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Research Article

EFFECT OF HYDROALCOHOLIC EXTRACTS OF *PARKINSONIA ACULEATA* L. AND *ANANUS COMOSUS* L. ON OXOTREMORINE INDUCED PARKINSONISM IN WISTAR ALBINO RATS

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ABSTRACT

Parkinson's disease is progressive neurodegenerative disease that occurs due to several causative factors although dopamine deficiency is prime characteristic of the disease. Environmental toxin, genetic abnormalities, drugs, oxidative stress are some of causative factor responsible for development of parkinson's disease. Current pharmacotherapy of this disorder is able to provide only symptomatic relief. Moreover their use is associated with lot of adverse effects. The importance of medicinal plants comes in this context. In present study oxotremorine (2 mg/kg) was used to induce the parkinsonism in wistar albino rats. Hydroalcoholic extracts of *Parkinsonia aculeata* L. seeds at the dose of (200 mg/kg) and *Ananas cosmosus* L. Fruits at the dose (250 mg/kg) were administered. The extracts of both the plants were administered prior to oxotremorine administration. At the interval of each hour tremor score, rigidity score, hypokinesia score, postural flexion score and postural immobility score were recorded. The result data shows that oxotremorine induces the tremor, rigidity, hypokinesia, postural flexion and postural immobility where as administration of hydroalcoholic extracts of *Parkinsonia aculeata* L. seeds at the dose of (200 mg/kg) and *Ananas cosmosus* L. fruits at the dose (250 mg/kg) significantly reduced the tremor score, rigidity score, hypokinesia score, postural flexion and postural immobility score at ($P < 0.05$).

Keywords: Tremor, Rigidity, Parkinsonia aculeata L. Ananas cosmosus L. oxotremorine

INTRODUCTION

Parkinson's disease 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 coordination of smooth movement. The disease generally affects persons aged between 55–64 years, although occasionally much younger individuals are also affected. The causes for degeneration of dopaminergic neurons in parkinson's disease (PD) are not well understood, emerging data from recent studies shows 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 parkinson's disease 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 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 the persons over age 65 prevalence of PD has been estimated at (1.8 %) individuals, increasing from (0.6 %) for persons between ages of 65 and 69 to (2.6 %) for those 85 to 89 years[1,2]. The population of elderly Indians has increased from 5.6 % in 1961 to 7.1 % in 2001. The elderly population in developing countries is expected to increase from 200-250 % compared to a mere 20-40 % in developed countries[3]. According to WHO health report 1999 Following the population census in India, the elderly population was only 24 million in 1961 but now it has been raised to 77 million in 2001. 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 Dopamine, the Dopamine-synthesizing enzymes tyrosine hydroxylase and dopa-decarboxylase, and the dopamine metabolites homovanillic acid, dihydroxy phenylacetic acid and 3-methoxytyramine.[4] Several groups have demonstrated evidence of 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 free radical damage to DNA further support free radical damage to proteins in Parkinson disease.[4] *Parkinsonia aculeata* L. is a large spinous shrub or small tree, native of America found throughout the drier part of India. *Parkinsonia aculeate* is a tree from the family Fabaceae.[5] Previous investigations shows that the leaves from the plant contains orientin, iso-orientin, vitexin, iso-vitexin, lucenin-II, vicenin-II, diosmetin 6-C-β glucoside, apigenin, luteolin, kaempferol, chrysoeriol, epiorientin, parkinsonin-A, parkinsonin-B, and parkintin.[6-9] *Parkinsonia aculeata* possess various pharmacological activity such as antibacterial effect of hexane and methanolic extract[10], anti diabetic effect of water soluble extract of aerial parts[11], antioxidant effect of 70 % hydroalcoholic extract[12], amoeibicidal activity of isolated rotenoid from the roots[13], hepatoprotective activity of 50 % ethanolic extract of leaves[14], Anti-spermatogenic activity of ethanolic extract of stem barks[15] and anti malarial activity of Crude extracts of aerial parts of leaves and flowers[16]. *Ananas cosmosus* L. is fruit bearing plant form the family Bromeliaceae. It contains various pharmacologically active phytoconstituents such as ananasate, beta-sitosterol, Chlorogenic acid, rutin, naringenin, bromelin, glysocides, flavonoids and neurotransmitters[17-19]. *Annanas cosmosus* L. reported to possess some medicinal properties such as anti diabetic activity,[20] antitumor property[21] anti-oxidant action[22], anti-inflammatory activity[23], immuno-modulatory activity[24] hepatoprotective action[25] platelet aggregation activity and anthelmintic activity.[26] Current pharmaco therapeutics for Parkinson's disease are L- dopa, carbidopa, Mono-amino oxidase inhibitors and anti-cholinergic drugs. All of these possess some sort of adverse effects on long term use. Current treatments 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 effectiveness of *Parkinsonia aculeata* L. seeds and *Ananas cosmosus* L. Fruits against oxotremorine induced parkinsonism.

