THE EFFECT OF *ABUTILON INDICUM* ON VARIOUS BIOCHEMICAL PARAMETERS ON STRESS INDUCED IN ALBINO RATS

Roshan . S *[1] Savadi .R.V [2]

1. Department of Pharmacy, Singhania University, Rajasthan.
2. K.L.E college of Pharmacy, Hubli, Karnataka.

*For correspondence: E-mail- roshansalfi@rediffmail.com.
roshansalfi@yahoo.com.

Summary

The present study was carried out to evaluate the effect of ethanolic extract of *Abutilon indicum* on swimming endurance and anoxia tolerance test in mice, cold induced stress and Immobilization in albino rats. The effect was assessed by swimming survival time and anoxia tolerance time, estimation of various biochemical parameters in cold, Immobilization stress like glucose, cholesterol, triglycerides and blood urea nitrogen (BUN), and by determining the weight of organ such as, liver, spleen, testes, adrenal gland, blood cell count (WBC) and also the differential count at a dose of 400mg/kg body weight per oral. It was found that ethanolic extract significantly ($p<0.001$) increases swimming time and anoxia tolerance time. Extract showed significant ($p<0.001$) decrease in blood glucose, cholesterol, triglyceride and BUN and also decreased the weight of organs. It also showed a significant ($p<0.05$) decrease in weight of adrenal gland. A significant ($p<0.01$) decrease in WBC count, polymorphs and monocyte and decrease in lymphocytes ($p<0.05$) and eosinophils was observed, compared to control group. Thus the obtained results revealed that the *Abutilon indicum* has got a significant adaptogenic activity.

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

Introduction

Hans Selye, has defined stress as the sum of all the non-specific changes caused by function or damage. It is fundamentally a physiological response, the primary object of which is to maintain life and re-establish the normal state. The general manifestations of stress are called the 'General adaptation syndrome' (G.A.S.), which comprises of 3 stages. Alarm reaction, resistance and exhaustion, respectively [1]
Fight or flight, alarm phase**.** This response is an alarm reaction triggered by messages in the brain. The pituitary gland releases adrenocorticotropic hormone (ACTH). ACTH causes the adrenal glands to secrete adrenaline, cortisol, and other stress hormones[2]. The heart beats faster to provide blood to the muscles and brain. The breath rate increases to supply extra oxygen to the muscles, heart, and brain.

Digestion and other functions not essential for maintaining the alarm reaction are halted. The liver rids itself of stored glycogen and releases glucose into the bloodstream. The body is now ready for any real or imagined danger.

Adaptation phase. If the stress factor continues (for example, in sport it might be heavy athletic training), our body learns to tolerate the stressful stimulus"adapt"and increase its resistance to the stress factor. The adaptation phase is usually a safe period and allows the body to endure ongoing stress [3]. The longer we can stay in the adaptation phase, the better.

Exhaustion phase. In this phase, the body fails to fight stress anymore and simply gives up. This stage is a result of chronic oversecretion of cortisol. This leads to adrenal exhaustion. Adrenal exhaustion accelerates the downward spiral to chronic poor health. Chronic headaches, nausea, allergies, nagging injuries, fatigue, dizziness, hypotension, low body temperature, depression, low sex drive, chronic infections, and cold hands and feet are just some of the symptoms that occur with adrenal exhaustion[4].

Abutilon indiums of family Malvaceae is found throughout tropical and sub tropical region in India, is known as Atibal in Sanskrit. The various parts of plant have claimed to have several traditional medicinal properties. The whole plant is studied for anti inflammatory, immuno stimulating effect, piles and gonorrhea treatment. Root and bark are used as aphrodisiac, anti diabetic, nervine tonic, and diuretic. Seeds are used as aphrodisiac, in treatment of urinary disorders [5]. The plant is reported to have analgesic[6], hypoglycemic [7], hepato protective [8], hyperlipidemic activity [9]. Also reported in the literature isolation of sesquiterpine lactone, isolation of Gallic acid, eugenol [10] wound healing [11] and anti bacterial activity[12]. The present study is an attempt to validate adaptogenic 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 mesh, 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 distill water using 1% Tween 80, used for pharmacological screening.

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 [13]

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 400mg/kg. Hence we selected 400mg/kg dose for pharmacological screening.

