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Pharmacological Evaluation of AnnonaSquamosa Leaves for Central Nervous System Depressant Activity in Swiss Albino Mice

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ABSTRACT

Trees and shrubs products have been used as principal ingredients that aid human health and well-being. Based on the literature review and traditional uses of plant *Annonasquamosa* (Family; *Annonaceae*), the present study was aimed to evaluate the CNS depressant activity of ethanol, petroleum ether and aqueous extracts of the leaves of *AnnonaSquamosa* in albino mice. The rotarod test and diazepam induced sleep test was used to evaluate the activity of extracts interfering with motor co-ordination and sleeping time at a dose of 200 mg/kg. 30 minutes after *i.p.* administration of extracts. The difference in the fall off time from the rotating rod and onset and duration of sleeping time between the control and the treated mice was recorded. It can be inferred from the results that the *A. squamosa*leaves extracts were exhibiting interesting aimed activity against the control group, however not on par with that of standard employed. Based on the results we can conclude that the ethanol crude extract of *A. squamosa*leaves exhibit significant CNS depressant activity.

Keywords: *A. squamosa*leaves extracts, CNS depressant activity, rotarod and diazepam induced sleep test in mice.

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INTRODUCTION

Trees and shrub products have long been used as principal ingredients that aid human health and well-being. Annonaceae is a large family of tropical and subtropical trees and shrubs, comprising 130 genera and about 2300 species [1-2]*Annonasquamosa* (Family; Annonaceae) locally known as bnahnona, seri kaya (Malaysia), and the plant is also known by its numerous regional names, *viz*., sitaphal, sharifa, sitapandu (Hindi, India), manonah, noinah, pommecannelle du Cap (Thailand); qu a na (Vietnam); mangcau ta (Combodia); makkhbieb (Laos); fan-li-chi (China); anon de azucar (Colombia); anonablanca (Honduras, Dominican Republic); anona de castilla (El Salvador); pommecannelle (Africa, French Guiana, French West, Guadeloupe); sweetsop (Jamaica, Bahamas); anon (Bolivia, Costa Rica, Cuba, Panama); fruta de condessa, pinha, ati (Brazil); ates or atis (Philippines); ate (Gabon); cachimancannelle (Haiti); rinon (Venezuela) saramulla, saramuya, ahate (Mexico); scopappel (Netherlands Antilles); cachiman (Argentina); cherimoya (Guatemala, Ecuador) [3-5] and sweet apple or custard apple (West Indies) [1, 6]*A. squamosa* is a woody, semi deciduas tree which grows widely in temperature and tropical parts of Asia[7]. Specifically *Annona*plant is widely found throughout the regions of India, Indonesia, Malaysia, Thailand, and China. It occupies a very important place in the field of medicinal plants and is widely used as a traditional medicine[6-7]. *viz*., a bark and leaves decoction of this plant is used for diarrhea and cold, while the root and leaves are used to treat dysentery, hysteria, fainting spells and seeds of the plant were screened for insecticidal activity [2, 6-7]. The plant contain annonaine an alkaloid which is believe to responsible for most of the activities of this plant[2,7], apart from this, aporphine alkaloids, falvonoids, glycoside, squamoline, bioactive acetogenin[7-8], fatty acid derivatives (Z)-2-hydroxy-3-(octadec-9-enoyloxy) propanoic acid and hexadecanoic acid-2,3-dihydroxy propyl ester [1] were isolated from this plant and screened for a wide spectrum of pharmacological properties, which are reported in the literature *viz.*,antidiabetic activity [4, 6, 9], antihyperlipidic activity [4, 6, 10-11], larvicidal activity against malaria vector *A. Stephensi*[12],diabetic wound healing activity [13], antibacterial and cytotoxic activity, genotoxic activity, antimicrobial activity, antioxidant activity [6, 11], anti-head lice activity [7, 11], anti-tumor activity, cyctotoxic activity [6, 11]insecticidal activity [6-7, 11], mosquitocidal activity, pesticidal activity, antiplasmodial activity, vasorelaxant activity, molluscicidal activity [6, 11], hepatoprotective activity, antiviral activity, antifertility activity, antithyroidic activity [11], analgesic and anti-inflammatory activity [6] As of date, there is (are) no relevant literature on the CNS depressant activity of *AnnonaSquamosa* leaf it was selected for the present study.