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, Gujarat, India.

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 concentrated and stored in desiccators for 3 days then preserved in a deep freezer at -4˚C[27]

Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in hydroalcoholic extract of fruits and seeds[27]

Study of extracts on Parkinson's disease

Animals

The albino rats (200-220 g) 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), Ministry of Social Justice and empowerment, Government of India. The study protocol was approved by institutional animal ethics committee with no. RBPMPC/12-13/IAEC/07/02

Oxotremorine induced Parkinsonism

Oxotremorine (2 mg/kg body weight) was used to produce the Parkinson's like symptoms.[28] Animals were divided into five groups each group containing six rats of either sex. The extracts of both the plants were administered one hour before the oxotremorine administration.

- Group-I: Disease control group [treated with Oxotremorine (2 mg/kg, s.c.)
- Group-II: Hydroalcoholic extract of [*Ananas comosus* Extract (ACEX) 250 mg/kg] + oxotremorine (2 mg/kg, s.c.)
- Group-III: Hydroalcoholic extract of [*Parkinsonia aculeata* Extract (PAEX) 200 mg/kg] + oxotremorine (2 mg/kg, s.c.)
- Group-IV: combination of both extract (ACEX + PAEX) (1:1)
- Group-V: Standard: Trihexphenidyl (2 mg/kg,) + Oxotremorine (2 mg/kg, s.c.)

The score was studied at the interval of one hour in all the groups of animals. The following scores were evaluated.[29]

Tremor

The animal was grasped by the trunk and held in the supine position; possible tremulous movements of the limbs were scored 2 when they were visible immediately and clearly; score 1 was assigned when tremulous movements were intermittent and/or of modest amplitude. Score 0 was assigned when no tremulous movements could be observed by this visual method. The animal was then placed back on the table and now gently palpated the four limbs. Tremor was scored 2 if it was detectable immediately and clearly by this method of palpation. A score 1 was assign when the tremor was intermittent and/or of modest amplitude; score 0 was assign when no tremor was detected. These manoeuvres was repeated 3 times in all, yielding 3 visual and 3 palpation scores, The median of the 3 visual scores and that of the 3 palpation scores were found and a final single score for tremor was obtained as the median of these two.

Rigidity

The animal was grasped by the hindquarter and held vertically, head up. Trunk rigidity was evaluated from the degree to which the trunk resisted gentle movements in the horizontal plane. Rigidity of the limbs was evaluated from

the degree to which the limbs resisted gentle bending and stretching.
Score 0: no clear evidence of increased muscle tone.
Score 1: any manoeuvre revealed muscle tone to be increased but less than severe.
Score 2: any of these manoeuvres revealed severe rigidity, compared to normal rats.

Hypokinesia
The animal was grasped by the trunk, manipulated for a few seconds and then placed back on the table.
Score 0: the animal was moved some distance over the table in response to this manipulation
Score 1: some movements of head and limbs were occurred, but there was no locomotion.
Score 2: the animal was failed to move the head or the limbs

Posture flexion
Flexion of the back was scored from observing the animal while it rested on the table. Flexion of limbs and digits were scored after the animal had been lifted off the table and turned on its back.
Score 0: no marked flexion was occurred; not markedly different from normal animals.
Score 1: flexion was marked but less than maximal.
Score 2: severe flexion was occurred.

Posture immobility
The animal was lifted off the table and held in the supine position in the hand. Righting movements was score according to the followings.
Score 0: Righting was not markedly impaired.
Score 1: Righting movements was clearly slow and/or impaired, but righting eventually occurred.
Scored 2: If they were absent or did not result in actual righting

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 were evaluated with use of computer software.

