinson disease. These changes appear to be most severe in the substantia nigra but can also be seen in some other areas of the PD brain. The cause of increased free radical release in PD is not known. Enzymes involved in the metabolism of glutathione do not appear to be significantly affected [3].

Parkinsonia aculeata L., is a large spinous shrub or small tree, native of America found throughout the drier part of India and commonly known an 'Vilayati Kikar". Parkinsonia aculeata is a tree from the family Fabaceae; common names include Mexican Palo Verde, Parkinsonia, Jerusalem thorn, or Jellybean tree [4]. Plantation of this species is raised in arid and semi arid area tract of western Uttar Pradesh, Rajashthan and Gujarat [5]. Pods are linear, torulose, striated, dehiscent 5 to 15 cm long and constricted between the oblong dark brown seeds which are 0.90 cm long pointed. Seeds are 1-5 in number, bean like and oblong in shape, 1 cm long, dark brown in colour. [6] Previous investigations showed that the leaves from the plant contains orientin, iso-orientin, vitexin, isovitexin, lucenin-II, vicenin-II, diosmetin 6-C-β-glucoside, apigenin, luteolin, kaempferol, chrysoeriol, epiorientin, parkinsonin-A, parkinsonin-B, and parkintin [7-10].

Ananas cosmosus L. is fruit bearing plants form the family Bromeliaceae and commonly known as pineapple. It contains the various pharmacologically active phytoconstituents such as ananasate, beta-sitosterol, Chlorogenic acid, rutin, naringenin, bromelin, glysocides, flavonoids and neurotransmitters [11-13]. Current pharmacotherapeutics for Parkinson's disease are L- dopa, carbidopa, Mono-amino oxidase inhibitors and anti-cholinergic drugs. All of these can possess some sort of adverse effects on long term use. Current treatment methods are focused only on relieving symptoms and delaying progression of the disease. To date, there is no known cure for PD, making prevention of PD as important as ever. Medicinal plants that are anciently used in treatment in various ailment form human mankind and it possess less adverse effects. Present study was undertaken to evaluate the efficacy of Parkinsonia aculeata L. seeds and Ananas cosmosus L. Fruits on parkinson's disease in rats.

MATERIAL AND METHODS

PROCUREMENT AND AUTHENTIFICATION OF HERBS

Fruits of Ananas Comosus L. were obtained from the local market and seeds of Parkinsonia aculeata L. were procured form the road side area near, Atkot region, Ta: Jasdan, Rajkot, Gujarat, India. Plants were authenticated at Department of Pharmacognosy Smt. R. B. Patel Mahila Pharmacy College-Atkot.

EXTRACTION

Fresh fruits and seeds were washed under running tap water followed by washing with distilled water to remove the surface debris. 250 g of peeled fruit pulps and seeds coarse powder were weighed and minced separately using a mixer grinder for fine maceration. The ground fruit and seeds powder then homogenized separately and extracted in 500 ml of methanol: water (1:1) as solvent for 7 days in dark at room temperature with intermittent shaking. After 7 days, the whole extracts were filtered using muslin cloth at first and then through a filter paper. The filtrate was then concentrated and stored in desiccators for 3 days then preserved in a deep freezer at -4°C [14].

PREPARATION OF SUSPENSION OF BOTH THE PLANTS EXTRACTS

Liquid suspension was formulated using hydroalcoholic extracts of Parkinsonia aculeata L. and Ananas comosus L. which had shown antiparkinson's activity. Combination of Extracts had shown the beneficial action thereby extracts were formulated as liquid suspension and tested for antiparkisnon's activity. The liquid formulation was prepared according to the following formula.

The 120 mesh size fine particles of the extracts were properly mixed by triturating. The powdered forms of extracts were wetted thoroughly with glycerine solution to reduce liquid–air interfacial tension. The suspending agent in the aqueous medium containing selected preservatives was then added into the wetted mass slowly, with continuous triturating. The formulation was prepared by using different concentrations of the selected plant extracts Parkinsonia aculeate L. (5.0 g), Ananas comosus L. (6.4 g). Finally the suspension was made up to the final volume with purified water by continuous trituration so as to get uniform product. The prepared suspension was then subjected to evaluation as per official standards.

Table 1: Preparation of formulation

Sr. No.	Name of component	Quantity
1	Hydroalcoholic extract of *Parkinsonia aculeate L.* seed extract.	2.5 gm
2	Hydroalcoholic extract of *Ananas comosus L.* Fruits extract.	3.2 gm
3	Sodium CMC	5.0 gm
4	Glycerine	5.0 ml
5	Methyl paraben	0.8 gm
6	Distilled water	Up to 100ml.

