Effect of *Pueraria tuberosa* tuber extract on chronic foot shock stress in Wistar rats

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ABSTRACT

The present study was undertaken to explore the protective effects of tuberous root extract of *Pueraria tuberosa* on chronic foot shock stress (CS) induced physiological, neurobehavioral and neuropathological alterations. Male Wistar rats (120-150 g) were divided into seven groups, consisting of ten animals in each. Group I served as normal, group II as positive control, while group III-VII as test drug treated. *P. tuberosa* tuber extract (PTE) was given to rats of groups III-VI at the doses of 50, 100, 200 and 400 mg/kg respectively, while group VII treated with *Withania somnifera* rhizome extract (WSE) (100 mg/kg) as reference drug. Group II (stress control) received only equivalent volume of distilled water (0.5 ml/100 g) orally. All the drugs were given orally once/ day for 14 consecutive days. The last dose was given 1 h before study. Simultaneously, all the animals (except group I) were subjected to 1 h of foot-shock (2 mA) through a grid floor for those 14 days in a standard conditioning chamber with the escape route closed [Chronic stress (CS)]. Thereafter, the rats were placed on open-field and plus maze apparatus for studying the behavioral patterns of them, and the anxiolytic effects of the putative drug. Sexual activities of the animals were also studied. Finally, the animals were sacrificed and their ulcer formation in gastric mucosa was noted. Weights of adrenals and spleen were also taken. Further, plasma corticosterone levels were estimated spectroflurometrically. Results indicated that, CS significantly altered the behavioral patterns, decreased the sexual urge and activities, damaged the gastric mucosal layers, enhanced plasma corticosterone levels and increased adrenal glands and spleen weights. PTE and WSE showed significant anxiolytic activity, protected the gastric mucosa, lowered plasma corticosterone level (indicating HPA axis inhibition) and negated the hypertrophy of adrenals and spleen. PTE also enhanced the sexual urge and activities in animals exposed to chronic stress. The findings suggest significant anxiolytic and anti-stress properties of PTE, confirming the clinical efficacy of the plant mentioned in *Ayurveda* (Indian system of traditional medicine).

Keywords: *Pueraria tuberosa,* anxiolytic, antistress, sexual behavior, plus-maze, corticosterone.

INTRODUCTION

The drugs of plant origin are gaining importance and are being investigated for remedies of a number of disorders including anxiety and stress. Since the introduction of adaptogen concept several plants have been investigated, which were used earlier as tonics due to their adaptogenic and rejuvenating properties in traditional medicine.[1] *Pueraria tuberosa* (Roxb. ex Willd. DC) (family: Fabaceae) is a perennial climber found throughout the Indian subcontinent in wet and damp areas.[2] The tuberous roots of this plant are used in Indian traditional medicine (*Ayurveda*) in general debility, nervous breakdown, spermatorrhoea, burning sensation, heart diseases, intrinsic hemorrhage, tuberculosis etc.[3] The chemical constituents have been identified as puerarin, diadzein, daidzin, â-sitosterol and sigmasterols.[4-5] Puerarin (isoflavones) has been reported to possess anti-fertility,[6] anti-hypertensive,[7] anti-hyperglycemic,[8] nootropic[9] and neuroprotective effects.[10-11] Previous studies indicated the potential actions of *P. tuberosa* as an antioxidant that normalizes the hypoxic stress induced elevation of lipid peroxides and catalase[12] and cold immobilization stress.[13] However adaptogenic activity of it is yet to be explored. This study was undertaken to explore the effect of the tuber extract of *P. tuberosa* on chronic stress induced physiological, neurobehavioral and neuropathological alterations.

MATERIALS AND METHODS

Adult male Wistar rats (120-150 g) were obtained from Central Drug Laboratories, Kolkata, Govt. of India and maintained according to recommended guidelines for the care and use of the animals (NIH publication No. 85-23, revised 1985). The institutional animal ethics committee approved all the experimental protocols. Animals were allowed to take food pellets and water *ad libitum*.

Table-1: Role of PTE and WSE on open-field exploratory behavior in chronic foot shock induced stress in rats

	Ambulation	Rearing	Self-grooming
Control	60.15±2.28	22.18±1.04	7.58±0.32
CS	86.20±3.15(a)***	36.05±2.38(a)***	2.40±0.19(a)***
CS+PTE (50 mg/kg)	79.15±2.18(b)	32.15±2.04(b)	3.08±0.28(b)
CS+PTE (100 mg/kg)	76.55±2.08(b)*	30.01±2.16(b)*	4.98±0.21(b)**
CS+PTE (200 mg/kg)	68.10±1.96(b)**	28.62±1.98(b)**	5.24±0.22(b)***
CS+PTE (400 mg/kg)	68.15±2.14(b)**	28.14±1.759b)***	5.98±0.26(b)***
CS+WSE (100 mg/kg)	63.29±2.05(b)**	26.53±1.75(b)***	7.15±0.28(b)***