Depression is a common serious illness, is a disorders of the brain were important neurotransmitters alters the brain cells that were communicate out of balance in depression. [14]. Today, depression is a significant contributor to the global burden of disease and affects people in all communities across the world. The World Mental Health Survey conducted in 17 countries found that on average about 1 in 20 people reported having an episode of depression and it is estimated to affect 350 million people globally [15]. Antidepressants primarily worked on neurotransmitters, especially serotonin, norepinephrine and dopamine [14, 16]Although there are many antidepressant drugs are available to treat depression, the search is still on for an antidepressant drug especially from the plant origin without side effects. Based on the traditional uses and in view of the above observation the present study was design to evaluate the CNS depressant activity of leaves extract of *AnnonaSquamosa* in albino mice. Diazepam was used as a standard throughout the experiment.

MATERIALS AND METHODS

Preparation of extract

The leaves of plant*Annonasquamosa* were collected from Taman Kemacahaya, Hulu Langat, Kajang, Selangor, Malaysia in. The leaves were sent to Institute of Bioscience, University Putra Malaysia for species authentication. The voucher specimen number is **SK 2089/09**.

Collected leaves were washed under running tap water, to remove adhering particles and shade dried at room temperature [4]After completion of drying, the leaves were size reduced until it becomes coarse powder [12] and stored in a well closed container until further used. Extraction was carried out for each solvent by cold maceration method[13] to obtain the respective crude extracts of distilled water, ethanol and petroleum ether. Maceration was done for each solvent approximately 7 days at room temperature. The soaking waste material was filtered off by using Whatmann filter paper to obtain the respective crude extract filtrates. The collected crude extract filtrates were stored in a refrigerator (2 – 8 °C) for subsequent use [4, 13]The refrigerated extract filtrates were then evaporated using water bath to dryness at 100 °C. A brown to dark brownish color residue with sticky mass was obtained. Suspensions of distilled water, ethanol and petroleum ether extract were prepared in saline using 0.5% carboxy methyl cellulose (CMC) as the suspending agent and were utilized for the CNS depressant activity.

Drug formulation and grouping

Animals

30 healthy male Swiss albino mice weighing between 20 to 25 gm were used for the experiment. The selected animals were divided into 5 groups of 6 animals in each group. Group 1, served as a 'control' group, Group 2, Group 3, Group 4 were treated with extracts and group 5 used as standard for rotarod test. All animals were housed in cages at controlled light and room temperature and were fed with standard diet and water.

Animal ethics

University animal ethics committee approved the experimental protocol & animals were maintained well in an animal house approved by AMU animal ethics committee (AMU/AEC/HSFBH/2009/8).

Rotarod test

The CNS depressant activity was evaluated by using rotarod test. The test purpose is to assess the activity of extracts interfering with motor co-ordination. Dunham, et al., {1956} reported that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod [17.] This test has subsequently been reported to be more effective to evaluate the study purpose[18]. The rotor apparatus (Retard Apparatus (ROTO-R D), IITC Life Sciences) consists of 5 compartments, which could allow one mice at a time into each compartment. The average falling time of mice from the roller before and after the treatment was calculated.

Procedure:

30 male Swiss albino mice were divided into 5 groups of six mice each Group 1 served as control group received 0.5% CMC while animals of group 2, group 3, group 4 were received ethanol, petroleum ether and aqueous extracts at a dose of 200 mg/kg respectively and group 5 was used as a standard throughout the experiment at a dose of 1 mg/kg (*i.p.*) [18]. Animals were received the test compounds intraperitoneally (*i.p.*) [18] and 30 minutes after *i.p.* administration the mice were placed on the rotating rod. The difference in the fall off time from the rotating rod between the control and the treated mice was recorded. [17-22]. before to this, all animals were underwent pre-test on the apparatus and the time were took for each mice to fall off the roller was recorded which served as a before treatment fall off time.

Diazepam induced sleep test

The purpose of this test is to study the sleep potentiating time of the extracts in experimental animals. 24 male Swiss albino mice were divided into 4 groups of six mice each. Group 1 received 10 mL/kg of 0.5% CMC while group 2, group 3, and group 4 were received ethanol, petroleum ether and aqueous extracts at a dose of 200 mg/kg by intraperitoneal route, respectively. 30

minutes later, each animal in all groups received diazepam (50mg/kg, *i.p.*) and then monitored for onset and duration of sleeping time. The duration of loss and reappearance of the righting reflex was taken as a measure of sleeping time and the time of each mice was recorded [23-26].

