Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 4 (8): 300-306, 2009
ISSN 1816-496X

Adaptogenic Activity of *Lagenaria siceraria*: An Experimental Study using Acute Stress Models on Rats

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Abstract: This study was conducted to evaluate the anti-stress potential of ethanolic extract of fruits of *Lagenaria siceraria* in rats. The present study was to investigate the influence of forced swimming endurance stress on swimming endurance time, organ weights and changes in biochemical parameters in rats. The purpose of the study was also to investigate the acute heat stress induced changes in biochemical parameters, adrenal gland weight and stress induced perturbations in blood cell counts in albino Wistar rats. These activities were tested at oral doses of 100-400 mg kg^{-1} of the extract using *Withania somnifera* as a standard reference drug. Pretreatment with the extract at different doses significantly ($p<0.05$) ameliorated the stress-induced variations in this biochemical parameters-serum glucose, triglyceride, cholesterol, BUN and cortisol levels, blood cell counts and organ weights in these stress models. The extract treated animals also showed increase in swimming endurance time. This ability of *Lagenaria siceraria* to prolong the swimming time and ameliorate the stress induced changes in both stress models, therefore, suggests an antistress and adaptogenic property.

Key words: *Lagenaria siceraria*, acute heat stress, forced swimming stress, antistress, adaptogenic

INTRODUCTION

Exposure to stressful situations is among the most common human experiences. In response to stressors, a series of behavioral, neurochemical and immunological changes occur that ought to serve in an adaptive capacity (Anisman, 1999). However, if those systems become overly taxed, the organism may become vulnerable to pathology. Likewise, the biological changes, if sufficiently sustained, may themselves adversely affect the organism's well being (Brown, 1993). The stress response, regarded as a positive adaptive process, comprises a set of functional and behavioral reactions to cope with challenging situations. A coordinated and adequate set of responses to stress is crucial for the survival of the organism in these situations. However, exaggerated responses to stress appear to be related to a wide range of physiological and psychological dysfunctions such as hypertension, stroke, depression, etc (Vogel, 1993).

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Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism in response to stress (Greenen *et al*., 1988; Tan *et al*., 1992). Swimming has got a number of advantages over other types of exercise such as treadmill running. The amount of work done during swimming exercise is far greater than that during the treadmill running of identical time duration. Swimming is not always a simple exercise stress, because emotional factors are difficult to be eliminated (Kramer *et al*., 1993; Nagaraja and Jeganathan, 1999). The forced swimming stress developed by Porsolt *et al*. (1977) has now become widely accepted model for studying physical stress in animals. Water temperature is another important factor in forced swimming test. By varying the water temperature, Richter (1957) found that rats could survive as long as 80 h in lukewarm water (36°C). Increasing or decreasing the water temperature above or below this point influences the overall behavior of the animal and changes the involvement of glucocorticoids (Abel, 1991).

The plant, *Lagenaria siceraria* (Mol.) Standl. (Family: Cucurbitaceae), known as bottle gourd, is a common fruit vegetable used throughout the India. Since, time immemorial the fruit is used as diuretic (Ghule *et al*., 2007), cardio-tonic, cardio-protective and nutritive agent (Hassanpour Fard *et al*., 2008). The fruit is also reported to have good source of vitamin B complex and choline along with fair source of vitamin C and β-carotene (Ghule *et al*., 2007). It is also reported to contain cucurbitacins, fibers and polyphenols (Kiritikar and Basu, 2001; Nadkarani, 1982). The LS fruit has been reported to possess antioxidant activity (Jiwjinda *et al*., 2002) hepatoprotective (Shirwaikar and Sreenivasan, 1996), hypolipidemic and antihyperlipidemic effects in normocholesterolemic and triton-induced hyperlipidemic rats (Ghule *et al*., 2006). The HPLC analysis of methanolic extract from plant shows the presence of flavone-C glycosides (Baranoswka and Cisowski, 1994). Lagenin, a novel protein has been isolated from lyophilized extract of seeds (Wang and Ng, 2000). No studies have shown anti-stress or endurance promoting activity of *Lagenaria siceraria*. This study therefore, reports on the anti-stress potential of ethanol extract of fruit of *Lagenaria siceraria* in different animal models.

