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Adaptogenic activity of methanolic extract of *Buchanania lanzan* leaves an experimental study in rat model

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ABSTRACT

Buchanania lanzan is a widely used plant with a long history of traditional medicinal use for the treatment of various diseases. The present study is aimed at evaluating the adaptogenic activity of the methanolic extract of Buchanania lanzan leaves using the swim endurance model in all groups under normal and stressed conditions. Urinary vanillylmandelic acid (VMA) and ascorbic acid were selected as non-invasive biomarkers to evaluate the antistress activity. The 24 h urinary excretion of vanillylmandelic acid (VMA) and ascorbic acid were determined by spectrophotometric methods. Daily administration of the extract at doses of 10,20,30,40 and 50 mg/kg body weight prior to induction of stress inhibited stress-induced urinary biochemical changes in a dose-dependent manner without altering the levels in normal control groups. The methanolic extract exhibited significant anti-stress activity.

INTRODUCTION

Adaptogens cause an adaptive reaction to a disease and are useful in many unrelated illness and appear to produce a state of non specific increased resistance during stress resulting in stress protection[1].The adaptogens not only help in coping with stress but help in enhancing the general state of well being. Studies on the mechanism of action of adaptogenic drugs revealed that they produce immunostimulation [2]. These drugs strengthen the defense mechanism against the free radical induced damage inducing stress.Plants with potent antioxidant activity have been reviewed for their immunomodulatory and adaptogenic plenty of research to prove that these drugs potent antioxidant activity [3]

Ayurveda uses the plant, plant products and active ingredients present in plants for treating various disorders. Several plants have been reported which have adaptogenic and rejuvenating properties and are being investigated for remedies for a number of disorders including antistress (adaptogenic) activity [4].

Buchanania lanzan is commonly known as Chironji; this plant is reported to posses cardio tonic, astringent, antioxidant activity and is also used in the treatment of skin diseases [5]. The parts of the plant are used for the treatment of various disorders. The oil from the seeds is used to reduce granular swelling of the neck [6, 7]. Ointment is made from the kernel which is used to relieve itch and prickly heat. The gum from the bark used for treating diarrhea and intercostals pains and leaves are used for promoting wound healing[8].The present study was aimed at evaluating the antistress activity of the methanolic extract of *Buchanania lanzan in-vivo*, in both normal and stress induced rats following a biochemical approach.

MATERIAL AND METHODS

Plant material: The plant material was collected from Yukka enterprises ,Navi Mumbai. The material was shade dried, pulverized and preserved in air tight containers.

Preparation of the extracts

The methanolic extract was prepared from the dried powder of the leaves using Soxhlet apparatus. This was then concentrated and dried to give dark brown mass.The extract was then subjected to preliminary phytochemical analysis using standard methods.

Chemicals

Vanillylmandelic acid (VMA) was purchased from Sigma-Aldrich, while ascorbic acid was purchased from SD fine chemicals. All other reagents used were of analytical grade.

Animals

All animal experiments were performed after obtaining approval from The Institutional Animal Ethical Committee and the guidelines for the animal care were strictly adhered to during the experimentation as recommended by committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt of India. Sprague-Dawley (SD) rats of either sex weighing 250-275 gm were used. The animals were maintained under standard conditions and were fed with commercial diet and water *adlibitum* during the entire experiment.

Phytochemical analysis

The extract was subjected to preliminary phytochemical screening for phytochemical constituents such as alkaloids, steroids, saponins, glycosides, and polyphenols.

Antistress activity

Rats of either sex weighing between 150-250 gm were divided into six groups (I, II, III, IV, V, and VI) each containing six animals. The 24h urine sample from each group was collected into two different beaker, one containing 5 ml of 10% oxalic acid for spectrophotometric determination of ascorbic acid at 550 nm and the other containing 0.5 ml 0f 6 N hydrochloric acid for the determination of vanillylmandellic acid (VMA) spectrometrically at 360nm .The