EVALUATION OF SUSPENSION

Prepared suspension was subjected to organoleptic evaluation such as color, odour and taste. Formulated suspension was subjected to sedimentation in stoppered measuring cylinder at the interval of 30 min. Up to 5 hrs. The suspension were placed in 100 ml. Graduated cylinder and rotated at 360 0 for 10 min. At the end uniformity of particle were observed. Particle size of suspension was evaluated by microscopically using stage and eye piece micrometer. About 100 particles were measured and average mean were calculated. Viscosity of suspension was measured by brook field viscometer and pH of suspension was measured by digital pH meter. [15]

EFFECTS OF EXTRACTS ON ROTENONE INDUCED PARKINSON'S DISEASE

Animals

The albino rats (200-220 gm) of either sex were used in the study. The study was performed in accordance with the guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol (RBPMPC/2012-13/IACE/07/02) was approved by institutional animal ethics committee.

Rotenone Induced Parkinson Disease

The exposure to pesticides like rotenone appears to correlate strongly with increased incidence of PD [16, 17]. The treatments were given daily starting from the first day of rotenone injection and continued thereafter for a total period of 28 days. Rotenone (1.5 mg/kg; s.c., dissolved in dimethyl sulfoxide and final volume make with sesame oil) was administered on alternate day of 28 days after 30 min of administration of extracts. Severity of rotenone induced motor abnormalities was evaluated using a quantitative neurological scale [18].

The animals were divided into five groups, each of contained six animals.

Group-I Normal control group (Vehicle treated group)

Group-II Disease control group [treated with Rotenone (1.5 mg/kg, s.c.)

Group-III Hydroalcoholic extract of (ACEX 250 mg/kg) + Rotenone (1.5 mg/kg, s.c.)

Group-IV Hydroalcoholic extract of (PAEX 200 mg/kg) + Rotenone (1.5 mg/kg, s.c.)

Group-V Test formulation containing both extracts + Rotenone (1.5 mg/kg,sc.)

General Behaviour Analysis

A general behavioural score was determined for each animal before the start of experiment and then regularly at an interval of 7 days before the dosing and last behavioural quantification were done after 24 hrs of last dose. Scoring was done according to the following scheme:

Score 0: Normal behaviour, Score 1: General slowness of displacement resulting from mild hind limb impairment,

Score 2: In-coordination and marked gait abnormalities,

Score 3: Hind limb paralysis,

Score 4: Incapacity to move resulting from forelimb and hind limb paralysis, and

Score 5: Recumbency.

Catalepsy Score

Catalepsy score was measured by using bar test. In the bar test, the animal was placed with both the front paws on a horizontal bar 3 cm above and parallel to the base in half rearing position. The amount of time spent with at least one forepaw on the bar was determined. When the animal removes its forepaw, the time was recorded. [19]

Locomotor Activity

Actophotometer was used to measure locomotor activity. Animals were initially allowed to get acclimatize in the chamber for a period of 2 min and then their locomotor activity was monitored for next 5 min. Total locomotor activity was calculated as mean of photo beam counts per 5 min/animal. [19]

Statistical Analysis

All the data were expressed as Mean ± SD. Statistical analysis was carried out using the one-way ANOVA with dunnet's test. The data was evaluated with use of computer software.

RESULTS

EVALUATION OF SUSPENSION

Prepared suspension had pleasant appearance and acceptable odour, colour and taste. As per the table 2 and table 3 average particle size of suspension was found to be 28.02 µm, viscosity of prepared suspension was found to be 59.23 where as pH was 5.03 and formulated suspension exhibit good redispersibility.

EFFECT EXTRACTS ON ROTENONE INDUCED BEHAVIOURAL CHANGES

As per the Figures-1 control group (II) animals shown the abnormal motor coordination during the weekly observation and at the end of the treatment they shown higher behavioural score. In Group III and Group IV animals treated with hydroalcoholic extracts of Fruits of Ananas comosus L. and seeds of Parkinsonia aculeate L. at the dose of 250 mg/kg and 200 mg/kg respectively exhibits improvement in motor coordination by reducing the behavioural score at significant level($P<0.05$). The observation in group was comparatively increased during every week intervals. While in group V animals treated with formulation of both the plant extract in the ration of 1:1 shows reduction in behavioural score during each week time period at the significant level ($P<0.01$).

