



Research Article

Phytochemical Investigation and Effect of *Abutilon Indicum* on Various Biochemical Parameters on Stress Induced In Albino Rats

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ABSTRACT

The present study was carried out to evaluate the effect of ethanolic extract of *Abutilon indicum* on swimming endurance test and cold induced stress in albino rats. The effect was assessed by swimming survival time and estimation of various biochemical parameters like glucose, cholesterol, triglycerides and blood urea nitrogen (BUN) and blood cell count (WBC) and also the differential count. In cold stress by determining the ulcer index and weight of organ such as, liver, spleen, testes, adrenal gland at a dose of and 400mg/kg body weight per oral. It was found that ethanolic extract significantly ($p < 0.001$) increases the swimming time, showed significant ($p < 0.001$) decrease in blood glucose, cholesterol, triglyceride, BUN and plasma cortisol levels. A significant ($p < 0.01$) decrease in WBC count, polymorphs, monocytes, lymphocytes and eosinophils. In cold stress significant ($p < 0.001$) decrease in ulcer index, protection increase in P^H of gastric juice. weight of organ was observed to control group. Thus the obtained results revealed that the *Abutilon indicum* has got a significant anti stress activity.

Key words: Swimming, Cold stress, *Abutilon indicum*, WBC, BUN

INTRODUCTION

Stress is a biological response to aversive conditions such as injury and emotional disturbances that tend to threaten the homeostasis of the organisms. Stress is involved in the pathogenesis of a

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variety of diseases that includes psychiatric disorders such as depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male impotence, cognitive dysfunction, peptic ulcer, hypertension and ulcerative colitis. Medical plants have been found to possess several phytochemical active compounds which possess wide range of biological activities, that are responsible for the observed curative effects of herbal medicines^[1,2].

Abutilon indicum (Malvaceae) is found throughout tropical and sub tropical regions of India, is known as Atibal in Sanskrit. The various parts of plant claimed to have several traditional medicinal properties. The whole plant is studied for anti inflammatory, immuno stimulating effect, piles and gonorrhoea treatment. Root and bark are used as aphrodisiac, anti diabetic, nervine tonic, and diuretic. Seeds are used as aphrodisiac, in treatment of urinary disorders^[3]. The plant is reported to have analgesic^[4], hypoglycemic^[5], hepato protective^[6], hyperlipidemic activity^[7]. Also reported in the literature isolation of sesquiterpine lactone, isolation of Gallic acid, eugenol^[8] wound healing^[9] and anti bacterial activity^[10]. The present study is an attempt to validate anti-stress activity of *Abutilon indicum*.

MATERIALS AND METHOD

The plant was collected from local area of Hubli-Dharwad, and authenticated by Dr. G.R. Hegde, Dept. of Botany, Karnataka University, Dharwad. A Voucher specimen Number (PG 506-1) has been deposited at KLE College of pharmacy, Hubli, Karnataka. Ethanolic extract *Panax ginseng* a gift sample obtained from Madhur Pharma, Bangalore (PAN-C00531).

The shade-dried plant pulverized to reduce to 60 meshes, powder was subjected to Soxhlet extraction with 95% ethanol, the ethanolic extract was concentrated in a rotary evaporator and dried in vacuum desiccator using sodium sulphite. The dried ethanolic extract was suspended in distilled water using 1% Tween 80, used for pharmacological screening.

Phytochemical screening: The extract was qualitatively tested for the presence of major phytochemical constituents^[11].

Experimental animals

Adult Swiss albino mice (20- 25g) and Wistar rats (150 -200g) of either sex were used for the study. The mice and rats were fed with standard pellet (Parnava Agro industries Ltd. Sangali, India) and water *ad libitum*. The animals were maintained under standard 12-hr light / dark cycle throughout the study. The study protocol was approved by IAEC. (No.CPCSEA/IAEC/PC-01/346).