RESULTS

Qualitative Phytochemical Analysis
The preliminary qualitative phyto-chemical analysis (Table 1) shows the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins, alkaloids, polyphenols and ascorbic acid in hydroalcoholic extracts of *Ananas comosus* L. fruits. While steroids, saponins, alkaloids, flavonoids, tannins and poly-phenols were present in hydro-alcoholic extracts seeds of *Parkinsonia aculeata* L.

Effects of extracts on oxotremorine induced Parkinsonism
Tremor
According to data depicted in Figure 1 Tremor score was highest in control group and administration of *Parkinsonia aculeata* L. seed and *Ananas comosus* L. fruits hydro-alcoholic extracts reverse the tremor score to significant level. ($P < 0.05$) Moreover administration of combined extracts (1:1) enhances the effectiveness by reducing the tremor score that was comparable with standard group.

Rigidity
Figure 2 shows that rigidity score was significantly higher compared to all treatment groups. While treatment of *Parkinsonia aculeata* L. seed and *Ananas comosus* L. fruits hydroalcoholic extracts were able to reduce the rigidity score at significant level. ($P < 0.05$) Combination of the both the plant extracts also show the synergistic action and reduces the rigidity score at ($P < 0.01$).

Hypokinesia
According to the data in Figure 3 hypokinesia score was also enhanced by the administration of oxotremorine while it was reverse by the treatment with of *Parkinsonia aculeata* L. seed and *Ananas comosus* L. fruits hydroalcoholic extracts at ($P < 0.05$) significant level. Moreover combination of both the plant extracts also shows synergism that was comparable with standard group.

Postural flexion
As data shown in Figure 4 postural flexion score were also increased by the administration of the oxotremorine while it was reduce at ($P < 0.05$) significant level by the administration of *Parkinsonia aculeata* L. seed and *Ananas comosus* L. fruits hydroalcoholic extracts. In group 4 combinations of plant extracts and standard group-5 postural flexion was also reveres.

Postural immobility
Figure 5 data indicated that postural immobility score was also increased during the treatment of oxotremorine in wistar albino rats. While it was reverse in Group II at ($P < 0.05$) significant level and Group- III treatment group at ($P < 0.05$) significant level as well as in group IV and Group V they were also reverse at ($P < 0.05$) significant level.

DISCUSSION

Oxotremorine induced Parkinson's like symptom is one of the tools for study of various chemicals that might become potential antiparkinson's agent. Oxotremorine is muscarinic agonist and its activity seems to mediate by activation of central cholinergic system.[30] In present study hydro-alcoholic extracts of *Parkinsonia aculeata* L. seed and *Ananas comosus* L. fruits were evaluated. Both of which contain many phytoconstituents phenolics, flavonoids, alkaloids, tannins as their phytoconstituents. Flavonoids of several classes are inhibitors of mono-amino-oxidase-A and mono-amino-oxidase-B, thus mediating an antidepressant or anti-parkinson's activity[31]. Recent study shows that consumption of polyphenols in diet can reduces the severity of parkinson's disease. There effects seems to be mediated through preventing the redox active transition metal from catalyzing free radicals formation or induction of the expression of antioxidant and detoxifying enzymes particularly in the brain.[32] Results shows that control group shows higher tremor, rigidity, hypokinesia, postural flexion as well as postural immobility score, where as treatment of 250 mg/kg of ACEX and 200 mg/kg of PAEX able to reverse all the score at significant level. Moreover combination of both the extracts were also shows the beneficial action by improvement in all scores. This improvement in the score is comparable with standard.

Table 1: Phytochemical Analysis of Hydroalcoholic Extracts of *Ananas comosus* L. Fruits and *Parkinsonia aculeata* L. Seeds

Phytochemicals	Phytochemical test	Fruit extract	Seeds extracts
		Ananas comosus	*Parkinsonia aculeata*
Carbohydrate	Barfoed's test	+	-
Proteins	Biuret test	+	-
Amino acids	Ninhydrin test	+	-
Steroids	Libermann burchard test	+	+
Glycosides	Legal test	+	-
Saponins	Foam test	-	+
Alkaloids	Hager's test	+	+
Flavonoids	Sinoda test	+	+
Tannins	$Fecl_3$ test	+	+
Polyphenols	Lead acetate test	+	+
Ascorbic acid	Colour reaction with $NaHCO_3$ and $FeCl_3$	+	-

