Evaluation of Anti anorectic activity of the Ethanolic extract of 
Lagenaria siceraria fruit in Animal model 

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KEYWORDS
Anorexia, Adaptogenic property, Lagenaria siceraria, restraint stress, LPS

A B S T R A C T

Anorexia nervosa is an eating disorder that predominantly affects young women. It is clinically diagnosed by a failure to maintain a healthy body weight, fear of becoming overweight, caloric restriction, and amenorrhea. Anorexia nervosa is the third most common illness among adolescent females. Physical stress and LPS administration significantly affect the feeding behavior in rodents. In previous studies reported Rhodiola rosea L. and Withania somnifera have shown anti anorectic effect due to its antistress and adaptogenic property. However there is no report on effect of ethanolic extract of Lagenaria siceraria fruit (EELS) on feeding behavior in experimentally induced models of anorexia in rats. To evaluate the anti anorectic activity of the ethanolic extract of Lagenaria siceraria fruit in female albino rats. In present study, total of 96 adult female wistar albino rats were taken whose weights ranged from 150-200 gm. Vehicle or EELS (200 and 400mg/kg, p.o.) was administered for 21 days in a) freely feeding rats b) 20 hrs food deprived rats c) stress induced anorexic rats and d) LPS induced anorexic rats. In freely feeding rats after administration of vehicle or EELS food was withdrawn for one hr and offer again. In 20 hr food deprived rats, vehicle or EELS was administered in 20 hrs fasted animals and food was kept for 4 hrs. In stress induced anorexic rats, vehicle or EELS was administered one hour later stress were applied daily for one hr and then food was kept. In LPS induced anorexic rats were administered with LPS (100µg/kg, i.p.), the food was withdrawn for four hour, later vehicle or EELS was administered, one hr later food was kept. All above procedures was continued for 21 days. Ethanolic fruit extract of Lagenaria siceraria fruit (200 and 400 mg/kg) dose dependently reduced the physical stress & LPS induced anorexia in female rats. As evidenced by increase in food consumption, number of attempts for food consumption and change in body weight. Whereas this changes was not observed in freely feeding and 20 hrs food deprived rats. Thus, it has been concluded that ethanolic fruit extract of Lagenaria siceraria possesses anti-anorectic activity in stress induced and LPS induced anorexic rats. However, further experiments are required to prove the mechanism of action and main active components of this extract are necessary.
Introduction

Anorexia nervosa (AN) is an eating disorder. It is a severe, very distressing and often chronic mental illness, which can lead to severe weight loss, chronic physical disabilities such as osteoporosis (bone loss with weakened bone structure and increased risk of fractures), growth retardation, infertility, impaired thinking and concentration, bowel and intestinal disorders and major disruptions to emotional, social and educational development. It can be life threatening (Charnock, 1998). People who are struggling with severe underweight and eating disorders, both of which can eventually lead to a complex state of malnutrition or death if left untreated. Eating disorders are considered a physiological or a psychological problem, depending on the disorder. One of the most common types of eating disorders is anorexia nervosa. Individuals with AN have a distorted body image, that is, they perceive themselves as being overweight despite their emaciated status, and they are often in denial of their condition (Forsythe, 1998; Schebendach and Reichert-Anderson, 2000). However, it is well worth stating that the literal meaning of anorexia is the loss of appetite, especially when prolonged. However, it is well worth stating that the literal meaning of anorexia is the loss of appetite, especially when prolonged.

Anorexia nervosa can lead to many medical outcomes that include a weakened immune system, anemia, delayed gastric emptying, organ damages, irregular heartbeats, changes in the central nervous system, low body temperatures, low blood pressures, pubertal delay in children, and the disruption of menstrual cycles in females (Schebendach and Reichert-Anderson, 2000). It is the third most common illness among adolescent females. It has been estimated that approximately 6% of anorexics die from anorexia nervosa (Forsythe, 1998).

Past research suggested that one in every 800-1000 females are associated with anorexia nervosa, but it is now estimated that one out of every hundred young females have the disorder (Forsythe, 1992). The most common period of onset seems to be during adolescence (Schebendach and Reichert-Anderson, 2000). Girls between the ages of 9-14 have reported a desire to be thinner and have attempted dieting (Halvarsson et al., 2002). More attention is given to females because the majority of the reported cases are primarily females; however, the rate of incidence in males is increasing (Forsythe, 1998). Males account for approximately 5-10% of all eating disorders (Carlat et al., 1997). Over five million Americans suffer from some form of eating disorder (Adolescent Medicine Committee & Canadian Paediatric Society, 1998) and it is suggested that incidences for males are lower than that for females because males tend to be less dissatisfied about their weight (Sisson et al., 1997). In addition, there is a higher prevalence among explicit groups in which diet restriction and regulation of body weight is of great importance (e.g., ballerinas, dancers, marathon runners, or models) (Carlat et al., 1997; Engstrom et al., 1999). Because eating disorders are more than merely medical complications, they are usually treated with the help of an interdisciplinary team (e.g., medical professionals, nurses, dietitians, and psychiatrists), family, relatives, and close friends (Adolescent Medicine Committee & Canadian Paediatric Society, 1998).

