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Adaptogenic Potential of *Pithecellobium Dulce* Fruit Extract: An Experimental Study

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Abstract:

Pithecellobium dulce fruit (Fabaceae) is a medicinal plant used in the treatment of many clinical conditions in India. Its adaptogenic potential activity has been investigated in this study using albino mice and rats. The methanolic extract of *Pithecellobium dulce* fruit was prepared and subjected to preliminary qualitative phytochemical screening. Flavanoids, Tannins and Alkaloids were found to be present. Acute toxicity studies were carried out in albino mice. The methanolic extract did not show the lethal effect at a dose of 2000mg/kg body weight with no signs of abnormalities or any mortality observed for 14 days period. Anoxia stress tolerance test, Swimming endurance test, Immobilisation stress models were used to investigate the adaptogenic potential of title plant. The results indicate that pretreatment with methanolic extract of fruit of *Pithecellobium dulce* exhibited significant adaptogenic potential activity at the tested doses of 125mg/ kg and 250 mg/ kg 500 mg/kg. On the basis of results, it was concluded that *Pithecellobium dulce* fruit possess adaptogenic potential activity.

Keywords: *Pithecellobium dulce* fruit, anoxia stress tolerance test, swimming endurance test model, Immobilization stress.

INTRODUCTION

Stress is a common phenomenon that is experienced by all individual. When stress becomes extreme, it is harmful for the body and hence needs to be treated. Stress plays an major role in the pathogenesis of a variety of diseases including hypertension, peptic ulcer, immunodepression, reproductive dysfunction and behavior disorder (Asad M et al., 2009). Evidence related that stress impairs learning and memory and encounter several disorders including anxiety and depression(Anuradha

H et al., 2008). Stress mapping or screening of biologically active constituents of natural origin, mainly from plant kingdom (Panossian A et al., 1999). Drugs having Antistress properties induce a state of non-specific resistance against stressful conditions.

Empirical use of medicinal herbs has been widely disseminated since ancient times to treat a wide range of diseases. In current days, the interest in alternative therapies has raised markedly across the globe(Muller JC

et al., 2009). Drugs derived from plant source are emerging as alternative therapies in the treatment of many diseases including psychiatric disorders(Kienzle S et al., 2002). Medicinal plants playing significant role in the search of novel pharmacotherapy to treat psychiatric illnesses(Zhang ZJ et al., 2004). Plants have always been an exemplary source of drugs and many of the available drugs have been derived directly or indirectly from them(Patil R et al., 2011).

Drugs like benzodiazepines, certain CNS stimulants such as amphetamines and caffeine as well as some anabolic steroids are routinely used by people to combat stress. The incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs(Sandhya KD et al., 2011). Alternative are clearly needed because of the inability of the current therapies to manage condition of disease(Marles RJ et al., 1995). The first drugs used to treat pathologic condition of the CNS were based on natural resources(Gomes NG et al., 2009). People from different area of world using herbal medicine to alleviate affective disorders(Mora S et al., 2009). The herbal formulations claimed to enhance physical endurance, mental functions and non-specific resistance of the body have been termed as adaptogen(Sandhya KD et al., 2011).

Various plants are being used in complementary and alternative medicines for management of stress(Gupta Vet al., 2010). The potential utility of safer and cheaper herbal medicines as Antistress agents have been reported as they can withstand stress without altering the physiological functions of the body.

Pithecellobium dulce (PD) commonly known as Seeme hunase in Kannada is a rich source of medicinal value having multidimensional curative properties. Different parts of the tree have been found

to possess medicinal properties; leaves are used for treating ulcers, aerial roots for gonorrhea whereas seeds and fruits are cooling and tonic. In India, milky juice (latex) of stem bark of *Pithecellobium dulce* is used for the treatment of dysentery, diarrhea, gum ailments, toothache and other hemorrhage(Battu Ganga Rao et al., 2018).

Phytochemical investigation of *Pithecellobium dulce* explored wide variety of constituents which are responsible for its wide range of pharmacological activities. They include Alkaloids, flavonoids, Glycosides, Saponins, fatty acids, steroids, Tannins and triterpenoids, furocoumarin, tiglic acid ester and some other esters(Battu Ganga Rao et al., 2018). Flavanoids have been acknowledged for their interesting medicinal properties(Battu Ganga Rao et al., 2018). Different parts of *Pithecellobium dulce* reported to possess anthelmintic, analgesic, immunomodulator, hypolipidemic, antidiabetic and antiallergic activities(Battu Ganga Rao et al., 2018). Flavonoids isolated from fruits of *Pithecellobium dulce* has been reported for Adaptogenic activity(Battu Ganga Rao et al., 2018). However, Antistress (adaptogenic) activity of fruits of title plant has not been scientifically validated till date. Hence the present study was undertaken to evaluate adaptogenic activity.