+: indicate the Presence of constituents -: Indicate absence of constituents

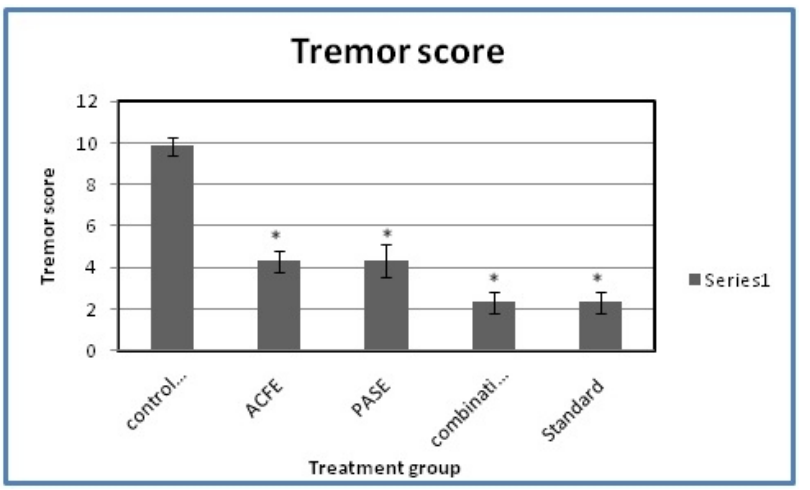

Figure 1: Effect of *Ananas comosus* L. Fruit extracts and *Parkinsonia aculeata* L. seed extracts on tremor score in oxotremorine induced parkinson's like symptom in rats

*indicated the significant difference from control group. ($P < 0.05$)

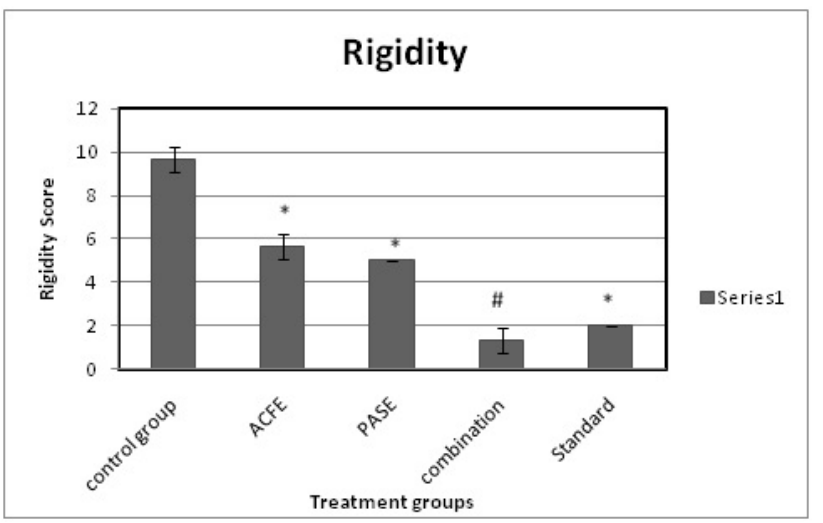

Figure 2: Effect of *Ananas comosus* L. fruit extracts and *Parkinsonia aculeata* L. seed extracts on rigidity score in oxotremorine induced parkinson's like symptom in rats

*indicated the significant difference from control group. ($P < 0.05$) and # indicated significant difference ($P < 0.01$) from control group

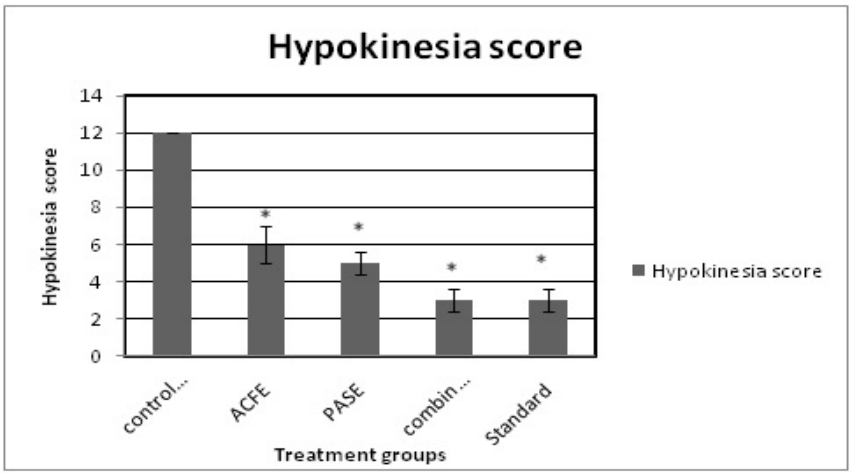

Figure 3: Effect of *Ananas comosus* L. Fruit extracts and *Parkinsonia aculeata* L. seed extracts on hypokinesia score in oxotremorine induced parkinson's like symptom in rats
*indicated the significant difference from control group. ($P < 0.05$)