experimental protocol was divided into four phases. In the first phase of the experiment, 24 h urine samples were collected in all the six groups and subjected to analysis for both VMA and ascorbic acid and the normal values were recorded for seven consecutive days. In the second phase, the animals in each group were subjected to fresh water swimming stress individually .In this method, rats were forced to swim until exhausted (three to four minutes) in cylindrical vessel of 60 cm height and 45 cm diameter containing water at room temperature (28◦C).Water depth was always maintained at 40 cm.The 24 h urinary levels of VMA and ascorbic acid under stressed conditions were determined again as described above daily for seven consecutive days. Third phase of experiment consists of administration of Methanolic Extract to same groups of animals after having recovered completely to normal condition. Groups II, III, IV, and V and VI were administered orally with Methanolic Extract at daily doses of 10, 20, 30, 40, and50 mg/kg body weight respectively for seven consecutive days while group I serving as control. The 24h urine samples were collected and the levels of both VMA and ascorbic acid were determined. The final phase of the experiment consisted of administrated of Methanolic Extract to the same groups of animals after a recovery period of one week. Groups II,III,IV,V,VI were administered orally with Methanolic Extract at doses of 10,20,30,40 and 50 mg/kg body weight respectively, and hour prior to the daily induction of stress for seven consecutive days while group I serving as control. The 24h urine sample were collected and analysed for VMA and ascorbic acid for seven consecutive days to study the influence of the Methanolic Extract on the stress induced biochemical changes.

RESULT AND DISCUSSION

Rats when forced to swim in a restricted space become immobile after a certain period of vigorous activity which indicates a state of mental depression. An increase in total swimming time indicates better stress tolerance. Adaptogens of plant origin reduce the reactivity of host defense system and decrease the damaging effects of the different stressors due to increased basal levels of mediators that are involved in the stress response[9].

Table-1 Effect of methanolic extract on 24hr urinary levels of VMA in normal and stress-induced rats

	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI
NORMAL(N)	171±22.2	170±30.46	165±31.7	160±20.7	172±33.5	171±24.6
STRESS(S)	302±23.11	300±22.1	291±22.2	295±24.33	301±19.2	299±33.17
NORMAL+ EXTRACT(NE)	160±35.4	169±19.12	152±30.3	160±28.4	171±29.1	169±24.2
EXTRACT+ STRESS(ES)	300±22.3 (control)	200±32.22	190±31.1	180±35.1	160±30.3	158±27.33

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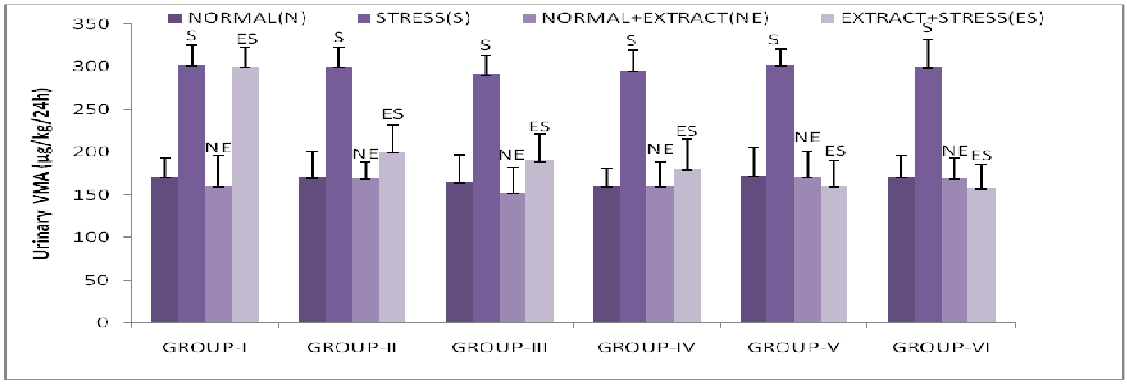
The urinary data of Vanillylmandelic acid and ascorbic acid observed during the different stages of the study are shown as in table 1 and 2 respectively. Induction of forced swim stress to the animals brings about a significant increase ($P<0.05$) in VMA level and decrease ($P<0.05$) in ascorbic acid excretion when compared to their basal excretion in normal condition. Both the parameters were regained within four days after withdrawal of stress. Daily treatment of to the animals under normal condition did not produced any change in the excretion of VMA and ascorbic acid compared to normal basal levels which indicates that there was no alteration in the excretion of VMA and ascorbic acid under normal condition. Daily administration of *the*

extract one hour prior to the induction of stress inhibited the increase in VMA and decrease in ascorbic acid excretion which was manifested during stress alone. The inhibition of both the parameters i.e. increase in VMA levels and decrease in ascorbic acid levels was significant at all dose levels ($p < 0.05$) in a dose dependent manner.