Anoxic stress tolerance test [14]

Albino mice of either sex weighing between (20±2g) were divided into 3 groups of six each. Group I control, Group II subjected to anoxia test and the oral administration of *Abutilon indicum* extract at the dose 400 mg/kg p.o (per oral) and Group III subjected to anoxia test and the oral administration of *Panax ginseng* extract at the dose 50 mg/kg p.o respectively for 21 days. Anoxia test was carried out on 7th, 14th and 21st day. One hour after the drug administration mice were placed in Hermetic vessel of 2000 ml air capacity was used for this test. Each animal was kept in the hermetic vessel and the time to show the first sign of convulsion was noted, it was immediately removed from the vessel and resuscitated if needed. After one week of drug treatment the animals were once again exposed to the anoxia stress. Similarly the animals were also observed at the end of 2nd and 3rd week with the same treatment and the time duration for anoxia stress tolerance was noted[15].The data obtained were subjected to statistical analysis.

Swim endurance test [14]

Swiss albino mice (20 ± 2 g) of either sex were randomly divided into 3 groups of 6 animals each consisting Group I control, Group II swimming test and subjected to the oral administration of extract at the dose 400 mg/kg, Group III subjected to anoxia test and the oral administration of *Panax ginseng* extract at the dose 50 mg/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 swim in cylindrical 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 and the data obtained were subjected to statistical analysis[15].

Cold stress

Albino rats (210-230gm) 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 induce and *Abutilon indicum* extract 400mg/kg p.o, and group - 4 cold stress induce and *Panax ginseng* 50mg/kg p.o. Cold Stress was induced in 2nd, 3rd and 4th groups in albino rats, by exposing animals to 4 ±1°C daily for 2 hrs for10

days[20]. On 11th day all the animals were sacrificed and blood was collected for estimation of biochemical parameters like, glucose[16], cholesterol, triglycerides, BUN[17], blood cell count[18] and weight of organs such as liver, spleen, testes and adrenal gland [19].

Immobilization stress

Albino rats (210-230gm) of either sex were divided into 4 groups of 6 animals each. Group -1 served as control, Group-2 served as immobilization stress control, Group-3 served as *Abutilon indicum* 400mg/kg treatment group and Group-4 served as *panax ginseng* 50mg/kg treatment. Immobilization stress was induced in 2nd, 3rd and 4th group animals by immobilizing the animals with head down in supine position, by fixing the animals to a board inclined position at an angle of 60° daily for 2hrs for the duration of 10 days[22]. On 10th day, the animals were sacrificed and blood was collected for estimation of biochemical parameter like glucose[16], cholesterol, triglyceride, BUN[[17], blood cell count[18] and weight of organs like liver, spleen, testes and adrenal gland [19].

Statistical analysis

All the values are expressed as mean ±SEM and data was analyzed by one-way ANOVA, using Graph pad INSTAT. The post-hock analysis was carried out by Dunnet's multiple comparison test to estimate the significance of difference between individual groups**.**

Results

The Anoxia tolerance test was determined by taking the appearance of convulsion as end point. The ethanolic extract of *Abutilon indicum* at a dose of 400mg/kg b.w has shown significant ($p<0.001$) increasing tolerance stress time in 14th and 21st day as compared with the control as shown in Table no 1

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 showed in Table 2

In cold stress induced, ethanolic extract of *Abutilon indicum* has significantly ($p<0.001$) reduced the elevated levels of biochemical parameters glucose, cholesterol, BUN and triglyceride when compared with stress control group, the results are shown in Table 3. 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 4. 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 as shown in Table 5.

Immobilization stress induced, the ethanolic extract of *Abutilon indicum* has significantly ($p<0.001$) reduced glucose, cholesterol, BUN and triglyceride when compared with stress control group, the results are shown in Table 6. The extract also reduced the blood cell count WBC's significantly ($p<0.01$) compared to stress control group Table 7. 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 as shown in Table 8.

Group	Mean duration of tolerance time (in min.) Mean± SEM		
	1st week	2nd week	3rd week
Control	4 ± 0.91	7.2 ± 0.71	12.3 ±.05
Ethanolic extract of *A.sativum*	2.2± 0.78*	6.3 ±1.37***	8.4 ± 0.18**
Ethanolic extract of *panax ginsang*	3.2 ± 0.72	6.9. ± 0.98	10.6 ± 0.81

Table 1 Effect of *Abutilon indicum* on anoxia tolerance test.