According to WHO, 80% of the population of developing countries rely on Traditional System of Medicine for the treatment of
common diseases such as anaemia, anorexia, chronic mental illness, severe weight loss, chronic physical disabilities, growth retardation, Reduced/ compromised immune system function, Intestinal problems (e.g. abdominal pain, constipation, diarrhoea) and loss of or disturbance of menstrual periods in girls and women. The most commonly prescribed drugs by Ayurvedic practitioner is, for the treatment of anorexia (i.e. loss of appetite). Herbal products are favoured in Ayurveda because the founders of Ayurveda recognized the possible synergistic and counterbalancing effects of herbs. But the traditional claim of EELS as an anti anorectic agent is not yet explored.

*Lagenaria siceraria* (Mol.) Standl. (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., 2004), 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 (Hassanpour Fard et al., 2008). It is also reported to contain cucurbitacins, fibers and polyphenols (Kirtikar and Basu, 1993; Nadakarni and Nadakarni, 1976).

LS fruit has been reported to possess antioxidant activity (Jiwjinda et al., 2002), hepatoprotective (Shirwaikar and Sreenivasan, 1996), hypolipidemic and antihyperlipidemic effects in normcholesterolemic and triton-induced hyperlipidemic rats (Ghule et al., 2006). HPLC analysis of methanol 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 which possesses immunoprotective, antitumour, antiHIV, and antiproliferative properties (Farnsworth, 1996). Phytochemical screening also revealed the presence of fucosterol and campesterols (Shirwaikar and Sreenivasan, 1996), flavoniods and saponins.

Since *Lagenaria siceraria* has a number of medicinal properties and is a potent anti-oxidant and immunoprotective, the present study was undertaken to evaluate the potential usefulness of *Lagenaria siceraria* fruit for anti anorectic activity in experimental animals.

**Materials and Methods**

**Plant material and extraction**

*Lagenaria siceraria* fruits were collected from the local farms of Udaipur District, Rajasthan in the month of November-December 2014, the botanical authentication was done by the authority of Department of Botany, Rajasthan Agriculture University, Udaipur.

The fresh and semi-ripe 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 70% ethanol at 60°C for 24 hrs using a Soxhlet apparatus. The solvent was evaporated under vacuum which gave semisolid mass (21% w/w) with respect to the dried powder.

The EELS was subjected to phytochemical screening (Farnsworth, 1996) for the detection of various phyto-constituents such as glycosides, steroids, tannins, alkaloids, flavonoids.

An acute toxicity of test compound was carried out on normal healthy rats by fixed dose (OECD-420 Guidelines) method.
Test drugs

1) Optimum dosage of oral suspensions containing 200 mg/ml and 400 mg/ml of the ethanol extract of L. siceraria fruit were prepared in 1% w/v gum acacia.

2) Lipopolysaccharide purchased from Sigma Aldrich, USA and dissolved in pyrogen-free isotonic saline which is administered by intraperitoneal route at the dose of 100μg/kg.

Animal Models

Wistar albino rats weighing 150-200 gm of female sex, 3 months of age were used for this study. The experimental animals were housed in clean and transparent polypropylene cages with six animals in each cage and maintained under standard laboratory conditions (12:12 hrs 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 Geetanjali Medical College, Udaipur was approved the study.

All the experimental procedures were carried out in accordance with committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines.

Study design

To investigate the effect of Lagenaria siceraria fruit extract on Feeding Behavior in

1) Freely feeding rats.
2) 20 hrs Food-deprived rats
3) Restraint stress induced anorexic rats.
4) Lipopolysaccharide (LPS) induced anorexic rats.

Effects of EELS on freely-feeding rats

To evaluate the general effects of EELS on food intake, attempts for food intake and body weight in freely-feeding rats. The animals (n=24) were divided into four groups that received oral administration of vehicle or EELS at 200 and 400 mg/kg. Their food was temporarily removed and offered again 1 hr later. This procedure was continued for 21 days.