MATERIALS AND METHODS

Plant Material and Extraction

Lagenaria siceraria fruits were collected from the local farms of Rangareddy District Andhra Pradesh in the month of October-November, the botanical authentication was done by the authority of Department of Botany, Osmania University, Hyderabad and voucher specimen No.MRCP/102 is lodged in present research laboratory for the future reference. The fresh and semi-riped fruits were sliced using a home slicer and the slices obtained were shade-dried, pulverized and passed through a 20 mesh sieve. The dried, coarsely powdered plant material was extracted with 99% ethanol at 60°C for 24 h using a Soxhlet apparatus. The solvent was evaporated under vacuum which gave semisolid mass (23% w/w) with respect to the dried powder. Oral suspensions containing 100, 200 and 400 mg mL^{-1} of the ethanol extract of *L. siceraria* were prepared in 1% w/v gum acacia.

Experimental Animals

Albino Wistar rats weighing 150-250 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60% humidity). Standard

pelletized feed and tap water were provided *ad libitum*. The Institutional Animal Ethical Committee (IAEC) of Malla Reddy College of Pharmacy, Hyderabad, approved the study.

Forced Swimming Endurance Test (Physical Stress)

Rats of either sex (200-250 g) were used for forced swim endurance stress. Group I rats received 0.1% gum acacia in saline; (vehicle control). Group II mice were treated with 0.1% gum acacia in saline and stress, (negative control). Group III rats were treated with Withania somnifera (100 mg kg^{-1} p.o.) and stress; (positive control). Group IV, V and VI mice were treated with ethanol extract at 100, 200 and 400 mg kg^{-1}, p.o. and stress. The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37×37×30 cm), filled with water to a height of 25 cm. Extracts were given to rats, once daily for period of 7 days. On 8th day the rats were allowed to swim till complete exhaustion and the endpoint was taken when the animal started drowning. The mean swimming time for each group was calculated. Then animals were killed and blood was collected by cardiac puncture to estimate biochemical parameters like serum glucose, triglycerides, cholesterol, BUN, cortisol. The weights of organs such as liver, adrenals, spleen were recorded after washing with alcohol (Kannur *et al*., 2006).

Acute Heat Induced Stress

Treatment groups were similar to forced swimming endurance stress. Albino rats (150-180 g) of either sex were subjected to stress. All the animals were subjected to heat stress by exposing them to a controlled temperature of 40±2°C daily for a period of 8 days. (Amarnath *et al*., 2006). After which the animals were sacrificed on the 9th day, blood was collected by cardiac puncture method and centrifuged at 5000 rpm for 10 min for separation of plasma for estimation of cortisol and glucose. Some of the blood was collected in heparinised tube for the Blood cell count (WBC and DLC).

Statistical Analysis

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

RESULTS AND DISCUSSION

The results of the study revealed that the extract possess antistress property as it significantly ($p<0.05$) increased swimming time (Fig. 1). Swimming endurance stress resulted in significant increase in adrenal gland weight and liver weight with concomitant decrease in spleen weight in stress control group, which was significantly ($p<0.05$) reverted by *Lagenaria siceraria* pretreatment at 100, 200 and 400 mg kg^{-1} (Table 1). Pretreatment of animals with *Lagenaria siceraria* at three doses also significantly ($p<0.05$) restored back forced swimming stress induced alterations in plasma cortisol, glucose, triglyceride, BUN and cholesterol (Table 2).

The extract at different doses offered significant protection against the heat stress induced changes in glucose and cortisol. Stress induced elevated blood cell counts of WBC i.e., lymphocytes, neutrophils, eosinophils and monocytes have been significantly ($p<0.001$) reduced by the ethanol extract in a dose dependant manner (Table 3).

