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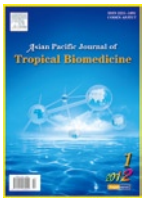

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Adaptogenic activity of *Trigonella foenum graecum* (Linn) seeds in rodents exposed to anoxia and immobilization stress

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ABSTRACT

Objective: To investigate the adaptogenic activity of methanolic extract of *Trigonella foenum graecum* seeds (METFGS) at 100, 250 and 500 mg/kg doses against anoxia stress tolerance in mice and immobilisation stress models. **Methods:** *Withania somnifera* (100 mg/kg, p.o.) was selected as reference standard and it showed significant effect in both stress models. The parameters like anoxia stress tolerance time was recorded in anoxia stress and estimation of biochemical marker levels and determination of organs weight were carried out in immobilisation stress model. **Results:** Concomitant treatment with METFGS at 500 mg/kg showed marked increase in anoxia stress tolerance time. But the lower doses (100 and 250 mg/kg) of METFGS produced mild increase in anoxia tolerance time. There was dose dependant significant reduction in biochemical parameters like serum glucose, cholesterol, triglycerides and BUN levels exhibited by test extract in immobilisation stress model as compared to stressed group. The stress induced increase in liver, adrenal gland weight and decrease in weight of spleen and testes were significantly reversed by the test extract at higher dose. But lower doses of extract caused the protective effect on weight of liver and adrenal gland only. **Conclusion:** The results from the present study indicate that METFGS possessed significant antistress activity.

1. Introduction

The human society has become complex and in many ways, more demanding. However, our physiological responces designed to cope with the ever-increasing adverse situations have not evolved appreciably during the past thousand years. The failure of successful adaptation during stressful situations has resulted in stress related illness that result from or are associated with dysregulaton of the stress response[1]. Varoious attempts have beeen made to counter the aversive effects of stres, ranging from yoga and meditation to antistress drugs, particularly the anxiloytic benzodiazepines (BDZ). However, despite claims to the contrary, these nonpharmacological and pharmacological methods appear to have limited utility[2]. An answer to this perplexing problem of countering stress incuced perturbations of physiological homeostasis came the plant kingdom. A group of plant based drugs, adaptogens. The actual word adaptogen was first used by a Soviet scientist, Dr. Nikolai Lazarev, who was funded by grants from the military was researching substances which produced a "state of nonspecific resistance (SNIR)"[3]. The idea was to find ways to enhance the productivity and performance of soldier, athletes and workers without using dangerous stimulants. Much of the early research into adaptogens was done by Dr. I. I. Brekhman who, in the late 1950's studied Panax ginseng.

Trigonella foenum graecum (TFG) has been used since ancient times in Indian folklore medicine for its many medicinal properties. TFG is reported to possess immunomodulatory, hypoglycemic, nootropic and anxiolytic activities[4-7]. In the present study, antistress activity is investigated in view of its reported anxiolytic and nootropic activity. In the present study METFGS showed significant antistress activity in swimming and cold stress models in our laboratory (unpublished data). Hence in the present investigation an attempt has been made to screen the adaptogenic property of METFGS against anoxia and immobilisation stress models.

2. Materials and methods

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2.1. Plant material and preparation of extract

The seeds of *Trigonella foenum graecum* (METFGS) were purchased from the local market of Harapanahalli in the month of May 2006 and authenticated by Prof. K. Prabhu, Dept. of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher specimen (SCSCP/PG/38/2006) has been deposited at the museum of college. The dried seeds were coarse powdered and defatted with petroleum ether (60 – 80 ℃) using Soxhlet extractor. The marc obtained was then subjected to extraction with methanol (64 – 65.5 ℃). The extract was concentrated using rotary flash evaporator. The dried extract was stored in airtight container in refrigerator below 10℃. Percentage yield of extract was 14.39%.

2.2. Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on METFGS for detection of phytoconstituents present following the standard methods described in practical pharmacogonosy by Dr. C.K. Kokate[8] and K.R. Khandelwal[9]

2.3. Animals

The male albino rats of wistar strain (150 – 200g) and albino mice of either sex (20 – 30 g) were used throughout the experimentation. The animals were procured from Nijalingappa Medical College, Bagalkot Karnataka. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandory condition; room temperature: 27 – 3 ℃, relative humidity: 65% – 10% and 12 hours. Light/dark cycle. All the animals were fed with rodent pellet diet (Gold mohr, Lipton India Ltd.,) and water was allowed *ad libitum* under strict hygienic condition. Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC).

2.4. Acute toxicity study

An acute toxicity of METFGS was conducted in female albino mice (20 – 30 g) maintained under standard conditions. The animals were fasted over night prior to the experiment. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adopted for toxicity studies[10].