After last dose, food consumption, number of attempts for food consumption was determined at 120th min. Food intake was measure by weighing of the food cups, with subtraction of the spillage from the total food intake. Experiments took place at 10:00 a.m., during the light phase of the cycle; the rats were not accustomed to eating at this time of the day, as shown by the low food intake in controls. At the end of study body weight were measured.

Effects of EELS on food intake in food deprived rats

To evaluate the effects of EELS on 20 hrs food deprived rats that had not been subjected to the stress conditions. For this purpose, 20 hrs food-deprived rats (n=18) received an oral administration of vehicle or EELS (200 and 400 mg/kg) and another group of non-deprived rats (n=6) served as the control. Food was given 1 hr after the drug or vehicle administration. This procedure continued for 21 days, after last dose consumption, number of attempts for food consumption was determined at 120th min. Body weight was measure at the end of study.

Effects of EELS on restraint stress-induced anorexia

To evaluate the effects of EELS on food consumption under restraint-stress...
conditions, rats (n=24) were subjected to 20hrs food deprivation, and then received oral administration of vehicle or EELSF (200 and 400mg/kg). One hour afterwards, restraint stress was induced in the rats by being restrained in cylindrical Plexiglas tubes for 60min, which has been reported to produce a marked inhibition of food intake. After the 60min restraint, the rats were returned to their home cage, where food was made available.

This procedure continued for 21 days, after last dose their food consumption, attempts for food consumption was determined at 120th min. Body weight were measure at the end of study. Another group of food deprived but non stressed rats (n=6) served as a control for the effects of the restraint stress; the rats of this group were oral administered with vehicle and returned to their own cages without being subjected to restraint.

**Effects of EELS on LPS-induced anorexia**

To determine the selectivity of the anti anorectic effect of EELS, a model of anorexia induced by LPS injection was used. Administration of low doses of LPS, a pathogenic agent, induces a moderate infection that is associated with a reduction in food consumption. Food-deprived rats (n=18) were injected intra peritoneally with 100μg/kg LPS, and 4 hrs later they received oral administration of 200 and 400 mg/kg EELS or its vehicle. Another group of food deprived rats (n=6) received the respective vehicles and served as controls. Sixty minutes after EELS administration, the rats were their food offered access to food pellets. This procedure continued for 21 days and their food consumption and attempts for food consumption was determined after last dose at 120th min. Body weight were measure at the end of study.

**Results and Discussion**

**Phytochemical screening of EELS**

Phytochemical screening revealed that EELS showed the presence of flavonoids, saponins, steroids and polyphenolic compounds.

**Effects of EELS on freely-feeding rats**

As shown in figure 1 the results indicates that EELS (200 and 400 mg/kg/day; p.o.) when administered for 21 days did not shown any significant changes in food consumption, number of attempts for food consumption and body weight when compared with normal control and freely feeding group respectively were summarized in Table 1.

**Effects of EELS on food-deprived rats**

As shown in figure 2 the results indicates that the EELS (200 and 400 mg/kg/day; p.o.) when administered for 21 days had not shown any significant effect on food consumption, body weight and number of attempts for food consumption as compare with 20 hrs food-deprived rats. But, food consumption, number of attempts for food consumption and body weight was significantly increased in 20 hrs food-deprived rats, as compared with normal control rats were summarized in Table 2.

**Effects of EELS on restraint stress-induced anorexia**

As shown in figure 3 the restraint stress induced anorexia group markedly reduced food consumption, number of attempts for food consumption and body weight, as compared with the normal control group that was not subjected to these stress conditions.
It revealed a significant treatment effect for restraint stress-induced anorexia. Treatment with EELS dose dependently increase food consumption, number of attempts for food consumption and body weight. This shows significantly reversed the anorectic effects of the restraint stress was summarized in Table 3.

**Effects of EELS on LPS-induced anorexia**

As shown in figure 4 and statistical evaluation shows that LPS treated animals shows decrease in food consumption, number of attempts for food consumption and body weight when compare with normal control group. Animals treated with ELSF (200 and 400mg/kg/day p.o for 21 days) shows highly significant increase in amount of food consumption. Also similar result was observed for number of attempts for food consumption and gain in body weight was summarized in Table 4.

The present investigation was carried out on the effect of EELS on feeding behavior in freely feeding rats, 20 hrs food-deprived rats, restraint stress induced anorexic rats, lipopolysaccharide induced anorexic rats.

The current study reveals that treatment with EELS prevents the physical stress & LPS induced anorexia in rats, suggestive of the anti-anorectic action of EELS. As evidenced by increase in food consumption, number of attempts for food consumption and change in body weight. Whereas this changes was not observed in freely feeding and 20 hours food deprived rats. These finding suggest that preventive effect of LS on stress and LPS induced anorexia is not related to non specific orexigenic action of LS.