Group	Mean duration of Swimming survival time (in min.) Mean± SEM		
	1st week	2nd week	3rd week
Control	12.3 ±1.91	19.2 ±0.71	22.4 ±1.05
Ethanolic extract of *Abutilon indicum*	13.6± 0.78**	23.6±137**	27.3± 1.18***
Ethanolic extract of *panax ginsang*	11.2 ± 0.98	16.8 ± 0.98	10.6 ± 0.81

Table 2 Effect of *Abutilon indicum* on swimming endurance test.

Parameter	Control	Cold stress	*Abutilon indicum* extract.	*Panax ginseng* extract.
Glucose	80.24 ± 0.812	112.2±2.91	96.45±5.21***	86.25±3.11
Cholesterol	40.21±1.882	58.4±1.727	43.29±2.93***	44.54±2.65
Triglyceride	71.24±0.712	105.24±2.64	83.14±1.95***	84.58±1.65
BUN	30.14±0.512	53.41±2.41	39.18±1.22***	34.58±1.87

Table 3 Effect of *Abutilon indicum* on biochemical parameter on cold stress induced in albino rats.

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

Table 4 Effect of *Abutilon indicum* on weight of organ on cold stress induced in albino rats.

Parameter	Normal	cold stress	*Abutilon indicum* extract.	*Panax ginsang* extract.
WBC's count	4824 ± 41.02	6840 ± 136.7	6214 ± 43.2***	5723±46.21***
Lymphocytes	48 ± 1.72	69 ± 1.29	64 ± 0.72**	56 ± 1.03**
Monocytes	1.00 ± 0.0	3.25 ± 0.19	1.75 ± 0.50**	1.5 ± 0.76**
Neutrophills	24 ± 0.40	35 ± 0.84	28 ± 0.50*	30 ± 0.22*
Eosinophils	1.25 ± 0.4	4.50 ± 0.25	2.25 ± 0.25*	1.0 ± 0.00*

Table 5 Effect of *Abutilon indicum* on blood cell count in cold stress induced albino rats.

Parameter	Normal	Immobilization stress	*Abutilon indicum* extract.	*Panax ginsang* extract.
Glucose	80.24 ± 0.812	118.2 ± 2.41	98.25 ±5.21***	87.15±4.91***
Cholesterol	40.21 ± 1.882	62.4 ± 1.98	49.69 ± 2.03**	44.79 ± 1.93**
Triglyceride	71.24 ± 0.712	110.24 ± 2.94	88.94 ± 1.76**	83.84 ± 1.46**
BUN	30.14 ± 0.512	51.41 ± 2.61	43.98 ± 1.22**	43.78 ± 1.42**

Table 6 Effect of *Abutilon indicum* on Biochemical Parameter in immobilization stress induced albino rats.

Parameter	Normal	Immobilization stress	*Abutilon indicum* extract.	*Panax ginsang* extract.
Spleen	3.57 ± 0.11	2.891 ± 0.12	3.08 ± 0.29**	3.11 ± 0..21**
Testes	1.681 ± 0.06	1.421 ± 0.02	1.341 ± 0.29**	1.421±0..03**
Liver	5.142 ± 62.5	6.818 ± 38.2	5.921 ± 13.8*	5.461 ±19.76*
Adrenal gland	0.250 ± 0.01	0.423 ±0.44	0.358 ± 0.01*	0.398 ± 0.22*

Table 7 Effect of *Abutilon indicum* on weight of organ in immobilization stress induced albino rats.

Parameter	Normal	Immobilization stress	*Abutilon indicum* extract.	*Panax ginsang* extract.
WBC's count	4824 ± 41.02	7540 ± 166.7	6124 ± 44.2***	5817±48.21***
Lymphocytes	48 ± 1.72	71 ± 1.29	64 ± 0.72**	59 ± 1.03**
Monocytes	1.00 ± 0.0	3.25 ± 0.19	1.75 ± 0.50**	1.5 ± 0.76**
Neutrophills	24 ± 0.40	35 ± 0.84	28 ± 0.50*	31 ± 0.22*
Eosinophils	1.25 ± 0.4	4.50 ± 0.25	2.25 ± 0.25*	1.0 ± 0.00*

Table 8 Effect of *Abutilon indicum* on blood cell count in immobilization stress induced albino rats.