2.5. Evaluation of antistress activity

2.5.1. Anoxia stress tolerance in mice[11-13]

Albino mice of either sex weighing 20 – 30 g were selected and divided into five groups of six each as Group I Control (Received only vehicle 1 mL/kg *p.o.*) , Group II Standard (*Withania somnifera*, 100 mg/kg *p.o.*) Group III METFGS (100 mg/kg *p.o.*) Group IV METFGS (250 mg/kg *p.o.*) Group V METFGS (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 one hour after the treatment stress was induced in all mice by placing each animal individually in the hermetic vessel of 1 lit. capacity to record anoxia tolerance time. The moment when the animal showed the first convulsions immediately removed from the vessel and resuscitated if needed. The time duration of entry of the animal into the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia tolerance. 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.

2.5.2. Immobilisation stress[11,12]

Adult male albino rats of 150 – 200 g were selected and divided into six groups of six animals each as Group I Negative control (Unstressed, untreated), Group II Positive control (Stressed, received vehicle), Group III Standard (*Withania somnifera* 100 mg/kg *p.o.*), Group IV METFGS (100 mg/kg *p.o.*), Group V METFGS (250 mg/kg *p.o.*) , Group VI METFGS (500 mg/kg *p.o.*) The treatment was made as stated above for 10 days 1 hour before the exposure of stress. Stress was induced by immobilizing rats with head down, supine position by fixing the forelimbs and hindlimbs to a wooden board inclined at an angle of 600 daily, 2 hours (11 am to 1 pm) for a period of ten days. The animals were sacrificed at the end of specified period and blood was collected by cardiac puncture 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). The weight of organs, such as liver, spleen, adrenal gland and testes after washing with alcohol was recorded per 100 g body weight of animal.

2.6. Statistical analysis

Results have been presented as Mean± SEM and the experimental groups were compared with control group in anoxia stress and positive control (stressed group) in immobilisation stress model. The statistical analysis was done using ANOVA followed by Turkey Karmmer Multiple Comparison Test. P value of 0.05, 0.01 and 0.001 were considered as statistically significant.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical investigation indicated the presence of flavonoids, tannins, saponins glycosides, alkaloids, steroids and carbohydrates in METFGS.

3.2. Acute toxicity study

The METFGS was studied for acute toxicity at dose of 2 000 mg/kg. The extract was found to be safe and no mortality of the animals observed. Hence 2 500 mg/kg was considered as LD_{50} cut off value as per fixed dose method of CPCSEA. So the doses selected for the screening of adaptogenic activity were 100, 250 and 500 mg/kg.

3.3. Adaptogenic (antistress) activity

3.3.1. Anoxia stress tolerance time

Anoxia stress tolerance time was significantly (P< 0.05) enhanced on 14th and 21st day in METFGS (500 mg/kg) treated group. There was increased anoxia tolerance time also seen after one week of treatment, but the result found

Table 1
Effect of METFGS on anoxia stress tolerance time in mice.

Group	Duration of anoxia stress tolerance (min)		
	7th Day	14th Day	21st Day
Control (vehicle)	145.80 ±4.32	146.80 ±4.20	148.60 ±3.86
Std. (Withania somnifera)100 mg/kg	185.44 ± 3.62** (21.49)	192.66 ± 4.18** (31.23)	201.20 ± 4.22** (35.39)
METFGS 100 mg/kg	149.30 ±3.21(2.40)	151.13 ±4.30(2.94)	152.91 ±4.51(2.90)
METFGS 250 mg/kg	152.60 ±3.18(4.66)	154.60 ±3.68 (5.31)	159.00 ±4.80 (6.99)
METFGS 500 mg/kg	160.00 ±3.38 (9.73)	164.00 ±4.54* (11.71)	168.80 ±5.20* (13.59)

Values are mean ± SEM (n = 6), The values in parenthesis are the % increase in anoxia tolerance, * P< 0.05, ** P < 0.01 as compared to control.

Table 2
Effect of METFGS on immobilisation stress induced biochemical parameters in rats.

Group	Biochemical estimations (mg/dl)			
	Glucose	Cholesterol	Triglycerides	BUN
Negative control (unstressed)	92.13 ±3.21	54.28 ±3.51	69.64 ±3.12	23.53 ±2.46
Positive control (stressed, received vehicle)	146.32 ±4.18	85.28 ±4.01	103.88 ±3.52	43.92 ±2.31
Std. (Withania somnifera) 100 mg/kg	98.24 ± 3.80**	58.78 ± 3.61**	76.10 ± 4.00**	27.13 ± 1.65**
METFGS 100 mg/kg	125.23 ±3.52*	65.13 ±3.27*	85.31 ±4.21*	34.63 ±2.10*
METFGS 250 mg/kg	113.15 ± 3.26**	64.88 ± 3.84*	83.44 ±4.12*	33.83 ±1.93*
METFGS 500 mg/kg	106.94 ± 4.41**	61.79 ± 4.19**	78.56 ± 3.83**	29.94 ±2.42**

Values are mean ± SEM (n = 6).
* P < 0.05, ** P < 0.01, *** P< 0.001 as compared to positive control.