Results were Mean±SEM with n=10 in each group. All the groups were compared statistically by ANOVA followed by Newman-Keul's multiple comparison tests using statistical software (spssV11.5). *P<0.05, **P<0.01 and ***P<0.001 and (a) and (b) means when compared to control and CS group correspondingly.CS= Chronic foot shock stress (2 mA) daily 1 h for 14 daysPTE= 70.0% hydroethanolic *P. tuberosa* tuberous root extract WSE= 70.0% hydroethanolic *W. somnifera* rhizome extract

Tuberous roots of *P. tuberosa* and rhizomes of *Withenia somnifera* were collected from the herbal garden of medicinal plants near Kolkata, identified by the experts of Botanical Survey of India, West Bengal and the voucher specimens (DB/NSU/01 and DB/NSU/02) kept in the departmental herbarium. The shade dried tuberous roots of *P. tuberosa* and rhizomes of *W. somnifera* were powdered and extracted with 70.0% ethanol in a soxhlet apparatus for 24 h. Thereafter, the extract was concentrated under reduced pressure below 40ºC by rotary evaporator (Hahn Shin Science Co., Korea) and lyophilized (Vertis, Italy) under freezing condition.[14] The yield of the ethanolic extract of *P. tuberosa* (PTE) was 14.0-18.0% (w/w) and *W somnifera* (WSE) was 8.0-12.0% (w/w). HPTLC analyses reported 0.6% puerarin in PTE.[12]

Animals (120-150 g) were divided into seven groups, consisting of ten animals in each. Group I served as normal, group II as positive control, while group III-VII as test drug treated. PTE was given to rats of groups III-VI at the doses of 50, 100, 200 and 400 mg/kg respectively, while group VII treated with WSE (100 mg/kg) as reference drug. Group II (stress control) received only equivalent volume of distilled water (0.5 ml/100 g) orally. All the drugs (except group I) were given orally once/day for 14 consecutive days. The last dose was given 1 h before study.

The rats (except group I) were subjected daily to 1 h of foot-shock (2 mA) through a grid floor in a standard conditioning chamber with the escape route closed. The intervals between the shocks were randomly programmed between 3 and 5 sec and 10 and 110 sec, respectively and continued for 14 days[Chronic stress (CS)].[15-16] The animals were then used for further studies like, open-field test, plus-maze test, sexual behavioral study, morphological studies and biochemical studies.

Open-field apparatus was made of plywood and consisted of squares (61×61cm^2) with high walls (61×61cm^2). The entire apparatus was painted black except for 6mm thick white lines, which divided the floor into 16 squares. Open-field was kept dark during the

Table-2: Role of PTE and WSE on plus maze test in foot-shock induced chronic stress in rats

	Time spent in open arm (sec)	Time spent in closed arm (sec)	Entries into open arm
Control	90.2±3.15	189.2±2.48	9.26±0.52
CS	50.4±2.13(a)***	238.8±3.95(a)***	2.80±0.32(a)***
CS+PTE (50 mg/kg)	52.9±1.33(b)**	231.4±2.22(b)	4.0±0.25(b)**
CS+PTE (100 mg/kg)	61.7±1.11(b)*	201.4±2.18(b)***	5.9±0.56(b)***
CS+PTE (200 mg/kg)	61.5±0.96(b)**	198.5±2.10(b)***	6.1±0.23(b)***
CS+PTE (400 mg/kg)	56.1±1.26(b)	192.6±2.45(b)***	6.2±0.24(b)***
CS+WSE (100 mg/kg)	69.6±1.24(b)***	180.2±2.55(b)***	6.5±0.34(b)***

Results were Mean±SEM with n=10 in each group. All the groups were compared statistically by ANOVA followed by Newman-Keul's multiple comparison tests using statistical software (spssV11.5). *P<0.05, **P<0.01 and ***P<0.001 and (a) and (b) means when compared to control and CS group correspondingly. CS= Chronic foot shock stress (2 mA) daily 1 h for 14 daysPTE= 70.0% hydroethanolic *P. tuberosa* tuberous root extract; WSE= 70.0% hydroethanolic *W. somnifera* rhizome extract

experiment. Each animal was placed in the test apparatus for 5min and the ambulation, rearing and self-grooming were noted.[17]

Anxiety was measured by elevated Plus-maze test. The maze had two opposite open arms (50×10cm^2), crossed with two opposite enclosed arms of the same dimension but having 40 cm high walls. The arms were connected with a central square, 10×10cm^2, giving the apparatus shape of a plus sign. The whole maze was elevated to a height of 50cm. Naïve rats were placed individually in the center of the maze, facing an open arm. Thereafter, number of entries and time spent on the open and enclosed arm were recorded during the next 5 min and compared with control behaviour.[18]

Sexual behavior study was performed by placing a male rat in a wooden-box in a dimly lit room for 10min with 2 oestronized female rats (sequentially treated with oestradiol valerate 5µg/rat, followed 48h later by hydroxyprogesterone 1.5mg/rat,s.c.). The parameters observed were the latency to initiate licking of genitalia, the total number of mounts and intromissions.[19]

After completion the behavioral study the animals were sacrificed. The stomach was opened and the gastric ulcer formation was scored[20] and the weight of spleen and adrenal gland was taken using electronic balance.[21] Further, plasma corticosteroid was estimated spectroflurometrically.[22] In brief, serum was mixed with dichloromethane and the non-polar layer was mixed with acid alcohol. The reading was taken at 530 nm with activation at 470 nm. The concentration was expressed as µg/100 ml of blood.