Discussion

Animals subjected to a period of stress produces characteristic changes in several hormones and parameters associated with the central nervous system and the 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[21].

All the body functions, including cellular respiration depends on the oxygen supply. Any lack of vital element will play havoc on all body mechanisms and increase in adaptation during stress by any drug could be considered as its major anti stress effect. During stress adaptogens are capable of increasing succinate dehydrogenase [SDH] in the brain. Decrease in brain neurotransmitters, i.e. nor epinephrine (NE), dopamine (DA), serotonin (5-HT) and acetylcholine (ACh) and hence the observed drug effective in this model may be effective by the modulation of above mentioned neurotransmitters[22]. The Extract *Abutilon indicum* significant increase in anoxia tolerance time is an indication of either resistance to it or reduction in cerebral oxygen consumption. Both these effect are quite useful to protect neuronal cell against oxidative stress, the enzyme is responsible for utilization and conservation of energy in the cellular system of the organism; which helps adaptive processes during stress. Under stressful conditions cortisol and corticosterone will be secreted by adrenal cortex[23]. Hyper secretion of cortisol helps in the maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis[24]. Treatment with *Abutilon indicum* extracts significantly reduced the hyperglycemia by reducing the hyper activity of adrenal cortex and also by maintenance homeostatic mechanism. This was further in agreement with plasma corticosterone levels. The mechanism by which stress raises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis resulting in increased liberation of catecholamines and corticosteroids[25]. In the present study a significant increase in plasma corticosterone was observed in cold stress. After treatment with *Abutilon indicum* it was reduced in cholesterol level might be increased due to the mobility of fat which could not be suppressed by extracts. The effect of stress on serum triglycerides has been shown to be variable probably catecholamines mobilize lipids from adipose tissues.

Cold stress models showed an increase in triglyceride levels. *Abutilon indicum* extracts were not able to suppress the stress induced increase in triglycerides levels. BUN levels were increased in cold stress as these are the end products of protein metabolism. In excess adrenocortical activity[26], due to increased metabolism of protein increases urea excretion, in the present study a similar effect was observed. Extracts of *Abutilon indicum* decreased the BUN levels as compare to stress control, indicating a diminished catabolism of protein under stressful conditions. Adrenal glands and liver weights were significantly increased in cold stress than control group. Stress induces adrenomedullary response in man to release adrenaline which in turn stimulates β2 receptors on the pituitary gland. It leads to greater release of ACTH that can stimulate the adrenal medulla as well as cortex[24]. Resulting in further release of adrenaline and increase in weight of adrenal gland to greater extent. Cortisol increases m-RNA levels in liver cells, increase in liver weight and it was well in correlation with the observed .increase in plasma corticosterone levels. Spleen contracts during stress and releases more amount of blood (RBC) into circulation hence its weight decreases. , extract of *Abutilon indicum* signifuicantly reduced the weight of adrenal glands, liver and spleen compared to stress group. Stress causes alteration in hematological parameters like increase in WBC and DLC counts, neutrophils were increased more significantly in cold stress. Extract reduced the WBC, lymphocytes, eosinophils and monocyte counts in cold stress [18].
The adaptogenic effect of *Abutilon indicum* so observed is attributed to the presence of flavonids, phenolic glycosides, hydrocarbons, proteins, and vitamins. The results suggested that the *Abutilon indicum* have got significant adaptogenic activity. Further studies on other adaptogenic models are ongoing.

Conclusion

Based on the results it is concluded that *Abutilon indicum* has the adaptogenic activity and could play an important role in the management of stress, which supports the traditional claim.