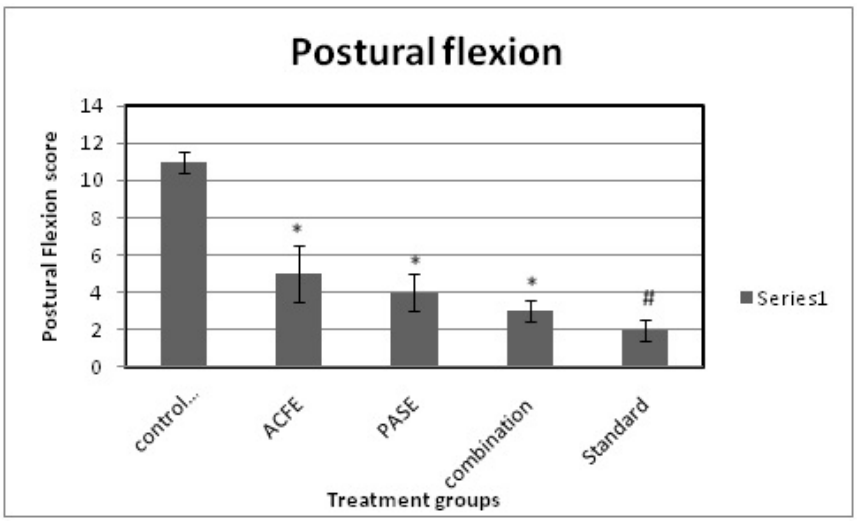

Figure 4: Effect of *Ananas comosus* L. Fruit extracts and *Parkinsonia aculeata* L. seed extracts on Postural flexion score in oxotremorine induced parkinson's like symptom in rats
*indicated the significant difference from control group. ($P < 0.05$) and # indicated significant difference ($P < 0.01$) from control group

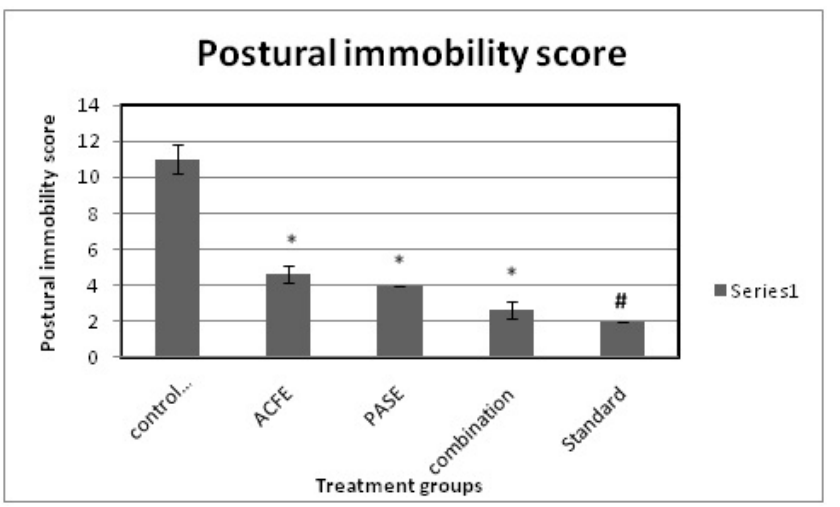

Figure 5: Effect of *Ananas comosus* L. Fruit extracts and *Parkinsonia aculeata* L. seed extracts on postural immobility score in oxotremorine induced parkinson's like symptom in rats
*indicated the significant difference from control group. ($P < 0.05$) and # indicated significant difference ($P < 0.01$) from control group

CONCLUSION

In present study hydro-alcoholic extracts of *Parkinsonia aculeata* L. seeds and *Ananas comosus* L. fruits shows the improvement in tremor, rigidity, hypokinesia, postural flexion and postural immobility score in oxotremorine induced parkinsonism in rats. Additionally combined extracts of both the plants were shows the beneficial action by further improvement in the scores. The effects might probably due to presence of flavonoids and polyphenols present in the extracts.

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