Acute toxicity study^[12]

The study was performed according to the acute toxic classic method (as per CPCSEA/OECD guidelines). Swiss albino mice were used for acute toxicity study. The animals were kept Fasting

for overnight providing only water, after which the test drug extract dissolved in Water was administered orally at the dose of 800 mg/kg and observed for 14 days. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory(heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion). The toxicity study carried out as per the guidelines of AOT- 421 using albino mice. The extracts were found to be safe till 600mg/kg. Hence we selected 400mg/kg dose for pharmacological screening.

Table 1 Effect of *Abutilon indicum* on swimming endurance test.

Group	Mean duration of Swimming survival time (in min.) Mean± SEM		
	1 st week	2 nd week	3 rd week
Control	22.3 ±1.91	39.8 ±2.11	54.4 ±3.85
Ethanollic extract of <i>Abutilon indicum</i>	18.6 ±1.78**	33.26±3.27**	47.3± 3.28***
Ethanollic extract of <i>panax ginsang</i>	21.4 ±2.18**	35.8 ± 3.98**	51.6 ± 3.11***

Swim endurance test^[13]

Albino rats (120 ± 150 g) of either sex were randomly divided into 4 groups of 6 animals each. Group 1 control, Group 2 swimming test control, Group 3 swimming test and treated with extract at the dose of 400mg/kg p.o. and Group 4 subjected to swimming test and treated with *Panax ginseng* extract at the dose of 100mg/kg p.o respectively for 21 days. Swimming test was carried out on 7th, 14th and 21st day. One hour after the drug administration mice were allowed to swimming in (24×24×18 cm) rectangular container filled with water maintained at 25 ± 2°C till they got exhausted and the moment they drowned head was considered as the endpoint The time was noted^[14]. On 21stday all the animals were sacrificed and blood was collected for estimation of biochemical parameters like, glucose, cholesterol, triglycerides, BUN and Plasma cortisol levels^[15], blood cell count^[16] the data obtained were subjected to statistical analysis

Cold stress

Table 2 Effect of *Abutilon indicum* on biochemical parameter on swimming test stress induced in albino rats.

Parameter	Control	Cold stress	<i>Abutilon indicum</i> extract	<i>Panax ginseng</i> extract
Glucose mg/dL	80.24 ± 0.812	112.2±2.91	96.45±5.21***	86.25±3.11***
Cholesterol mg/dL	40.21±1.882	58.4±1.727	43.29±2.93***	44.54±2.65***
Triglyceride mg/dL	71.24±0.712	105.24±2.64	83.14±1.95***	84.58±1.65**
BUN mg/mL	30.14±0.512	53.41±2.41	39.18±1.22***	34.58±1.87***
Plasma cortisol (µg/100 ml)	13.04 ± 0.24	21.41±0.56*	18.63±0.59*	15.63±0.09**
WBC Cells mm ⁻¹	4824 ± 41.02	6872 ± 143.7	5013 ± 14.6***	5217±48.21***
Lymphocytes Cells Cumm ⁻¹	48 ± 1.72	64 ± 1.09	52 ± 0.52**	59 ± 1.03**
Monocytes Cells Cumm ⁻¹	1.00 ± 0.0	3.25 ± 0.19	1.25 ± 0.50**	1.5 ± 0.76**
Neutrophills Cells Cumm ⁻¹	24 ± 0.40	35 ± 0.84	25 ± 0.40*	31 ± 0.22*
Eosinophils Cells Cumm ⁻¹	1.25 ± 0.4	4.50 ± 0.25	1.0 ± 0.25*	1.0 ± 0.25*