Literature Review

Anonymous et al.,(1969)*Pithecellobium dulce* Benth. (Leguminosae) is a small to medium sized, evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. It is known as Vilayati babul in Hindi and Kodukkapuli in Tamil.

Ahmad. G,Yusuf Amin K.M, KhanA.N et al.,(1998) Adaptogens are the plant-derived biologically active substances that appear to induce a state of non-specific increase of resistance of the organism to diverse assaults

that threaten internal homeostasis and improve physical endurance.

Rai, Gitika Bhatia G., Sen T. and Palit G et al., (2003) Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, viz; hypertension, peptic ulcer, diabetes, immunosuppression, reproductive dysfunctions and behavioural disorders like anxiety due to involvement of the central nervous system (CNS), endocrine system, and metabolic system.

Battu Ganga Rao et al. (2018) The genus *Pithecellobium* includes some 650 species of plants occurring in most tropical and subtropical forest throughout the world. The genus is remarkable for the large variation in the habits of its species.

Watsika Vichaidrtand Panumart Thongyoo. (2019) Antioxidant: Study of the aqueous extract of *Pithecellobium dulce* leaves revealed phenolics including flavonoids and showed potent free radical scavenging activity.

Sharma Shweta, Mehta B.K et al., (2013) *Pithecellobium* species (leguminosae) are widely distributed in the tropics, chiefly in Asia and America. *Pithecellobium dulce* Benth, a most versatile medicinal plant, has attracted a worldwide prominence in recent years, owing to its wide range of medicinal properties and diverse utility. All plant parts of the *P. dulce* elaborates a vast array of biologically active compounds and have been demonstrated to exhibit antidiabetic, locomotor, anti venom, free radical scavenging, protease inhibitor, anti inflammatory, anti bacterial, anti mycobacterial, abortifacient, spermicidal, anti convulsant, anti ulcer, anti diarrheal, anti fungal, anti tubercular, anti tumor and anti oxidative properties. Here the compounds present in different parts of *P. dulce* and biological activities of their extracts or the chemical constituents as reported in the literature since 1962 to 2013 have been reviewed.

Bhattacharya K.S, Muruganandam V.A et al., (2003) *Withania somnifera*: (WS) Dunal is classified in Ayurveda, the ancient Hindu system of medicine, as *arasayana*, a group of plant-derived drugs reputed to promote physical and mental health, augment resistance of the body against disease and diverse adverse environmental factors, revitalise the body in debilitated conditions and increase longevity. These attributes are remarkably similar to the properties ascribed to adaptogens like *Panax ginseng* (PG) in contemporary medicine.

Materials and methods (Research Methodology)

Plant material

The fruits of *Pithecellobium dulce* was identified and authenticated by Dr. Ramchandra Naik. M Professor & HOD, Dept. of Botany S.B Arts & KCP Science College, Vijayapur, Karnataka. Then sufficient amount of fruits of *Pithecellobium dulce* were collected from in and around the garden of Vijayapur city, Karnataka and the sample has been preserved in the herbarium of the college.

Preparation of extract

The fruits were shade dried at room temperature and ground to coarse powder and then extracted with methanol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator. The yield of the extract obtained was 7.4 %. The obtained crude extract was stored in airtight container in refrigerator below 10 °C for further studies.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on test extract for the detection of phytoconstituents by following literature reported methods (Kokate CK et al., 1994) (Khandelwal KR et al., 2004).

Experimental animals

The Wistar albino rats of 150 - 200 gm and Swiss albino mice 20 - 30 gm of either sex was used in the experimentation. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry condition as follows.

- Room temperature: $27 \pm 3^\circ$
- Relative humidity: $65 \pm 10\%$,
- 12 r light/dark cycle

All the animals were fed with rodent pellet diet (VRK Nutritional Solutions, Pune, India) and water *ad libitum* under strict hygienic condition. Study protocol was approved from Institutional Animals Ethics Committee (IAEC) before initiation of the experiment.

Acute toxicity study (LD₅₀)

An acute toxicity of methanolic extract of *Pithecellobium dulce* fruits was performed on female albino mice (20-30 gm). The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 423) method was adapted for toxicity studies (Shivakumar H et al., 2006). 1/40th, 1/20th and 1/10th LD₅₀ cut off value of the extract were selected as screening doses for the anti-stress activity.