Table 3
Effect of METFGS on organs weight in immobilisation stress induced rats.

Group	Organs weight (gm/100 gm b.w.)			
	Liver	Adrenal gland	Spleen	Testes
Negative control (unstressed)	3.445 ±0.13	0.013 ±0.001	0.399 ±0.03	1.648 ±0.060
Positive control (stressed, received vehicle)	5.820 ±0.15	0.030 ±0.002	0.232 ±0.02	1.261 ±0.032
Std. (Withania somnifera) 100 mg/kg	3.801 ± 0.14**	0.017 ± 0.002**	0.372 ± 0.03**	1.560 ± 0.051**
METFGS 100 mg/kg	5.213 ±0.16*	0.023 ±0.002*	0.265 ±0.02	1.387 ±0.048
METFGS 250 mg/kg	4.357 ± 0.13**	0.021 ±0.002*	0.293 ± 0.01	1.420 ± 0.050
METFGS 500 mg/kg	4.302 ± 0.18**	0.018 ± 0.002**	0.364 ±0.03*	1.510 ± 0.043**

Values are mean ± SEM (n = 6, * P < 0.05, ** P < 0.01 as compared to positive control.

statistically not significant. However the effect of METFGS at doses of 100 and 250 mg/kg on anoxia stress tolerance time in mice was not statistically significant at the end of 1st, 2nd and 3rd week of treatment. The results are shown in Table 1.

3.3.2. Immobilisation stress

Effect on biochemical parameters – Immobilisation stress caused marked increase in serum glucose, cholesterol, triglycerides and BUN in rats. This stress induced elevated levels of biochemical parameters were significantly reversed by METFGS in dose dependant manner. The results are given in Table 2.

Effect on organs weight – METFGS at dose of 500 mg/kg offered significant protection against change in the weight of liver, adrenal gland, spleen and testes when compared to stress group. However METFGS at doses of 100 and 250 mg/kg produced significant decrease in weight of liver and adrenal gland but failed to increase spleen and testes weight significantly. The results are presented in Table 3.

4. Discussion

In the present investigation METFGS has been evaluated for the antistress activity against different types of stress viz. Anoxia and immobilisation models. The well known adaptogen *Withania somnifera* was taken as a reference standard in the present study.

In case of anoxia stress tolerance test METFGS at dose of 500 mg/kg exhibited significant antistress activity as indicated by increase in duration of anoxia stress tolerance time. The test drug also produced mild increase in anoxia tolerance at doses of 100 and 250 mg/kg which was however, not significant statistically.

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 mobilise lipids from adipose tissues. The effect of stress on serum triglycerides has been shown to be variable. The increase in release of catacholamines leads to elevated levels of glucose and BUN. In immobilisation stress model, the test extract reduced the elevated levels of serum biochemical parameters in dose dependant manner[14-17].

Stress induces adreno-medullary response in man. Adrenaline in turn stimulates β_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 blood cells (RBC) during stress. So

its weight decreases during stress[18-20]. This stress induced changes of organs weight were significantly reversed by the test extract at higher dose, whereas the lower doses (100 and 250 mg/kg) of extract could able to exhibit protective effect on weight of liver and adrenal gland. But failed to produce statistically significant increase in spleen and testes weight in immobilisation stress model.

Experimental studies have confirmed the adaptogenic properties of ginseng and the effects are apparently due to presence of saponin glycoside content in the root. Saponin glycosides are the main chemical constituents present in the seeds of fenugreek4 and this may be responsible for significant adaptogenic activity in our study. Literature survey indicate that flavonoids and tannins were reported to possess variety of pharmacological activities including antistress activity[21,22]. In the present investigation also preliminary phytochemical screening on METFGS gave positive tests for flavonoids and tannins, this could be the reason for significant adaptogenic property of test extract.

Increased generation of oxidative free radicals (OFR) or impaired antioxidant defense mechanism have been implicated in chronic stress induced perturbed homeostasis including immunosuppression, inflammation, diabetes mellitus, peptic ulceration and other stress related disease[23]. The seeds of fenugreek have shown to exert significant antioxidant activity[24] induced by augmented activity of OFR scavenging enzymes namely superoxide dismutase (SOD), catalase (CA) and glutathione peroxidase (GPx). Thus at least part of observed adaptogenic (antistress) effect of METFGS may be due to the antioxidant activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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