Albino rats (150-180gm) of either sex were divided in to 4 groups of 6 animals each. Group -1 served as control, Group-2 served as cold stress control , Group-3 served as cold stress induced and

treated with *Abutilon indicum* extract 400mg/kg p.o. and group - 4 cold stress induced and treated with *Panax ginseng* 100mg/kg p.o. Cold Stress was induced in 2nd, 3rd and 4th groups in albino rats, by exposing animals to $4 \pm 1^\circ\text{C}$ daily for 2 hrs for 10 days^[17]. On 11th day all the animals were sacrificed and the organs such as liver, spleen, testes and adrenal gland removed and weighed^[18]. The stomach was removed and split open along the greater curvature. The numbers of discrete ulcers were noted by the help of magnifying glass. The animals were dissected and the stomach carefully keeping the esophagus closed opened along the greater curvature the gastric contents were collected in a tube and centrifuged at 3000 rpm for 5 min, the volume of supernatant was expressed as ml/100g body weight. The mucosa was flushed with saline finally the ulcers were observed macroscopically. The observation was made for any ulceration or inflammation in the stomach. The stomachs were opened along the greater curvature and the mucosa was exposed for evaluation. Ulcer index, percentage protection and the P^H of gastric juice was determined statistically^[19].

Table 3 Effect of *Abutilon indicum* on weight of organ on cold stress induced in albino rats.

Parameter	Control	Cold stress	<i>Abutilon indicum</i> extract	<i>Panax ginseng</i> extract
Spleen mg/100g	3.57 ± 0.11	2.591 ± 0.12	3.08 ± 0.29**	3.38 ± 0.26**
Testes mg/100g	1.681 ± 0.06	1.121 ± 0.02	1.341 ± 0.29**	1.431 ± 0.21**
Liver g/100g	5.142 ± 62.5	6.718 ± 38.2	5.921 ± 13.8***	5.101 ± 10.8***
Adrenal gland g/100g	0.250 ± 0.01	0.489 ± 0.44	0.358 ± 0.01*	0.318 ± 0.01*

Statistical analysis

Analysis of data was performed using statistical package for social version 11.0 (SPSS) computer software. Descriptive statistics were adopted to display data in mean of \pm SEM ANOVA was used to compare the mean value obtained between the different groups. Differences were considered significant whenever the P value are reported as mean \pm SEM. ***p<0.001, **p<0.01 and *p<0.05.

RESULTS

Preliminary phytochemical analysis of the extract revealed the presence of alkaloids, carbohydrates, steroids, tannins and flavanoids.

Table 4 Effect of *Abutilon indicum* on gastric ulceration in cold stress induced albino rats.

Parameter	Ulcer index	% Protection	pH of Gastric juice
Control	-	-	6.03± 0.20
Cold stress	12.5 ± 0.7	18.8±3.29	3.0± 0.20
<i>Abutilon indicum</i> Extract	5.7 ± .5*	8.8 ± 1.8*	4.9± 0.17*
<i>Panax ginseng</i> Extract	4.6 ± 1.44*	3.9±1.01*	5.4± 0.80*

In Swimming endurance test ethanolic extract of *Abutilon indicum* at a dose of 400mg/kg b.w has shown significantly ($p < 0.001$) increased in the swimming time as compared to control (Table 1). In cold stress induced, the ethanolic extract of *Abutilon indicum* has significantly ($p < 0.001$) reduced the elevated levels of biochemical parameters glucose, cholesterol, triglyceride, BUN and cortisol levels when compared with stress control group (Table 2). The extract has also reduced the blood cell count WBC's significantly ($p < 0.01$), except lymphocytes and Eosinophils ($p < 0.05$) compared to stress control group (Table). Determination of weight of organs showed that the extract has significantly ($p < 0.01$) reduced the weight of liver, spleen and testes, however it showed no effect on weight of adrenal gland (Table 3).

Cold stress increases the incidence and severity of gastric ulcers. In the present study showed significantly reduced Ulcer incidence (%), increase in P^H of Gastric juice (Table 4).

DISCUSSION

Animals when subjected to a period of stress produce characteristic changes in several hormones and parameters associated with central nervous system and hypothalamic-pituitary-adrenal axis (HPA). HPA changes include an increase in cortisol, a reduced sensitivity of the HPA to feedback down-regulation, and a disruption in the circadian rhythm of cortisol secretion. Central nervous system changes include the stress-induced depletion of catecholamine neuro transmitters such as

nor epinephrine and dopamine. An acute increase in beta-endorphin levels is also observed under stressful conditions^[20].