EFFECTS OF EXTRACTS ON ROTENONE INDUCED CATALEPSY SCORE

According the data of Figure 2 at the starting day there was no any significant changes in catalepsy score amongst the all group. While in group II control group at 7th day and all consecutive days' catalepsy score was increased representing the motor disturbance. In Group III and Group IV animals were treated with hydroalcoholic extracts of Fruits of Ananas comosus L. and seeds of Parkinsonia aculeate L. at the dose of 250 mg/kg and 200 mg/kg respectively shows improvement in motor coordination by reducing the catalepsy score at significant level ($P<0.05$) compared to control group II. The observation in both groups was comparatively increased during every week interval. While in group V animals were treated with formulation of both the plant extract in the ration of 1:1 shows reduction in catalepsy score

Table 2: Sedimentation of formulation at 30 min. Time Interval

Sr. no.	Time (min.)	Ultimate ht. (ml.)	Sedimentation ratio	Sedimentation ratio
1	30	100	75	1.333333333
2	60	100	72	1.388888889
3	90	100	68	1.470588235
4	120	100	63	1.587301587
5	150	100	59	1.694915254
6	180	100	58	1.724137931
7	210	100	55	1.818181818
8	240	100	51	1.960784314
9	270	100	49	2.040816327
10	300	100	48	2.083333333

Table 3: Study of Evaluation parameter of formulation

Parameter	Observed value
Particle size (µm)	28.02
viscosity (cps)	59.23
pH	5.03
Redispersibility	Good

during each week time period at the significant level when compared to control group.

EFFECT OF EXTRACTS ON ROTENONE INDUCED LOCOMOTOR ACTIVITY

According the data of Figure 2 at the start of experiment day there was no any significant changes in locomotors activity amongst the all group. While in control group during every week locomotors activity was found to be reduced exhibiting the parkinson's like symptoms. While in group III and Group IV animals were treated with hydroalcoholic extracts of Fruits of Ananas comosus L. and seeds of Parkinsonia aculeate L. shows restoration of locomotion compared to control group at the significant level (P<0.05).

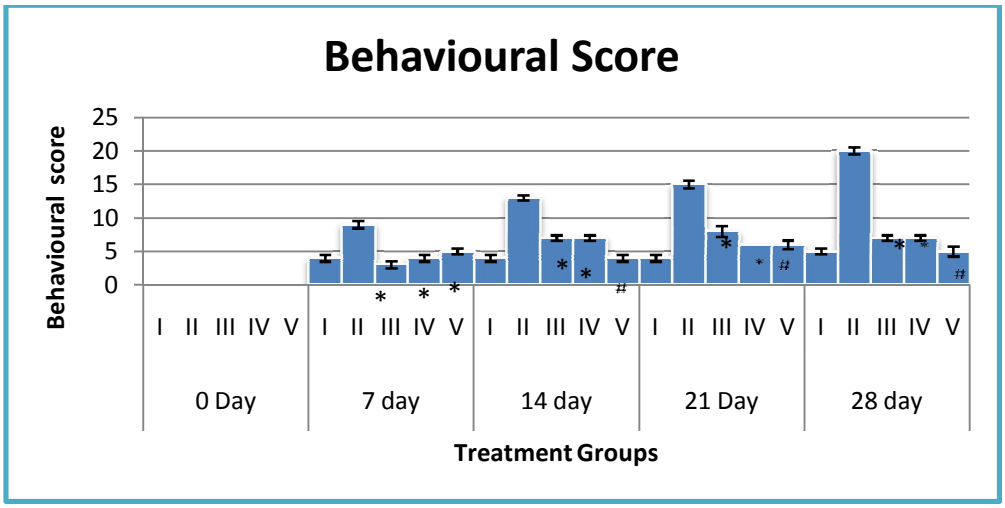

Figure: 1 Effect of *Parkinsonia aculeata L.* and *Ananas comosus L.* on rotenone induced behavioural score. * indicate significant difference from control group at (P<0.05) # indicate significant difference from control group at (P< 0.01)