Evaluation models for adaptogenic activity of methanolic extract of *Pithecellobium dulce* fruit extract

Anoxia Stress Tolerance Test

Albino mice of either sex weighing 20-30 gm were selected and divided into five groups of six each.

Group I - Control, received distilled water

Group II - Std. (*Withania somnifera*, 100 mg/kg, p.o.)

Group III - MEPDF (125 mg/kg, p.o.)

Group IV - MEPDF (250 mg/kg, p.o.)

Group V - MEPDF (500 mg/kg, p.o.)

Animals were treated as shown above for the three weeks. At the end of 1st, 2nd and 3rd week i.e. on 7th, 14th and 21st day 1 hr. after the treatment. Stress was induced in all the groups of animals by placing each mouse individually in the air tight bottle of 250 ml capacity to record anoxia time. The moment when the animal showed the first convulsions removed immediately from the bottle and resuscitated if needed. The time duration of animal entry into the air tight bottle and the appearance of the first convulsion were recorded as anoxia time. Appearance of convulsion was very sharp end point, as delay by minute of removal of the animal from the vessel may lead to death of the same.

Swimming Endurance test in mice

Albino mice of either sex weighing 20 -30 gm divided into five groups of six animals each for the test as below

Group I - Control, received distilled water

Group II - Std. (*Withania somnifera*, 100 mg/kg, p.o.)

Group III - MEPDF (125 mg/kg, p.o.)

Group IV - MEPDF (250 mg/kg, p.o.)

Group V - MEPDF (500 mg/kg, p.o.)

Treatment was given to mice for 7 days. On seventh day 1 hr. after treatment, all the mice were subjected to swimming endurance test. The mice were allowed to swim individually in swimming tank 30 cm height with 20 cm diameter containing water of 25 cm height maintained at $26 \pm 1^\circ$ C temperature. The end point was taken when the animals remained at the bottom of swimming tank for 10 sec. The mean swimming time for each group was calculated.

Immobilization Stress in rats (Vinod Pawar et al., 2011)

In the present study, adult albino rats of either sex weighing 150 – 200 gm were divided into six groups of six animals each.

Group I -	Normal control
Group II	- Stress control
Group III	- Standard
<i>(Withania somnifera</i> , 100mg/kg, p.o.)	
Group IV	- MEPDF (125 mg/kg, p.o.)
Group V	- MEPDF (250 mg/kg, p.o.)
Group VI	- MEPDF (500 mg/kg, p.o.)

The treatment was made as stated above for 10 days 1hr. before the exposure of stress. Stress was induced by immobilizing rats with head down, supine position by fixing the forelimbs and hind limbs to a wooden board inclined at an angle of 60°, daily 2hrs. for a period of ten days.

Biochemical estimations

At the end of 10th day one hour after drug treatment the blood was collected from retro orbital plexus in sodium citrated tubes under mild ether anesthesia using disposable syringe and needle for estimation of biochemical parameters, such as serum glucose (GOD-POD method), cholesterol (CHOD-PAP method), triglycerides (GPO-Trinder method), BUN (Blood Urea Nitrogen, GLDH-UREASE method) using Erba Chem Semi-auto analyzer and ready reagent kits.

The rats then scarified and their organs such as liver, spleen and adrenal glands were removed. The weight of organs such as liver, spleen and adrenal glands after washing with alcohol was recorded per 100 g body weight of animal.

Results & Discussion

Preliminary phytochemical screening

Preliminary phytochemical investigation on methanolic extract of *Pithecellobium dulce* fruit indicated the presence of flavonoids,

alkaloids, quercitin, kaempferol, dulcitol, afezilin and tannins.

Acute toxicity study

MEPDF was studied for acute toxicity at dose of 2000 mg/kg i.p. in female albino mice. The extract did not cause any mortality (0/3 mice died) of the animals at dose of 2000 mg/kg, even at repeated dosing using 3 new mice. Hence, 5000 mg/kg was taken as LD₅₀ cutoff value as per fixed dose method of OECD guideline number 423.

The screening doses selected for the anti-stress activity were:

125 mg/kg - 1/40th dose of LD 50 cut off value, 5000 mg/kg b.w.

250 mg/kg - 1/20th dose of LD 50 cut off value, 5000 mg/kg b.w.

500 mg/kg - 1/10th dose of LD 50 cut off value, 5000 mg/kg b.w.

Preparation of stock solution of methanolic fruit extract of *Pithecellobium dulce*

Appropriate concentration of stock solution of the test extract was prepared by suspending in 2% w/v of tween 80 in distilled water. This stock solution was administered orally at 1ml/100g body wt. of mice and 0.5ml/100g body wt. of rats.

