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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF *ANNONA SQUAMOSA* L. SHOOT EXTRACT USING ANIMAL MODELS

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ABSTRACT

Objective: *Annona squamosa* L. (Annonaceae) is a small ever green tree is cultivated throughout india for its fruits. This plant has been used for the treatment of a variety of diseases. hyperthyroidism and lipid-peroxidation. The plant also posses analgesic activity, anti-inflammatory activity, anti-microbial activity, cytotoxic activity, anti-oxidant activity, anti-lipidimic activity, anti-ulcer activity, molluscicidal properties, genotoxic effect, vasorelaxant activity, anti-tumour, hepatoprotective activity, larvicidal activity, insecticidal activity, anthelmintic activity, etc **Methods:** This study was undertaken to evaluate the possible antidepressant effect of *Annona squamosa* L. Shoot extract (ASSE) using Tail suspension test(TST) & Forced swim test (FST). 36 albino rats of either sex weighing between 200-250gm were randomly selected and divided into 6 equal groups. Group-I (control) received polyethyleneglycol (1ml/100gm), Group-II, III & IV received ASSE in doses of 100,200,400 mg/kg orally (P.O.) respectively. Group V & VI (positive control) received Fluoxetine & Imipramine at doses of 20mg/kg & 15mg/kg p.o respectively. Drug treatment was given for seven & fourteen successive days. 60 minutes after last dose of drug or standard the immobility period was recorded. **Results:** ASSE produced significant antidepressant like effect at dose of 200 & 400 mg/kg administered for 7

& 14 consecutive days as indicated by reduction in immobility times of Rat in TST & FST (P<0.05). The efficacy of ASSE at 200mg/kg was found to be comparable to that of Fluoxetine & Imipramine at doses of 20mg/kg & 15mg/kg. **Conclusion:** The results of the present study indicate that ASSE possesses significant antidepressant activity compared to that of both Fluoxetine & Imipramine.

KEYWORDS: *Annona squamosa* L., Forced swim test, Tail suspension test, Antidepressants, Immobolity time.

INTRODUCTION

Annona squamosa L. (Annonaceae), commonly known as the custard apple tree is a native of West Indies. But the cultivation is present throughout India, because of its edible nature.[1] It is a fruit tree considered as a native of Central America also and hence have a wider cultivation throughout the regions of tropics. The taste of the pulp of the fruit is really sweet because of its higher sugar content of about 58% of dry mass, and hence it is found clear that the fruit pulp possess a high calorie value. This plant was reputed to contain several medicinal properties. Folkloric record reported the use of *Annona squamosa* as an insecticidal, an anti-tumor agent, anti-diabetic, antioxidant, anti-lipidimic and anti-inflammatory agent which has been characterized due to the presence of the cyclic peptides. In addition, the crushed leaves were sniffed to overcome the hysteria and fainting spells, and they were also applied on the ulcers and wounds. A leaf decoction was taken in the case of dysentery. Plant have proved that they possess a wide variety of compounds like acetogenins which were responsible for anti-feedant, anti-malarial, cytotoxic and the immunosuppressive activities. Diterpenes which was isolated from the *Annona squamosa* possess the anti-HIV principle and the anti-platelet aggregation activity. *Annona squamosa Linn* is a multipurpose tree with edible fruits & is a source one of the medicinal &industrial products. *Annona squamosa Linn* is used as an antioxidant, antidiabetics, hepatoprotective, cytotoxicactivity, genetoxicity, antitumour activity, antilice agent. The partially purified flavonoids were reported from the same source as the responsible agent for the anti-microbial and othe pesticidal activities. Some lignans and other hydroxyl ketones were also found to be present in this plant.[1]

Taxonomic Classification

Annona squamosa L.

Kingdom : *Plantae*

Subkingdom: *Tracheobionta*

Super division: *Spermatophyta*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Sub class: *Magnoliidae*

Order: *Magnoliales*

Family: *Annonaceae*

Genus: *Annona L.*

Species: *Annona squamosa*

Traditional uses: The plant is attributed with the medicinal properties that include anti-fertility and antitumour activities which were observed in mice and rats. The young leaves of *Annona squamosa* were used extensively due to its anti-diabetic activity.[2]

Depression is a major clinical illness affecting 9.5% of population. Changes in the monoamine neurotransmitters have been observed in patients of depression.[3] The use of plant products for the treatment of human ailments has been a natural approach to health care since the beginning of civilization. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research,worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a varietyof animal models.[4] Thus the present study has been undertaken to evaluate the antidepressant activity of *Annona squamosa shoot* extract (ASSE) in rats employing tail suspension test (TST) & forced swim test (FST). Standard antidepressant drugs such as Fluoxetine (SSRI), and Imipramine (TCA) have been employed to standardize the animal models of depression.

