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ISOLATION, PHYTOCHEMICAL INVESTIGATION ON METHANOLIC EXTRACT OF *SCUTIA MYRTINA* AND EVALUATION OF ANTI-DIABETIC AND CNS STIMULANT IN ANIMAL MODELS

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ABSTRACT

The present paper deals with isolation, characterization and evaluation of Anti-diabetic and CNS Stimulant activities of methanolic extract of *Scutia myrtina*. Extraction was carried out with soxhlet apparatus using solvents- petroleum ether and methanol. Flavonoids are isolated using the solvent toluene:ethylacetate (5:1). The methanolic extract of whole plant of *Scutia myrtina* at the dose of 400 mg/kg was administered orally once a day to the groups for 21 days. The plant extract significantly ($p<0.001$) decreased the levels of Glucose, Cholesterol, Triglycerides, SGOT and SGPT and also significantly ($p<0.001$) increased the level of Total protein. The methanolic extract produced significantly ($p<0.001$) 20.11% increase in locomotor activity when compared to the Normal Control. The methanolic extract had no significant effect on rota rod. The extract significantly ($p<0.05$ & p0.001) decreased the no. of entries and the time spent in the open arm in the elevated plus maze. The extract also significantly ($p<0.001$) increased the no of entries in the Y maze. It also significantly ($p<0.001$) decreased the immobility time and increased the frequency of swimming and climbing in forced swim test.

Keywords: Anti-diabetic, CNS Stimulant, Isolation, Phytochemical analysis, *scutia myrtina*.

INTRODUCTION

Scutia myrtina (Rhamnaceae) is a large, evergreen straggling shrub or liane, sometimes a small tree distributed by forest border and on denuded slopes. Fruit edible. In India the leaf is used as an ointment to hasten childbirth (South African national list of trees). The aerial part of the plant was used for stomach problems, salpingitis.The root and leaves of the plant traditionally used as an antihelmintic. In eastern Tanzania the root of this plant is used for the treatment of bilharzias, intestinal worms and fever. The leaves and root bark of the *Scutia myrtina* decoction is used for gonorrhea, bilharzias, and intestinal worms in Tanzania. The tribal peoples of KolliHills of Tamil Nadu India used the whole plant for the treatment of tumor, inflammation and liver disorders (Ramanathan sambath kumar *et al.*, 2010). Used for diarrhea (root), dysentery (root), tonic, poison by fish (flower) by some of the islanders of Indian Ocean (S.K. Jain *et al.*, 2005). Root of the Scutia boiled in goat bone soup and used for getting strength (M. Ichikawa). To treat measles (Parker *et al.*, 2007). Root, stem bark or pieces of stem is used as stimulant (Listagem, 2010). Traditionally used as anti-diabetic (Sandhya.S *et al.*, 2011). Used as carbonization (MPMK, 1978). Anti-diabetic and CNS stimulant activity has not been reported. The present study describes the procedure of isolation and structural elucidation of flavonoids and to establish the traditional use of *Scutia myrtina* Anti-diabetic and CNS stimulant.

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MATERIALS AND METHODS

Plant Material:

The plant *Scutia myrtina* is widely found throughout India. It is available in Tirupathi hills and the collected plants were authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Tirupathi.

Preparation of Extract: The entire plant of *Scutia myrtina* was dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve number 40 and retained in sieve number 60 and stored in an air tight container for further use. The dried powder material of the plant was defatted with petroleum ether (40^0 c) for 48 hours in soxhlet apparatus. The defatted plant material thus obtained was further extracted with methanol for 72 hours in the soxhlet. The solvent was removed by distillation under reduced pressure and the resulting semisolid mass was dried (Ramanathan Sambath Kumar *et al.*, 2011; S. De1 *et al.*, 2010).

Phytochemical analysis: The extract was subjected to Preliminary Phytochemical Screening for the presence of different chemical groups (V.B. Patania; V.K. Srivastava). The plant contains phtochemicals such as alkaloids, glycosides, tannins, flavonoids, triterpenoids, fixed oils and fats. The phytochemicals which are present in the plant were indicated in the Table No.1

Isolation: Glass column of 1m × 2.5 cm is taken and the stationary phase is silica gel G 100-120 mesh. The column is prepared by wet packing technique. The

methanolic extract of *Scutia myrtina* is used for the isolation and the mobile phase is toluene:ethylacetate (5:1). The sample which is usually mixture of component is dissolved in minimum quantity of mobile phase. 90 fractions are collected and monitored by TLC (12, 13). The fractions which have components are concentrated and the crystals are collected. The crystals are identified by UV, IR, NMR, MASS.

Pharmacological activities

Animals: Male albino rats of Wistar strain weighing about 150 – 200 gm were used for the study. The animals were got from Nandha College of Pharmacy and Research Institute, Erode, and were approved by Ethical Committee and approval no. is NCP/IAEC/PG-20/2012. The animal house was well ventilated and animals had 25 ± 2 ºC. The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were fed with rat pellets feed supplied by M/s Hindustan Lever Limited, Bangalore, India and filtered water ad libitum. Animals described as fasted were deprived of food for 16 hr but allowed free access to water.