References

1. Selye H. Experimental evidence supporting the conception of adaptation Energy. Amer. J. Physiol 1938;123: 758.
2. Chrousos GP, Mc Carty R, Pacak K, Cizza G, Sternberg E, Gold PW, Kventansky R. Stress: basic mechanisms and clinical implications. Ann NY Acad Sci 1995;771:396-418.
3. Brekhman II, Dardymov II. New substances of plant origin which increase non-specific resistance. Ann. Rev. Pharmacol 1969;9:419.
4. Panossian A, Gabrielian E, Wagner H. On the mechanism of action of plant adaptogens with particular references on cucurbitacin R diglucoside. Phytomedicine 1999;6:147-155.
5. Kirtiar KR, Basu BD. Indian medicinal plants, Delhi India. sri satguru publication, 3rd revised and enlarged edition, Vol.I, 1983, pp-321-324.
6. Bagi MK, Kalyani GA, Dennis TJ, Kumar AK, Kakrani HKA A preliminary pharmacological of *Abutilon indicum* analgesic activity. Fitoterapia 1985;56 (3**):**169-171.

7. Seetharam G, Halageri S, Setty R, Bheemachar. Hypoglycemic activity of *Abutilon indicum* leaf extract in rats. Fitoterapia 2000;73(2):156-159.
8. Roshan S, Ali Sadath, Saleem MA, Patel MJ, Ali Asgar. Effect of *Abutilon indicum* on carbon tetrachloride induced hepatic damage in albino rats. J Pharmacutical Research 2004;3(4):61-62.
9. Ahamed M, Amin S, Islam M, Takahashi M, Okuyama E. Analgesic principal from *Abutilon indicum*. Die pharmazie 2000;55(4):314-316.
10. Ahamed ZA, Sharma PV. Two sesquiterpine lactone from *Abultion indicum*. Phytochmistry 1989;3525.
11. Roshan S, Sadath Ali, Abdullah Khan, Tazneem B, Purohit MG. Wound healing activity of *Abutilon indicum*. Pharmacog magazine 2008;4(15):85-88.
12. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants, J Ethnopharmacol 2006;107(2):182-188.
13. Nimbekar, Patil, Patki. Pharmacological evaluation of antistress and androgenic activity of polyherbal formulatiom AP 30000 containing *Panax Ginseng*. Indian drugs 2001;27-31.
14. Tomar VS. Effect of gerifort a herbal compound drugs on anoxia stress tolerance in animals. Indian Drugs 1984;233-235.
15. Shivprasad, Kharya, Rana. Antioxidant and Adaptogenic effect of an herbal preparation, Triphala. J Natural Remedies 2008;82-88.
16. Nagaraja HS, Jeganathan PS. Forced Swimming stress induced alterations in ingestive behavior in rats. Ind J Physiol Pharmacol 2003;41(1):94-100.
17. Vinay Agarwal, Bhayesh Gupta, Ushan Singhal, Bajpal SK. Examination stress changes in serum cholesterol, triglycerides and total lipids. Ind J Physiol Pharmacol .41(1): 404-8(1997).
18. Charles OE, Michael U. Adikwu1, Chukwuemeka S. Nworu, Festus BC, Okoyeand Damian C. Dimegwu O. Adaptogenic potentials of *Camellia sinensis* leaves, *Garcinia kola* and *Kola nitida* seeds Scientific Research and Essay 2007;2 (7): 232-237.
19. Sandip R, Sardesai, Marjorie AE, Jolly FM. Effect of stress on organ weight in rats. Ind J Physiol Pharmacol 1993;37(2):104-8.
20. Bhattacharya SK, Ghosal S. Experimental evaluation of anti stress activity of Zee stress. Ind J Indg Med 1994;2:1-8.
21. Gregory S, Kelly ND. *Rhodiola rosea* A Possible Plant Adaptogen. Alternative Medicine Review 2001;(6)3: 291-296.
22. Deore SL, Khadabadi SS. Screening of antistress properties of *Chlorophytum borivilianum* tuber Pharmacologyonline 2009;1:320-328.
23. Bijlani RL, Sud S, Gandhi BM, Jandon BN. Relationship of examination stress to serum lipid profile. Ind J Physiol Pharmacol 1985;30(1):22-30.
24. Bhatwadekar AD, Chintawar SD, Logade NA, Somani RS, Veena Kasture S, Kasture SB. Antistress activity of *Butea monosperma* flowers. Ind J Pharmacol 1999;31:153-5.
25. Mishra KK, Pandey HP. A study on physiological changes in certain psychosomatic disorders with reference to cortisol, blood glucose and lipid profile. Ind J Pharmacol 1996;40(2):151-154.
26. Takano T. Additive effect of nitrogen oxides and cold stress on circulating leukocyte counts in rats. Toxicol lett 1983;17:289-91.