Statistical analysis

Theresultswere expressed as mean ± S.D. All statistical Comparisons were made by Dennett's test after conducting one-way ANOVA.

RESULTS AND DISCUSSION

Table 1: Effect of *Annonasquamosa* leaves extracts on fall off time in rotarod test:

Extracts/Groups	Fall off time (sec)		
	Before treatment	After treatment	Percentage decrease
Control	39.5±7.77	39.5±7.77	-
Ethanol	45±9.89	11.5±2.12 *	74.44
Petroleum Ether	42±11.31	23.5±9.19 *	44.04
Aqueous	51±15.55	31.0±8.48	39.21
Diazepam	37.5±2.12	17.1±1.4**	54.66

Values are given as mean ± SD; experimental groups were compared with control. *: $p<0.05$ and **: $p<0.01$ was considered statistically significant.

Table 2: Effect of *Annonasquamosa* leaves extracts on diazepam induced sleep in mice:

Extracts/Groups	Dose (mg/kg) *i.p.* route	Onset of Sleep (min)	Duration of Sleep (min)
Control	50	12.0±1.4	57.0±1.4**
Ethanol	200	09.5±0.7	76.5±4.9*
Petroleum Ether	200	10.5±0.7	69.0±1.4**
Aqueous	200	11.5±0.7	66.5±9.19*

Values are given as mean ± SD; experimental groups were compared with control. *: $p<0.01$ and **: $p<0.001$ was considered statistically significant.

The effect of *Annonasquamosa*leaves extracts on motor co-ordination (skeletal muscle relaxation) was evaluated using rotarod test. The difference in the time spent by the animals on rotating rod (fall off time) between the control and the treated animals was recorded 30 minutes after extract administration at the dose of 200 mg/kg (*i.p.*) and reported in Table 1. The fall off time decreased (percentage decrease) significantly from 39.5±7.77 seconds in the control group to 11.5±2.12 (74.44%), 23.5±9.19 (44.04%) and 31.0±8.48 (39.21%) seconds in the experimental animals treated with ethanol, petroleum ether and aqueous extracts, respectively. Ethanol extract showed highly significant skeletal muscle relaxant activity in experimental animals 30 minutes after extract administration as compare to control that received 0.5% CMC only.

The effect of *Annonasquamosa* leaves extracts on sleep induced by diazepam in mice was evaluated and reported in Table 2. The result of diazepam induced sleep test shows that the onset

of action was decreased significantly in the animal groups treated with the ethanol, petroleum ether and aqueous extracts when compared with the group1 served as control group. The duration of action increased significantly from 57.0±1.4 minutes in the control group to 76.5±4.9, 69.0±1.4 and 66.5±9.19 minutes in the experimental animals treated with ethanol, petroleum ether and aqueous extracts at the dose of 200 mg/kg (*i.p.*), respectively. Among all the studied extracts, the ethanol extract showed greater decreased onset of action and longer duration of action compared to animals of control group that received 0.5% CMC and diazepam only.

It can be inferred from the rotarod test and diazepam induced sleep test results that the *Annonasquamosa*leaves extracts were exhibiting interesting CNS depressant activity against the control group, however not on par with that of standard employed. It is generally known that crude extracts might contain wide verities of pharmacologically important phytoconstituents *viz.*, alkaloids, falvonoids, glycoside, squamoline, bioactive acetogenin[6-7], fatty acids[1], terpenoids, phenolics, and N-oxides [27]. The presence of the mentioned compounds abundantly in all types of plants [28] might help explain the observed CNS depressant activity of *Asquamosa*leaves extracts in experimental animals.

CONCLUSION

The results obtained in the present study indicate that ethanol crude extract of *A. squamosa*leaves exhibit significant CNS depressant activity. This study showed the basic about the CNS depressant potential of *A. squamosa*leaves extracts with variable responses. However, further studies are recommended to substantiate new biologically potent active CNS depressant compound(s) from the plant *A. squamosa*.

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