Previous preclinical studies suggest that application of chronic stress cause marked reduction in food consumption (Roberto Ciccocioppo et al., 2002). Also it suggests that central mechanisms involved in the stress-induced inhibition of food consumption, certain peptides and neurotransmitters are thought to be involved in the response (Porter et al., 1998).

It is well established that monoamines and corticotropin-releasing factor influence feeding behavior and mediate behavioral and physiological responses to stress. Several investigators have attributed stress-induced anorexia to activation of corticotropin-releasing factor and/or serotonin (5-hydroxytryptamine, 5-HT) pathways (Sihimizu et al., 1989). Both of these transmitters are elevated in response to stress in a number of brain areas, including those that are involved in the regulation of feeding behavior (Roberto Ciccocioppo et al., 2002). In consistent with these reports, in the present study application of physical stress significantly decreased the food consumption, number of attempts for food consumption and body weight (Exner et al., 2000; Roberto Ciccocioppo et al., 2002). Treatment with EELS dose dependently reversed the anorectic effect followed by application of physical stress.

Similar effect was demonstrated in previous studies treatment with Rhodiola rosea L. and Withania somnifera reverse the anorectic effect followed by physical stress, by virtue of their antistress and adaptogenic properties (Roberto Ciccocioppo et al., 2002; Rybkin et al., 1997). Similarly ethanolic fruit extract of LS shows antistress, adaptogenic and antioxidant effect in foot shock and forced swim induce stress (Lakshmi and Sudhakar, 2011, 2009; Erasto et al., 2009). This gives an idea that in present study anti anorectic effect of EELS treatment on stress and LPS induce anorexia may be due to its antistress, adaptogenic and antioxidant property.
Table 1 Effects of oral administration of vehicle or Ethanolic Extract of *Lagenaria siceraria* fruit (200 and 400 mg/kg, p.o., for 21 days) on Food consumption, Number of attempts for Food consumption and change in Body weight in Freely Feeding (FF) rats

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Control</th>
<th>FF</th>
<th>FF + ELS 200mg/kg</th>
<th>FF + ELS 400mg/kg</th>
<th>Control</th>
<th>FF</th>
<th>FF + ELS 200mg/kg</th>
<th>FF + ELS 400mg/kg</th>
<th>Control</th>
<th>FF</th>
<th>FF + ELS 200mg/kg</th>
<th>FF + ELS 400mg/kg</th>
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<tr>
<td>Mean ± SEM</td>
<td>8.306 ± 8.15 ± 8.36 ± 8.16 ± 21 ± 20.5 ± 21.6 ± 20.8 ± 21.4 ± 20.43 ± 21.34 ± 22.5 ±</td>
<td>0.4559 ± 0.3022 ± 17.04 ± 1.065 ± 0.9916# ± 1.563 ± 1.033 ± 1.045 ± 0.9387# ± 1.197 ± 1.647 ±</td>
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</table>

# P < 0.05 when compare with control group

Table 2 Effects of oral administration of vehicle or Ethanolic Extract of *Lagenaria siceraria* fruit (200 and 400 mg/kg, p.o., for 21 days) on Food consumption, Number of attempts for food consumption and changes in Body weight in 20 hrs Food Deprived (FD) rats

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Control</th>
<th>FD</th>
<th>FD + EELS 200mg/kg</th>
<th>FD + EELS 400mg/kg</th>
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<tr>
<td>Mean ± SEM</td>
<td>9.05 ± 9.90 ± 9.89 ± 9.95 ± 22.5 ± 22.83 ± 23.83 ± 25 ± 1.155 ± 23.43 ± 23.21 ± 24.5 ± 25.21 ±</td>
<td>0.6696 ± 0.4504# ± 0.4779 ± 0.6927 ± 1.088 ± 1.078# ± 1.138 ± 0.8106 ± 0.9676# ± 1.024 ± 0.7078 ±</td>
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**Table 3** Effects of oral administration of vehicle or Ethanolic Extract of *Lagenaria siceraria* fruit (200 and 400 mg/kg, p.o., for 21 days) on Food consumption, Number of attempts for food consumption and change in Body weight in Stress induce anorexic rats

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Food Consumption (Grams)</th>
<th>Number of attempts for Food consumption</th>
<th>Body Weight (Grams)</th>
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<tr>
<td>Mean ± SEM</td>
<td>9.25 ± 0.564</td>
<td>2.198 ± 0.2288**</td>
<td>13.03 ± 1.178**</td>
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<td>P – Value</td>
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</table>