Fig 1: Effect of methanolic extract on 24hr urinary levels of VMA in normal and stress-induced rats



Each bar indicates the mean excretion of six animals Control is CMC (carboxy methyl cellulose)+ DMSO (dimethyl sulfoxide).Herein group-1 is the control.

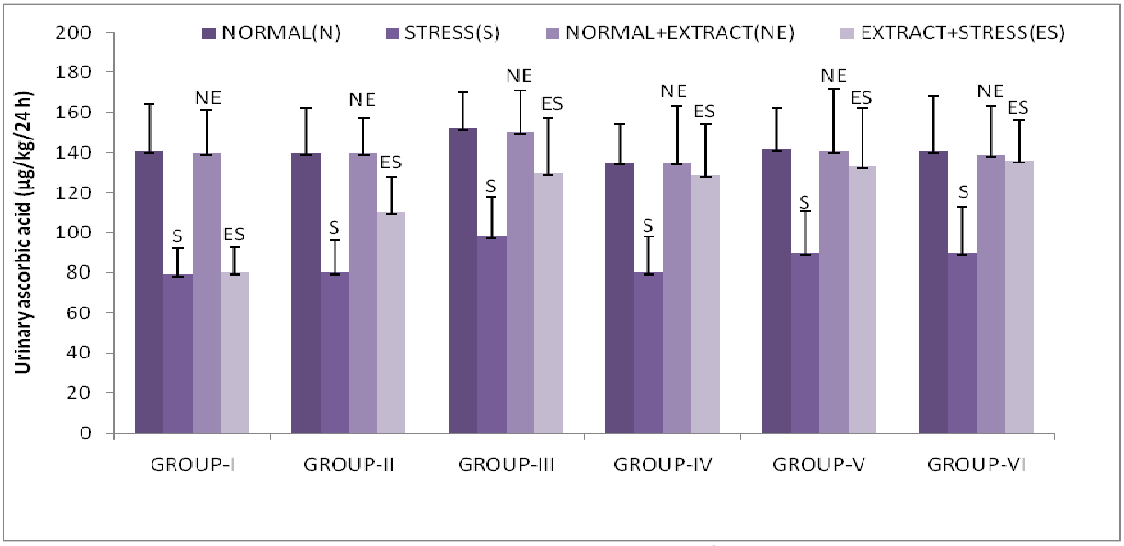
Review of literature has revealed that noradrenaline is released during stress, which is metabolized to vanillyl mandelic acid (VMA) peripherally and 3-methoxy 4-hydroxyphenyl glycol (MOPEG) centrally, VMA is the major metabolite of sympathetic amines and hence taken as indirect biochemical index non invasive biochemical marker and to represent the increase in peripheral sympathetic activity during stress [10].

It has also been reported that the tissue levels of ascorbic acid decreased when stress is applied. Ascorbic acid which is a free radical scavenger helps in scavenging the free radicals responsible in stress resulting in its decreased urinary concentration [11]. Hence in our investigation ascorbic acid excretion in urine was taken as an indirect biochemical index to indicate the influence of stress on catecholamine synthesis in rats and to evaluate the antistress (adaptogenic) activity of the *Buchanania lanzan* extract on prior administration of stress induction. Literature survey has revealed that the anti-stress activity of some of the potential medicinal plants could be attributed to their antioxidant effect [12, 13].

Table 2: Effect of methanolic extract on the 24hr urinary level of Ascorbic acid in normal and stress-induced rats

	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI
NORMAL(N)	141±23.3	140±22.1	152±18	135±19	142±20.2	141±27.1
STRESS(S)	141±13.3	80±16.3	98±20	80±18	90±21	90±23
NORMAL+ EXTRACT(NE)	140±21.1	140±17	150±21	135±28	141±31	139±24
EXTRACT+ STRESS(ES)	80±12.9 (control)	110±18	130±27	129±25	133±29	136±20

Fig 2: Effect of methanolic extract on the 24hr urinary level of Ascorbic acid in normal and stress-induced rats



Each bar indicates the mean excretion of six animals

Buchanania lanzan has been reported to posses anti oxidant activity [14].Hence it can be concluded that the antioxidant activity of the plant may be one of the contributing factor for the adaptogenic activity of the plant.

CONCLUSION

In conclusion, the present study provides scientific support for the antistress (adaptogenic) activity of the methanolic extract of the leaf of *Buchanania Lanzan.* Further investigations are required to characterize the active constituent(s) responsible for observed activities of the extract.

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