Anoxia stress tolerance time in mice

In the anoxic tolerance test, the time taken for the mice to exhibit clonic convulsions was taken as the end point. The graded doses (125, 250, 500 mg/kg) of the test extract demonstrated dose and duration dependent significant delay in clonic convulsions on 7th, 14th and 21st day compared to control group. Adaptogenic effect of the higher dose (500 mg/kg) of the test extract was found to be less effective than that of the standard drug. The results are presented in Table-1.

Swimming endurance test in mice

There was dose dependent significant increase in swimming performance time

observed in mice seven days pretreated with graded doses (125, 250 and 500 mg/kg) of the test extract. The percentage increase in swimming performance time was found to be 22 to 46. However, the effect of test extract on swimming performance time was found to be less potent than the reference standard drug, *Withenia sominifera*. The results are tabulated in Table -2.

Immobilization Stress

Effect on biochemical parameters

Immobilization stress adversely affected the serum concentration of various biochemical parameters. The induction of Immobilization stress significantly elevated the serum cholesterol, triglycerides, BUN and glucose levels in stress control rats compared to normal control group. Animals pretreated for ten days with test extract at different dose levels (125, 250 and 500 mg/kg) showed significant and dose dependent fall in all the biochemical parameters, as compared to the stress control animals. The results are displayed in Table -3.

Effect on weight of organs

Immobilization stress significantly increased the weight of liver, adrenal glands and decreased the spleen weight. Ten days pretreatment with graded doses of MEPDF significantly and dose dependently ameliorated the Immobilization stress induced altered organs weight. The results are represented in Table -4.

Discussion

In the current study, anti-stress activity of MEPDF was demonstrated at different dose levels (125, 250 and 500 mg/kg) against anoxia stress tolerance test, swimming endurance time and immobilization stress models in experimental animal like mice and rat.

Anoxia is a more severe stress. All the body functions including cellular respiration depends on oxygen supply to them. Any lack of this vital element plays major role on all body mechanisms. Increase in adaptation

during anoxic stress by any drug could be considered as its major anti-stress effect (Corda MG et al., 1911) (Bhargava K P et al., 1981) (Singh RH et al., 1979). The results of the anoxic tolerance test showed that MEPDF significantly delayed the latency of post anoxic convulsions in experimental animals, thereby confirm its adaptogenic activity.

Increase in swimming endurance time has reported in mice when pre-treated with adaptogenic agents (Bhatwadekar AD et al., 1999) and the test has been utilized to investigate the adaptogenic activity of different agents, based on the fact that swim endurance reflects physical endurance (Krupavaram B et al., 2007). In the present investigation the results indicate clearly that the extract of *Pithecellobium dulce* have the properties whereby they increased the physical endurance as well as the overall performance in mice.

Experimental animals exposed to an Immobilisation stress resulted in hyperglycemia, this is because during stressful condition adrenal cortex secretes excess cortisol (Krupavaram B et al., 2007). Excessive secretion of cortisol maintains the internal homeostasis through the process of gluconeogenesis and lipogenesis (McEwen BS et al., 2008). The results of the current study revealed that the extract of the *Pithecellobium dulce* exhibited promising effect in controlling hyperglycemia indicating the ability to prevent the alterations on adrenal cortex and helping in maintaining the homeostasis.

The mechanism by which stress rises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis (HPA) resulting in liberation of catecholamines and corticosteroids. This could lead to increase in blood cholesterol level, since epinephrine is known to mobilize lipids from adipose tissues. The effect of stress on serum

triglycerides has been shown to be variable. The increase in release of catecholamines leads to elevated levels of glucose and BUN (Alexander N et al., 2010) (Sapolsky RM et al., 2006). In Immobilisation stress model, the test extract reduced the elevated levels of serum biochemical parameters in dose dependent manner.

Stress induces adreno-medullary response in man. Adrenaline in turn stimulates Beta 2 receptors on the pituitary glands causing greater release of ACTH, which can stimulate the adrenal medulla as well as cortex. So adrenal gland weight increases. Cortisol increases mRNA levels in liver cells, this lead to increase in weight of liver. Spleen constricts to release more red blood cells (RBC) during stress, so its weight

decreases during stress (Nimbakar SR et al., 2001) (Kannur DM et al., 2006) (Schimmer BP et al., 2006). The rats pretreated with *Withania Somnifera* and MEPDF significantly reversed altered organs weight of adrenal glands, liver and spleen thus supports the anti-stress effect of MEPDF against immobilization stress model.

The literature reports indicated that extracts of medicinal plants containing flavonoids and tannins known to possess significant adaptogenic activity (Jiban D et al., 2011). In our study also flavonoid and tannin contents of crude extract of the title plant (evidenced by phytochemical analysis) may be responsible for observed adaptogenic (anti-stress) activity.