MATERIALS AND METHODS

PREPARATION OF *ANNONA SQUAMOSA SHOOTS* EXTRACT (LEAVES AND LATERAL BUDS)

shoots were collected and shade dried. They were crushed into coarse powder and extracted with 90% methanol using soxhlet's apparatus for 24 hrs. The extract was concentrated under pressure and then dried in air. The concentrated ethanolic extract was suspended in poly ethylene glycol. Freshly prepared solution was used for each experiment.

PLAN OF STUDY

ANIMALS

About 36 albino rats of either sex weighing between 200 -250 gms. procured from disease free animals were used for the present study. Animals had free access to food and water and maintained under standard laboratory conditions with a natural light and dark cycle. The animals were acclimatized for at least five days before behavioural experiments. Experiments were carried out between 9.00 and 15.00 hrs. Experimental protocol was approved by the institutional animals' ethics committee before the start of the study.

DRUGS & CHEMICALS

ASSE, Fluoxetine Hydrochloride (Ranbaxy Lab.), Imipramine Hydrochloride (Sigma Aldrich).

VEHICLE

Polyethylene Glycol (PEG).

STUDY DESIGN

The animals were selected randomly for each experiment and divided into 6 equal groups. Drugs (PEG, ASSE, Fluoxetine, Imipramine) administered orally (P.O.) for 7&14 successive days as depicted in (**Table 1)**

Table 1: Protocol of the study (Approved by IAEC)

GROUP	*DRUG*	*DOSE(P.O.)*
1	PEG	1 ml / 100 gm.
2	ASSE (Aq)	100 mg/kg
3	ASSE(Aq)	200 mg/kg
4	ASSE(Aq)	400 mg/kg
5	Fluoxetine	20 mg/kg
6	Imipramine	15 mg/kg

Sixty minutes after last dose, immobility period was recorded in two different animal models of depression like:

Forced Swim Test (FST) (5)

Tail suspension test (TST) (6)

Laboratory Models For Testing Antidepressant Activity

Forced Swim Test (FST): FST(5) or behaviour despair was proposed as a model to test for antidepressant activity by Depression was produced by forcing the animal to swim

individually in a glass jar containing fresh water of 15cm height and maintained at 25^{o}C. This constituted pretest session. Twenty-four hour later each animal was again forced to swim. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups as mentioned in the above table.

Tail Suspension Test (TST)*[6]*: The total duration of immobility induced by tail suspension was measured according to the method d. Depression was produced by suspending the animal from the edge of a table 50 cm above the floor by an adhesive tape placed approx. 1cm. from the tip of the tail. Immobility time was recorded during a 6 min. period. Changes in the immobility duration were studied after administering drugs in separate groups of animals. The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug.

Acute toxicity study: Acute toxicity study was done according to OECD (Organization for Economic Co-operation and Development) Guideline, fixed dose method; with starting dose of 2000mg/kg body weight was adopted. Starting dose of 2000mg/kg (per oral) of each was given to 5 animals (albino rats), animals were kept for observation of behavioural change and death up to 72h.

STATISTICAL ANALYSIS

All the results are expressed as Mean ± SEM. All the groups were analysed using student's 't' test.

RESULTS

The observation of acute toxicity study indicated that there was no death in 2000mg/kg dose after 72hr. ASSE(Aq) at the dose of 100 mg/kg had no beneficial effect on immobility period of rats in both the models of depression i.e. FST & TST. The decrease in immobility period in both the models was observed starting from 200 mg/kg. But the increase in dose from 200 to 400 mg/kg did not produce any further reduction in immobility period, suggesting the ceiling effect at 200 mg/kg. At the dose 200 mg/kg, ASSE(Aq) showed antidepressant effect which is comparable to that of Imipramine and Fluoxetine at the dose of 15 & 20 mg/kg respectively **(Table 2).**

Table 2: Effect of *Annona Squamosa Shoot* Extract (Asse) On Immobility Period (Secs) Of Rats Using Forced Swim Test

Group	Drug	Dose	Immobility PERIOD (Secs)		
			Pre Treatment	Post Treatment (7 days)	Post Treatment (14 days)
1	PEG	1ml/100gm	201.13 ± 3.01	196.4 ± 1.31	195.04 ±0.16
2	ASSE(Aq)	100mg/kg	200.51 ± 1.14	192.51 ± 1.15*	190.01 ± 1.01*
3	ASSE(Aq)	200mg/kg	203.08 ± 1.05	151.61 ± 1.14b**aδ	152133 ± 0.05b**aδ
4	ASSE(Aq)	400mg/kg	192.2 ± 1.82	150.241± 0.01b*aδ	152.07 ± 1.12b*aδ
5	FLUOXETINE	20mg/kg	196.18 ± 1.70	120.64 ± 0.16b	102.71 ± 0.13b
6	IMIPRAMINE	15mg/kg	190.33 ± 1.17	136.91 ± 1.11b	124.6 ± 1.06b