Rodents when forced to swim in a restricted space become immobile after an initial period of vigorous activity indicating the stress. Pretreatment with adaptogen increase swimming endurance in rats^[21]. Increase in total swimming time of *Abutilon indicum* treated rats showed significant improvement in the swimming time. Stress increases total leukocyte count, eosinophils and basophils. Plant adaptogen are smooth prostressors which reduce the reactivity of host defense system. The mode of action of adaptogens is basically associated with stress system. Adaptogen increase the capacity of stress to respond to the external signals of activating and deactivating mediators of stress response subsequently^[21,22]. The stress induced increase in total WBC count, which is decreased by ethanolic extract of *Abutilon indicum* indicating its antistress and adaptogenic activity are similar to the changes produced by reference drug ethanolic extract of *Panax ginseng*.

During stress ACTH is released which acts on adrenal cortex where by cortisol and corticosterone will be secreted. Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves, which in turn increases blood glucose level. The increased cortisol levels and increased blood glucose level are reversed by anti-stress agents^[21-23].

A Blood glucose level increase in response to stress is due to release of Glucocorticoids as a result of HPA axis stimulation to compensate initial demand of energy. The acute demand of glucose is fulfilled by the increase in glucogenolysis from liver during stress^[24], which is found to be significantly reduced by *Abutilon indicum* and it also significantly reduced stress induced plasma cortisol level exhibiting anti-stress activity which is comparable to standard *Panax ginseng*.

The mechanism by which stress raises serum cholesterol, triglycerides and BUN levels in stress induced animals is due to the enhanced activity of hypothalamo-hypophyseal axis resulting in increased liberation of catecholamines and corticosteroids^[25]. *Abutilon indicum* as well as the standard *Panax ginseng* significantly reduced the elevated serum cholesterol, triglyceride and BUN levels which might be due to the inhibition of stimulation of sympathetic nervous system.

The increase in adrenals in stressed animals is due to the stress induced adrenomedullary response leading to increased production of corticotropic hormone that leads to increase in weight of adrenals^[25]. *Abutilon indicum* and *Panax ginseng* has significantly reduced the liver and adrenal gland weight this could be due to the reversal of stress induced adrenomedullary response and hence decrease production of corticotropic hormone. Pretreatment with *Abutilon indicum* significantly increased the spleen weight. This might be due to the inhibition of recruitment of lymphocytes to blood from spleen.

Stress causes alteration in hematological parameters like increase in WBC and DLC counts, neutrophils^[24]. Cold stress typically increases total leukocyte count, eosinophils and basophils^[25]. Plant adaptogens are smooth prostressors which reduce the reactivity of host defense system and decrease the damaging effects of various stressors due to increased basal levels of mediators involved in the stress response^[26]. The stress induced increased total WBC count is decreased by ethanolic extract of *Abutilon indicum*, thus the plant possesses antistress, adaptogenic activity. *Abutilon indicum* and *Panax ginseng* significantly reduced the WBC, lymphocytes, eosinophils and monocyte counts in cold stress.

Stress-induced ulcers are caused by number of factors both physical and psychological. Increase in gastric motility, vagal overactivity, mast cell degranulation, decreased mucosal blood flow, and decreased prostaglandin synthesis are reported to be involved in the genesis of stress-induced ulcers^[27]. The result showed the ability and the efficacy of the ethanolic extract of *Abutilon indicum* in preventing gastric ulceration in cold stress induced rats suggesting its anti-ulcer activity. A variety of biological activities including Anti-stress activity were reported with flavonoids, tannins and phenolic glycosides. *Abutilon indicum* contains biologically active chemicals that include flavonoids, saponins, alkaloids, proteins, fixed oils and proteins. The anti stress activity may be due to the presence of these constituents where as standard drug *Panax ginseng* an established adaptogenic drug too contains glycosides, steroids and flavonoids.

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