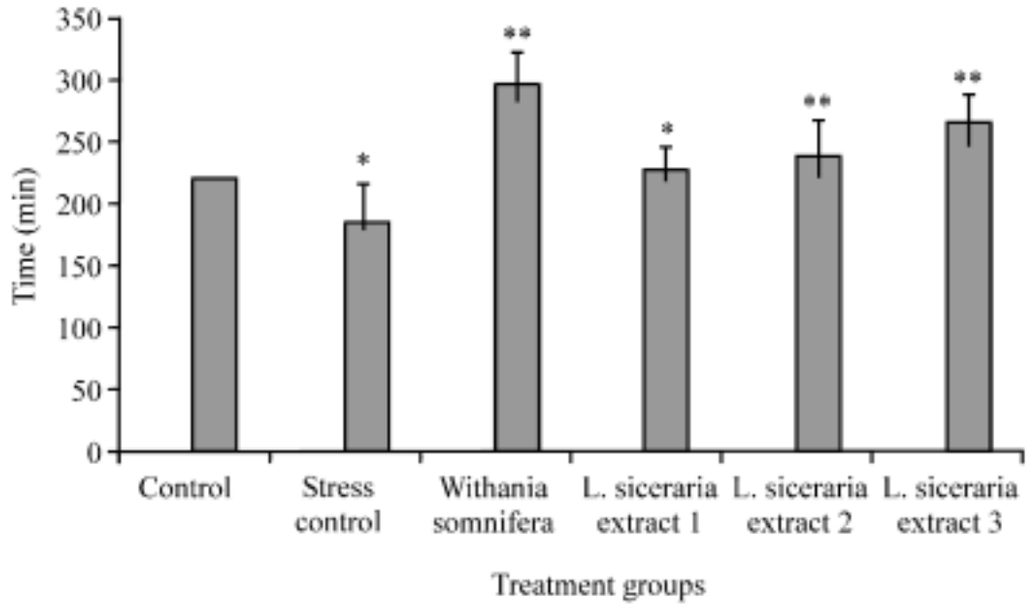

Fig. 1: Effect of ethanolic extract of *Lagenaria siceraria* in forced swimming endurance test in rats (Swimming time). The mean increase in duration of swimming time was noted. $^{*}p<0.05$ significant as compared to control, $^{**}p<0.05$, significant as compared to stress control, statistical test employed is ANOVA followed by dunnet's t test

Table 1: Effect of ethanol extract of *Lagenaria siceraria* on organs weight in forced swimming endurance stress in rats

	Organs weight		
Treatment	Spleen (mg/100 g)	Liver (g/100 g)	Adrenal glands (mg/100 g)
Control	271.85±0.99	3.56±2.31	17.25±0.26
Swimming stress (Control)	192.62±1.52	5.89±1.84	39.42±0.65
Withania somnifera (100 mg kg^{-1} p.o.)	285.80±0.84*	3.76±1.72*	15.26±0.91*
L. siceraria ext (100 mg kg^{-1} p.o.)	207.12±0.76	4.92±1.99	31.20±0.59
L. siceraria ext (200 mg kg^{-1} p.o.)	256.34±0.54*	4.25±0.98*	26.49±0.36*
L. siceraria ext (400 mg kg^{-1} p.o.)	269.50±1.19*	3.91±0.76*	19.84±0.57*

The values are expressed as Mean±SEM, n = 6. Significance at $^{*}p<0.05$ when compared to control as determined by ANOVA followed by Dunnet's t test

Table 2: Effect of ethanol extract of *Lagenaria siceraria* on biochemical parameters in swimming endurance stress in rats

Groups	Cortisol (μg dL^{-1})	Glucose	Cholesterol (mg dL^{-1})	Triglycerides	BUN (mg mL^{-1})
Control	87.62±1.56	97.860±1.63	87.42±3.63	132.63±2.85	35.87±2.69
Swim stress					
Control	153.29±3.95	163.22±2.95	184.62±1.89	194.06±5.02	49.62±1.98
Withania somnifera	91.18±2.57**	102.30±3.48**	84.62±3.59*	125.15±0.89*	31.84±3.78*
L. siceraria					
Extract 1	135.61±1.85*	148.49±1.11*	119.85±4.05*	172.81±2.64*	45.09±1.25*
Extarct 2	119.72±3.87**	125.72±1.57**	94.35±3.82**	151.12±3.98*	39.21±1.81*
Extract 3	98.84±3.35**	104.61±1.85**	76.93±1.56**	129.69±2.63**	36.07±1.34*

The values are expressed as Mean±SEM, n = 6 in each group. $^{*}p<0.05$ significant as compared to control, $^{**}p<0.05$, significant as compared to stress control, statistical test employed is ANOVA followed by dunnet's t test

Rodents, when forced to swim in a restricted space become immobile after an initial period of vigorous activity. This immobility signifies behavioral despair, resembling a state of mental depression (Thiebot *et al*., 1992; Archana and Namasivayam, 1999). The increase in total swimming time of *Lagenaria siceraria* treated mice indicates better stress tolerance. Increased swim duration in rats pretreated with *Lagenaria siceraria* are similar to the changes produced by reference drug Withania somnifera. During stress, blood glucose level increases (Dominiczak, 1999) which is found to be significantly reduced in *Lagenaria siceraria* treated rats. Lowering of stress induced hyperglycemia is an indication of

Table 3: Effect of ethanolic extract of *Lagenaria siceraria* on some parameters in heat induced stress