Values as Mean ± SEM, n=6, 1. a = p < 0.05, b = p < 0.001 as compared to pre treatment value, 2. * = p < 0.001, ** = p < 0.05 when compared to standard (Both Fluoxetine & Imipramine), 3.α = p < 0.001when compared to control, 4. □ = p < 0.001 when ASSE (100) is compared to ASSE (200) and ASSE(400).

At the dose 200 mg/kg, ASSE showed antidepressant effect which is comparable to that of imipramine and Fluoxetine at the dose of 15 & 20 mg/kg respectively. The comparable anti depressant effect of ASSE with that of TCA (imipramine) and SSRI (fluoxetine) suggest possible involvement of either nor-adrenergic or serotonergic system.

Table 3: Effect of *Annona Squamosa Shoot* Aqueous Extract (ASSE) On Immobility Period (Secs) Of Rats Using Tail Suspension Test

Group	Drug	Dose	*Pre Treatment*	*Post Treatment After*		
				4days	7days	14days
1	PEG	1ml/100gm	195.09 ±1.11	194.41±0.97	193.02 ±0.54	190.16±1.12
2	ASSE(Aq)	100mg/kg	189.40 ± 1.14	187.12±1.18	182.07 ±1.12	180.01±1.17
3	ASSE(Aq)	200mg/kg	190.01± 1.44	188.06±1.77	156.17±1.46*bα	150.78±1.21*bα
4	ASSE(Aq)	400mg/kg	188.15 ± 0.11	181.09±0.12	165.04 ± 1.21*bα	154.12±1.42*bα
5	Fluoxetine	20mg/kg	182.43 ±0.71	180.56±1.08	113.50± 0.95 *c	114.61±1.02*c
6	Imipramine	15mg/kg	192.5 ±1.14	190.31±1.02	125.12± 1.13 *c	120.51±1.14*c

Values as Mean ± SEM, Student's t test n = 6, 1.* P<0.001 when compared to pre treatment, 2.a=P<0.05, b=P<0.01, c=P< 0.001 when compared to control, 3.α =P<0.05, β=p<0.001 When compared to standard.

DISCUSSIONS

In the present study, ASSE (Aq) (200 mg/kg) produced significant antidepressant effect in in FST & TST. These models of depression are widely used to screen new antidepressant drugs. The tests are quite sensitive and relatively specific to all major classes of antidepressant drugs

including TCAs, SSRIs, MAOI, Atypical antidepressants. The forced swimming test is the most widely used tool for assessing antidepressant activity pre-clinically. The widespread use of this simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents.[7] It has been argued that TST (Tail Suspension Test)is less stressful than FST (Forced swim test) and has greater pharmacological sensitivity. The results obtained from TST are in concordance with the validated FST by Porsolt et al. Environmental factors and hereditary factors play a major role in producing deficient monoaminergic transmission in central nervous system thereby producing symptoms of depression.[13] The plant is reported to contain glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, amino acids. The various chemical constituents isolated from leaves, stems and roots of the plant including anonaine, aporphine, coryeline, isocorydine, norcorydine, glaucine. Leaves contains 4-(2-nitro-ethyl 1)-1-6-((6-o-β-Dxylopyranosy1- β-D-glucopyranosyl)-oxy)benzene, Anonaine, Benzyltetrahydroisoquinoline, Borneol, Camphene, Camphor, car-3-ene, Carvone, β- Caryphyllene, Eugenol, Farnesol, Geraniol, 16-Hetriacontanone, Hexacontanol, Higemamine, Isocorydine, Limonine, Linalool acetate, Menthone, Methyl anthranilate, Methylsalicylate, Methylheptenone, p-(hydroxybenzyl)-6,7-(2- hydroxy,4-hydro)isoquinoline, n-Octacosanol, a- Pinene, b-Pinene, Rutin, Stigmasterol, β-Sitosterol, Thymol and n-Triacontanol. Alkaloids, proteins & amino acids are absent in the leaf extract.[8] May be facilitating monoaminergic transmission there by producing antidepressant effects.

CONCLUSION

Hence *Annona squamosa shoot* extract (ASSE) possesses antidepressant effect in animal models of depression which was comparable to that of Imipramine and Fluoxetine as demonstrated in this study. The phytochemical analysis, separation of active ingredients and further investigation in this line methanolic extrac is essential to establish its therapeutic benefits.

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