Table 1: Effect of MEPDF on anoxia stress tolerance time in mice

Groups	Treatment	Dose (mg/kg)	Duration of anoxia stress tolerance time (min)		
			7 th Day	14 th Day	21 st Day
I	Control	--	28.3 ± 0.96	30.1 ± 2.02	34.1 ± 2.01
II	Std. (<i>Withania somnifera</i>)	100	55.5 ± 3.9***	57.2 ± 2.18***	60.1 ± 3.1***
III	MEPDF	125	33.3 ± 1.9*	40.3 ± 1.9*	42.3 ± 2.1*
IV	MEPDF	250	36.1 ± 2.6*	41.6 ± 2.1**	44.3 ± 1.5*
V	MEPDF	500	42.3 ± 3.1***	46.1 ± 3.1***	48.1 ± 3.1***

The values are expressed as Mean ± SEM, (n=6).

Where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.

Table 2: Effect of MEPDF on swimming endurance time in mice

Groups	Treatment	Dose mg/kg	Swimming endurance time (min)	% increase in swimming time
I	Normal control		----	---
II	Stress control		228.4 ± 13.93	----
III	Standard (W S)	100	418.6 ± 9.54***	49.95
IV	MEPDF	125	266.8 ± 12.10**	16.61
V	MEPDF	250	330.2 ± 16.47***	33.48
VI	MEPDF	500	394.9 ± 11.19***	45.88

The values are expressed as Mean ± SEM, (n=6),

Where @ $p < 0.001$ * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to stress control.

Table 3: Effect of MEPDF on serum biochemical changes in immobilization stress in rats

Groups	Treatment	Dose mg/kg	Biochemical estimations			
			Glucose mg/dl	Total Cholesterol mg/dl	Triglyceride mg/dl	BUN mg/dl
I	Normal Control	--	98.3±4.9	52.6±3.8	58.3±2.9	22.2±1.2
II	Stress Control	Vehicle	149.3±4.9 [@]	138.6±3.1 [@]	99.5±3.1 [@]	37.3±1.2 [@]
III	Standard (W S)	100	108.3±6.1 ^{***}	58.3±2.9 ^{***}	59.9±2.6 ^{***}	24.3±1.1 ^{***}
IV	MEPDF	125	130.5±3.1 [*]	123.8±1.9 ^{**}	82.3±1.9 ^{***}	32.4±0.8 [*]
V	MEPDF	250	123.5±2.9 ^{***}	101.3±1.8 ^{***}	77.1±1.9 ^{***}	29.9±1.2 ^{***}
VI	MEPDF	500	119.8±2.5 ^{***}	71.5±1.9 ^{***}	66.3±1.8 ^{***}	26.3±0.9 ^{***}

The values are expressed as Mean ± SEM, (n=6),

Where[@] $p < 0.001$, $*$ $p < 0.05$, $**$ $p < 0.01$, $***$ $p < 0.001$ as compared to stress control.

Table 4: Effect of MEPDF on organs weight in immobilization induced stress rats

Groups	Dose mg/kg	Organs weight (gm/100 gm b.w.)		
		Liver	Spleen	Adrenal glands
Normal control		4.00 ± 0.22	0.251 ± 0.01	0.158 ± 0.02
Stress control		7.19 ± 0.90 [@]	0.060 ± 0.04 [@]	0.496 ± 0.02 [@]
Standard (W S)	100	3.56 ± 0.14 ^{***}	0.226 ± 0.02 ^{***}	0.280 ± 0.02 ^{***}
MEPDF	125	5.32 ± 0.17 ^{**}	0.085 ± 0.02 [*]	0.416 ± 0.03 [*]
MEPDF	250	4.56 ± 0.15 ^{***}	0.115 ± 0.01 [*]	0.370 ± 0.01 ^{**}
MEPDF	500	4.10 ± 0.16 ^{***}	0.140 ± 0.02 ^{**}	0.315 ± 0.02 ^{***}

Values are expressed as Mean ± SEM, (n=6)

Where, $*$ $p < 0.05$, $**$ $p < 0.01$, $***$ $p < 0.001$ as compared to stress control and

[@] $p < 0.001$ compared to normal control.

Conclusion

In conclusion, the methanolic extract of *Pithecellobium dulce* fruit exhibited dose dependent significant adaptogenic activity by increasing the capacity to tolerate stress in experimental animal as well as restoring the altered biochemical parameters and organs weight. Thus the fruit extract of the title plant acts as an adaptogenic agent in management of stress and stress related diseases.

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