### P<0.001 when compare with control group and **P<0.001 when compare with vehicle treated group

**Table 4** Effects of oral administration of vehicle or Ethanolic Extract of *Lagenaria siceraria* fruit (200 and 400 mg/kg, p.o., for 21 days) on Food consumption, Number of attempts for food consumption and change in Body weight in Lipopolysaccharide (LPS) induced anorexic rats

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Food Consumption (Grams)</th>
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<th>Body Weight (Grams)</th>
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<td>9.07</td>
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<td>Mean ± SEM</td>
<td>9.19 ± 0.7827</td>
<td>2.098 ± 0.4567##</td>
<td>16.65 ± 1.389**</td>
</tr>
<tr>
<td>P – Value</td>
<td>-</td>
<td>P&lt;0.001</td>
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### P<0.001 when compare with control group and **P<0.001 when compare with vehicle treated group
Figure 1 Effect of oral administration of vehicle or Ethanolic extract of Lagenaria siceraria fruit (200 and 400 mg/kg p.o, for 21 days) in freely feeding rats on (a) food consumption, (b) number of attempts for food consumption, (c) body weight

Data represent Mean ± SEM of rats
Figure 2 Effect of oral administration of vehicle or Ethanolic extract of Lagenaria siceraria fruit (200 and 400 mg/kg p.o, for 21 days) in freely feeding rats on (a) food consumption, (b) number of attempts for food consumption, (c) body weight.

Data represent Mean ± SEM of rats
#P < 0.05, significant when compare with control group
Figure 3 Effect of oral administration of vehicle or Ethanolic extract of Lagenaria siceraria fruit (200 and 400 mg/kg p.o, for 21 days) in freely feeding rats on (a) food consumption, (b) number of attempts for food consumption, (c) body weight

Data represent Mean ± SEM of rats
#P < 0.001, significant when compare with control group and **P < 0.001 significant when compare with vehicle treated group
**Figure 4** Effect of oral administration of vehicle or Ethanolic extract of *Lagenaria siceraria* fruit (200 and 400 mg/kg p.o, for 21 days) in freely feeding rats on (a) food consumption, (b) number of attempts for food consumption, (c) body weight.

Data represent Mean ± SEM of rats

#P < 0.001, significant when compare with control group and **P < 0.001 significant when compare with vehicle treated group
Lipopolysaccharide (LPS) a endotoxin derived from the cell walls of dead and disintegrating gram-negative bacteria trigger many of the host’s responses to bacterial infection (Feingold ET AL., 1995). Doses of LPS mimicking the clinical aspects of bacterial infections reduce food consumption after parenteral administration in a variety of animal. The food consumption reduction during bacterial infection is result to complex neural, neurohumoral and endocrine interactions between bacterial products and endogenous mediators in the periphery as well as in the brain (Porter ET AL., 1998). It’s well known that cytokines that they release such as interleukins and tumor necrosis factors, prostaglandins, interleukin, leptin and interferon are responsible for a number of pathological features like fever that shows decrease in food consumption, number of attempts for food consumption and body weight (Lugarini et al., 2002).

In the present study also administration of LPS decreased food consumption, number of attempts for food consumption and body weight like previous reports of CRF-induced anorexia (Roberto Ciccocioppo et al., 2002). Treatment with ethanolic fruit extract of LS dose dependently reversed LPS induce anorexia. Lugarini et al has suggested that treatment with NS-398 reverses the anorectic effect of LPS by inhibition of inflammatory mediators (Roberto Ciccocioppo et al., 2002).

Similarly ethanolic fruit extract of LS have shown potent anti inflammatory activity in various animal models and they suggested that this effect would be due to inhibition of inflammatory mediators (Ghule et al., 2006). In View of these reports, in the present study we suggest that anti anorectic effect of ethanolic extract of Lagenaria siceraria in LPS induce anorexia may be due to inhibition of inflammatory mediators.

Conclusion

In the present study, treatment with EELS significantly increased the feeding behavior in Restraint stress induced and Lipopolysaccharide induced anorexic rats. These changes supported by relevant activities like anti-stress, adaptogenic, anti inflammatory and antioxidant properties of the plant.

Based on improvement in food consumption, number of attempts for food consumption and body weight when compare with control group and as well as vehicle group, it is concluded that the ethanolic extract of Lagenaria siceraria fruit possesses anti anorectic effect in physical stress and lipopolysaccharide (LPS) induce anorexia. Such Scientific evaluation studies are essential for the global acceptance of Herbal based drugs as an integral part of modern drug therapy.

However, further experiments are required to prove the mechanism of action and main active components of this extract are necessary.

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References

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