Groups	Cortisol (μg dL^{-1})	Glucose (mg dL^{-1})	WBC (Cells mm^{-3})	Blood cell counts DLC No. of cells $Cumm^{-1}$ L	N	E	M
Control	95.84±1.85	74.82±5.91	7349±23.98	5704±24.19	1565±11.24	69.84±1.36	10.62±0.34
Heat stress (Control)	163.91±2.95*	182.05±6.84*	9842±21.23*	6956±24.26*	2782±14.56*	92.21±3.96*	12.15±1.01*
W. somnifera (100 mg kg^{-1} p.o.)	85.82±3.04**	92.82±5.46**	7801±24.23**	5967±13.34**	1753±21.89**	71.19±2.32**	10.81 ±0.23**
L. siceraria ext (100 mg kg^{-1} p.o.)	125.21±3.33	122.28±4.91*	9123±26.25*	6563±15.45*	2461±11.74*	87.18±4.23*	11.99±0.99*
L. siceraria ext (200 mg kg^{-1} p.o.)	113.48±2.85*	109.54±3.89*	8562±14.25**	6637±17.98*	1835±12.36*	79.47±2.54 **	10.62±0.67*
L. siceraria ext (400 mg kg^{-1} p.o.)	91.46±1.7**	82.56±4.6**	7250±18.64**	5544±28.24**	1622±13.23**	74.21±6.12*	10.31±1.28 *

The values are expressed as Mean±SEM, n = 6 in each group. *p<0.001 significant as compared to control, **p<0.001, significant as compared to stress control, statistical test employed is ANOVA followed by dunnet's t test

antistress, adaptogenic activity of plant (Sen *et al*., 1992). In response to stress, ACTH is released, which acts on the adrenal cortex to stimulate the synthesis and release of cortisol (Sadock and Sadock, 2003). Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves (Tache and Selye, 1976) which in turn increases blood glucose level. The increased cortisol levels are reversed by anti-stress agents (Sen *et al*., 1992). *Lagenaria siceraria* significantly decreased stress induced elevated levels of cortisol and blood glucose levels. The reference drug in this study, Withania somnifera, also produced similar results.

The marked increase in serum cholesterol, triglycerides and BUN levels in stress induced animals is due to stimulation of Hypothalamo-Pitutary Axis (HPA) and sympathetic system, resulting in, liberation of catecholamines and glucocorticosteroids, which inhibits the immune system at multiple sites like liver, kidney (Schimmer and Parker, 2006). *Lagenaria siceraria* as well as reference standard drug *Withania somnifera* significantly ($p<0.05$) reduced the elevated serum cholesterol, triglycerides and BUN levels, which may be due to inhibition of stimulation of sympathetic nervous system.

The increase in weight of 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 (Krupavaram *et al*., 2007). *Lagenaria siceraria* and *Withania somnifera* has significantly ($p<0.05$) reduced the liver, adrenal gland weight, this may be due to the reversal of the stress induced adrenomedullary response and hence, decreased production of corticotropic hormone.

The decrease in weight of spleen may be due to recruitment of lymphocytes to blood from spleen which results in squeezing of the spleen (Rai *et al*., 2003). The pretreatment with the *Lagenaria siceraria* and reference standard *Withania somnifera* significantly ($p<0.05$) increased the spleen weight. This may be due to inhibition of recruitment of lymphocytes to blood from spleen.

Heat stress increases the total leukocytes count, eosinophils and basophils. 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 (Panossian *et al*., 1999). Since, the stress induced increased total WBC count is decreased by the extract of *Lagenaria siceraria*, it indicates antistress, adaptogenic activity.

A variety of biological activities including adaptogenic activity were reported with flavonoids, tannins and phenolic glycosides (Krupavaram *et al*., 2007). *Lagenaria siceraria* contains biologically active chemicals that include flavonoids, glycosides, saponins,

alkaloids, fixed oils, triterpenes, proteins and steroids (Ghule *et al*., 2007). The adaptogenic activity may be due to these constituents, where as standard drug Withania somnifera an established adaptogenic drug too contains glycosides, steroids and flavoniods (Krupavaram *et al*., 2007).

CONCLUSION

In conclusion, present results suggest that administration of *Lagenaria siceraria* is capable of increasing the capacity to tolerate non-specific stress in experimental animals as evident from the restoration of a large number of parameters studied during different types of stress and acts as an Adaptogenic agent. It may also be useful in the management of adverse changes associated with the stressors that alter and impair the normal functioning of the organism.

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