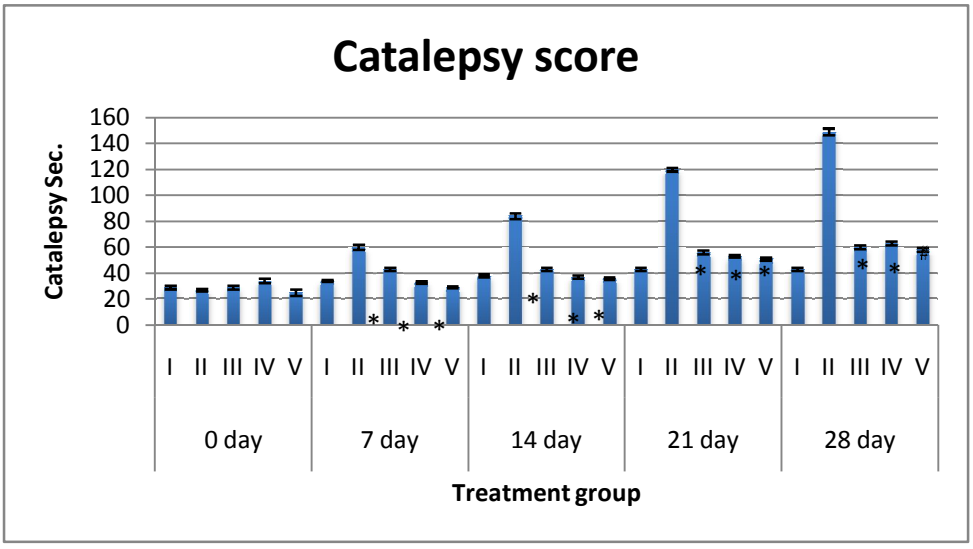

Figure: 2 Effect of *Parkinsonia aculeate L.* and *Ananas comosus L.* on rotenone induced catalepsy score. * indicate significant difference from control group at (P<0.05) # indicate significant difference from control group at (P< 0.01)

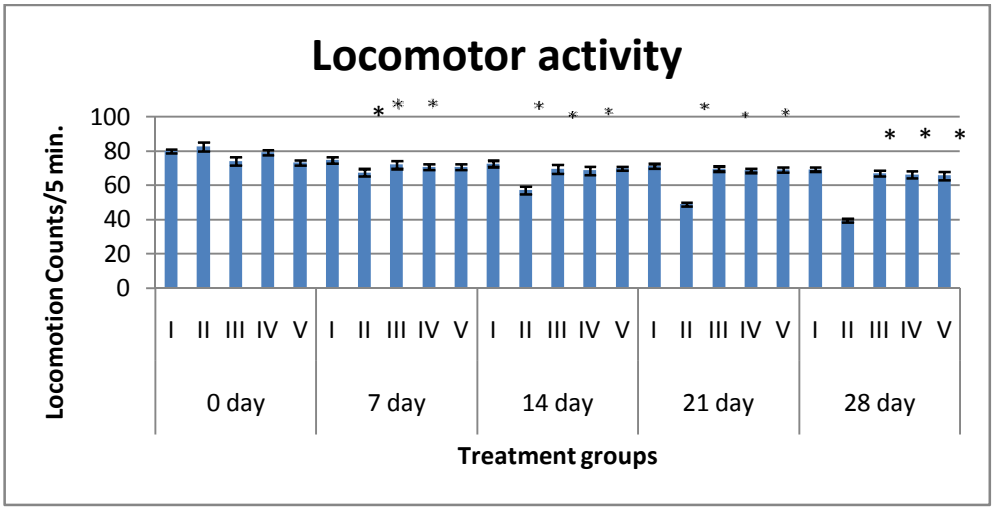

Figure: 3 Effect of *Parkinsonia aculeate L.* and *Ananas comosus L.* on rotenone induced locomotor activity. * indicate significant difference from control group at (P<0.05)

In Group V animals were treated with formulation of both the plants extracts in the ratio of 1:1 shown higher locomotion compared to control group.

DISCUSSION

Rotenone is rotenoid alkaloid. It is a pesticide and herbicide. It is highly lipophilic and hence readily crosses biological membranes and accumulates in sub cellular organs like mitochondria. Rotenone administration in rodents provides a valuable tool for studying mechanisms of oxidative stress induced dopaminergic damage [20]. Oxidative stress produced due to mitochondrial dysfunction, particularly mitochondrial complex-I dysfunction plays an important role in the Parkinson's pathogenesis. [21] In the present study, rotenone administration to rats caused a significant increase in catalepsy, decrease in locomotor activity and decrease in muscle activity which are the symptoms of parkinosn's disease. Seeds of Parkinsonia aculeate L. and fruits of Ananas comosus L. contains various phytoconstituents including flavonoids, phenolics. Flavonoids can regulate the oxidative stress generated during the pathogenesis of disease. The current data thus suggested damage to the motor control system (DA-ergic neurons) and development of Parkinson's disease like behavioral symptoms in rats exposed to rotenone. Pretreatment of rats with ACEX at the dose of 250 mg/kg and PAEX at the dose 200 mg/kg resulted in a significant decrease in catalepsy, increase in locomotor activity and increase in muscle activity. Moreover the prepared formulation when administered to group-V was also able to reduce the catalepsy score, increases locomotion and improves the muscle activity. The antiparkinson's activity of both the plants may be due to phytoconstituents present in both the plant extracts.

CONCLUSION

The present study shows that hydroalcoholic extracts of both the plants exhibit the significant difference in behavioural score, catalepsy score and locomotor activity compared to control group. Additionally prepared suspension in the ratio (1:1) of both the plant extracts shows synergistic action and exhibit the significant difference from control group. So it reveals that combination of hydroalcoholic extracts of both the plant shows more significant results compared to alone treatment.

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