Anti-diabetic activity: The experimental rats were divided into five groups of five animals in each group. The animals were fasted overnight before the experimental schedule began but allowed free access to tap water.

Group I: The rats received 2 ml Normal Saline. These animals serve as normal controls.

Group II: The rats were made diabetic by an intraperitonial injection of Single dose of (150 mg/kg body weight) Alloxan monohydrate in normal saline and served as diabetic control.

Group III: The diabetic rat given Glibenclimide, 0.5 mg/kg, i.p. once a day

Group IV: The diabetic rat given *Scutia myrtina* methanolic extract (SCME) 400 mg/kg orally once a day

Blood samples were collected from the tip of the tail on 0^{th}, 7^{th}, 14^{th} and 21^{st} day. By without sacrificing the animals, from the tail vein by snipping off the tip of the tail and blood glucose were checked by one Touch select simple Blood glucose monitoring system.

On 21^{st} day the blood samples were collected through the retro-orbital puncture of eye of animals under mild chloroform anesthesia in Eppendorff's tube (1 ml) containing 50µl of anticoagulant (10% trisodium citrate) and serum was separated by centrifugation at 3000rpm for 15 min. The biochemical parameters Cholesterol, Triglycerides, Serum protein, SGPT and SGOT are determined by using the commercial kit available (Ecoline, Manufactured by Merck specialties, Private limited, Ambernath) (Alm, 2004; Sama Venkatesh *et al., 2010*).

CNS Stimulant activity

Group I: Animals treated with vehicle (0.5% sod. CMC) and treated as normal control (NC).

Group II: Animals treated with standard drug caffeine (10 mg/kg, i.p.) and treated as standard (STD).

Group III: Animals treated with *Scutia myrtina* extract (400 mg/kg) and treated as test (SCME).

Locomotor activity using actophotometer: Albino rats of either sex (20 - 25 g) were randomly divided into three groups of six animals. The rats were placed individually inside the chamber of actophotometer for 10 min and basal activity score was noted and after 30 min rats are again placed in actophotometer for 10 min and the activity was monitored. Percentage increases in activities were calculated (Indu Dhillon *et al.,* 2009; Angad Verma *et al.,* 2010).

Motor coordination using Rota-rod: Rota rod apparatus (Dolphin make) is a four panel techno device with timer. Animals (4 at a time) were placed on rod rotating at 20-25 rpm speed. The fall off time was recorded in all the groups before and 30 min after drug administration (Owolabi *et al.,* 2008; Uma Bhosale *et al.,* 2011).

Elevated plus maze (EPM): This test has been widely validated to measure anxiety in rodents. This apparatus was made of Plexiglas and consisted of two open arms (30cm × 5cm) and two closed arms (30cm × 5cm) with 25cm walls. The arms extended from a central platform (5cm ×5cm). The maze was elevated 38.5cm from the room floor. Each animal was placed at the center of the maze, facing one of the enclosed arms. Number of entries and the time spent in enclosed and open arms was recorded for 5 min test. Entry into an arm was defined as the animal placing all four paws onto the arm. All tests were taped by a video camera. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution) (Naik *et al.,* 2011; Preeti Kothiyal *et al.,* 2011).

Exploratory behavior using Y maze

Runaway test: This test is used to study the effect of a drug on spontaneous activity and motor coordination. The mice were placed individually in a symmetrical Y–shaped runway (33 cm x 38 cm x 13 cm) for 3 min and the number of the maze with all 4 ft (an 'entry') were counted (Ramanathan Sambath Kumar *et al.,* 2008; J.M. Sonpetkar *et al.,* 2012).

Modified forced swimming test: Rats were placed individually in a transparent glass cylinder (12 cm in diameter, height 25 cm), which was filled with water to a height of 15 cm. Two swim sessions were conducted. An initial 15-min pre-test followed 24 hr later by a 6 min test. In the pre test session, the mice which have not yet treated were forced to swim in a glass cylinder for 15 min. In the second session, each mouse received a respective dose of sample 1 hour prior to test, and placed in the cylinders again for 6 min. The following behaviors were recorded during the last 4 min.

1. Immobility: floating in water without swimming.
2. Swimming: active movements of extremities and circling in the container.
3. Climbing: active movements of forelimbs on the container wall (Joy Harris Hoskeri *et al.,* 2011; Vikram *et al.,* 2011).

Statistical evaluation: Statistical evaluation was done using one way analysis of variance (ANOVA) followed by Dunnet' T-test. Statistical significance was set as $p<0.001$, $p<0.01$, $p<0.05$.

RESULT AND DISCUSSION

The lamda max was found to be 242, 275, 306 cm; melting point: 150^0c; ^{1}H-NMR (ppm in $CDCl_3$); 7.5-7.8 (s, 10H- benzene); IR (KBr/ cm^{-1}); 1600 & 1487 (Aromatic C=C), 3072 (Aromatic CH), 1134 (C-O-C), 1745 (Ketone C=O); MS m/z 222. From the spectral data the isolated fraction might be flavonoid type of compound. Table no3 showed the levels of glucose in rats in different groups. The glucose level was significant ($p<0.01$) high in alloxan control rats compared with normal control. But the level of glucose was significant ($p<0.05$) decreased in diabetic rats treated with extract as compared with diabetic control rats. On repeated administration of the extract for 21 days, a significant decrease in the glucose level was observed in the diabetic rats as compared to diabetic control. There was no significant difference normal control and rats treated with only with extract.

It was evident from the table that untreated diabetic rats has elevated blood glucose levels and that the methanolic extract of whole plant were able to correct this metabolic deviation from the Diabetic Control significantly since there was no significant difference between Normal Control and Diabetic Control. So the whole plant extract has anti-diabetic activity.

Biochemical Parameters in experimental rats was depicted in Table no 4. In alloxan induced diabetic rats, there was significant ($p<0.001$) decrease in total protein when compared to Normal Control and the level was restored to nearly normal after the treatment. There was no significant difference between Normal Control and rats only treated with extract.

In alloxan induced diabetic rats, there was a significant ($p<0.001$) increase in Cholesterol, Triglycerides, SGOT and SGPT in serum compared to Normal Control. The plant extract used in the experimental study significantly ($p<0.001$) decreased the levels of Cholesterol, Triglycerides, SGOT and SGPT. This shows that the plant extract had favorable effect on lipid metabolism of diabetic rats.

Diabetes Mellitus is a multifactorial disease, which is characterized by hyperglycemia and lipoprotein abnormalities. Diabetes Mellitus leads to various metabolic aberrations in the animals viz. increased blood glucose, decreased protein content, increased cholesterol, Triglycerides, SGOT and SGPT.

The increased levels of transaminases (SGOT, SGPT) which are active in the absence of insulin because of the availability of amino acids in the blood of diabetics are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes. In the present study, the whole plant extract significantly decreased the enzymatic activities. Hence restoration of the SGOT, SGPT to the normal levels may also indicate revival of insulin secretion to normal levels.

The methanolic extract produced significant ($p<0.001$) increase in locomotor activity when compared to the Normal Control. The methanolic extract showed 20.11% increase in activity where as the standard drug Caffeine showed 34.69% increase in activity (Table no 5).

The extract and the caffeine (standard) had no significant effect on the mean time spent on the Rota rod (Table no 6). The extract's CNS stimulant effect was further confirmed by its ability to maintain the animals on the Rota rod, thus indicating muscle co-ordination.

In the Elevated plus maze (Table no 7), the rats treated with caffeine and extract showed significant ($p<0.01$ & $p<0.001$) decrease in the no. of entries in open arm and the time spent in the open arm compared with the control. Caffeine and the plant extract showed stimulant and anxiogenic effect.

In Y–maze test, the animals treated with methanol extract of *Scutia myrtina* at the dose of 400 mg/kg and caffeine showed a Significant ($p< 0.001$) marked increase in the no. of entries compared with control (Table no 8).

In modified forced swim test, the animals treated with caffeine and plant extract showed significant ($p<0.001$) reduction in immobility time and increased the frequency of climbing and swimming (Table no 9).

The results obtained in this study indicate that the extract possesses CNS stimulant properties which probably act via competitive antagonism at adenosine receptors leading to increase in nor-epinephrine secretion and enhanced neural activity in numerous brain areas.

CONCLUSION

The extracts obtained were subjected to various phytochemical tests, to identify the active constituents. Methanolic extract of the whole plant showed the presence of Alkaloids, Glycosides, Tannins, Flavonoids and Triterpenoids. The isolated fraction was subjected for the spectral studies (UV, IR, ^{1}HNMR and MASS), which indicated that the isolated fraction might be flavonoid type of compound. In alloxan induced diabetic rats, there was a significant ($p<0.001$) increase in Cholesterol, Triglycerides, SGOT and SGPT in serum compared to Normal Control. The plant extract used in the experimental study significantly ($p<0.001$) decreased the levels of Cholesterol, Triglycerides, SGOT and SGPT. This shows that the plant extract had favorable effect on lipid metabolism of diabetic rats. It shows that Flavonoids present in the extract may be possibly responsible for the pharmacological action. It also increased the locomotor activity compared to standard drug Caffeine. The extract and the caffeine (standard) had no significant effect on the mean time spent on the Rota rod. In the Elevated plus maze the rats treated with caffeine and extract showed decrease in the no. of entries in open arm and the time spent in the open arm compared with the control. In Y maze the extract and caffeine showed increase in the no. of entries compared with control. In modified forced swim test, the animals treated with caffeine and plant extract showed reduction in immobility time and increased the frequency of climbing and swimming. The pharmacological studies of extract showed that, extract possess CNS Stimulant activity.

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