The results were expressed as mean±SEM of two independent experiments. The statistical significance was determined by One-Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison tests. P<0.05 was considered to be statistically significant.

RESULTS

In the present study, CS resulted marked behavioral changes such as enhanced ambulation and rearing responses but lowered self-grooming activities in rats. While, rats pretreated with PTE and WSE significantly and dose dependently lowered open field ambulation, rearing, and increased self-grooming in comparison to CS rats (Table-1).

In the present study, it was observed that CS produced fear responses and anxiety in rats and due to anxiousness, open arm entries and time spent in open arm were declined to normal control. Treatment with PTE and WSE induced significant increases in the both the number of entries and time spent in the open arms, while, the number of entries and time spent in the closed arms were declined (Table-2).

It is evident from the present study that CS significantly inhibited the male sexual response indices, inducing an increase in latencies in licking female genitalia with decrease in the number of mounting and intromission. PTE (50-400 mg/kg) reversed these changes dose dependently. This effect was also noted with WSE (100 mg/Kg) using the same paradigm of CS in rats (Table-3).

CS induced ulceration in stomach, hypertrophy of spleen and adrenal glands and enhanced level of plasma corticosteroids (Table-4). PTE (50-400 mg/kg) inhibited CS induced gastro-duodenal ulcerations and plasma corticosterone level significantly and dose dependently and also negated the hypertrophy of spleen and adrenal glands (Table-4). WSE also corroborated these findings.

Table-3: Role of PTE and WSE on sexual behaviour in foot-shock induced chronic stress in rats

	Latency (min)	Number of actions	
	Licking	Mounting	Intromission
Control	2.9±0.45	6.2±0.42	5.4±0.32
CS	7.8±0.61(a)***	1.3±0.18(a)***	0.7±0.15(a)***
CS+PTE (50 mg/kg)	6.9±0.88(b)	1.8±0.24(b)	1.5±0.12(b)
CS+PTE (100 mg/kg)	5.3±0.22(b)**	2.6±0.14(b)	2.1±0.19(b)
CS+PTE (200 mg/kg)	5.0±0.47(b)***	3.4±0.26(b)***	2.6±0.16(b)*
CS+PTE (400 mg/kg)	4.8±0.59(b)***	3.8±0.32(b)***	2.8±0.14(b)***
CS+WSE (100 mg/kg)	3.5±0.86(b)***	4.8±0.38(b)***	3.8±0.17(b)***

Results were Mean±SEM with n=10 in each group. All the groups were compared statistically by ANOVA followed by Newman-Keul's multiple comparison tests using statistical software (spssV11.5). *P<0.05, **P<0.01 and ***P<0.001 and (a) and (b) means when compared to control and CS group correspondingly. CS= Chronic foot shock stress (2 mA) daily 1 h for 14 daysPTE= 70.0% hydroethanolic *P. tuberosa* tuberous root extract; WSE= 70.0% hydroethanolic *W. somnifera* rhizome extract

DISCUSSION

Acute or short duration of stress appears to have limited aversive effects on the individual since the body sets in motion or array of physiological, biochemical and endocrine responses to counter stress effects. However, chronic and excessive stress, and the inability of the organism to cope with the stress, appears to induce the syndromal state described by Syle in 1936. As such, a workable model of experimental stress must incorporate the factors of chronic, unpredictability and the inability to escape from the stressor. The experimental model used in this study (*i.e.,* chronic foot shock) fulfilled these criteria and showed in earlier studies, significant neurobehavioral, neurophysiological and neuropathological perturbations that could be attenuated by putative anti-stress agents.[23-24]

Behavioral psychopharmacology deals with the evaluation of new psychotropic drugs. The open field test and plus maze test are designed to measure behavioral responses such as locomotor activity, hyperactivity, and exploratory behaviors. Plus maze and Open field are also used as a measure of anxiety.[17,25] In the present study, CS created marked behavioral changes such as enhanced ambulation and rearing responses, while lowered self-grooming activities in rats. All these behavioral activities noted in CS rats were due to alteration of neurophysiological responses related to stress and anxiety. While, rats pretreated with PTE and WSE significantly and dose dependently lowered open field ambulation, rearing, and increased self-grooming in comparison to foot shock induced stressed rats. It might be either due to faster habituation to the novel environment or due to reducing their physiological responses to anxiety and stress.

Further, we observed that CS produced fear responses and anxiety in rats and due to anxiousness; open arm entries and time spent in open arm were declined, compared to normal control. Treatment with PTE and WSE induced significant increases in the both the number of entries and time spent in the open arms, while, the number of entries and time spent in the closed arms were declined than CS rats. The results obtained in this study suggested that the tuberous root of *P tuberosa* possess anxiolytic activities.

Stress was reported to induce significant suppression in male sexual function.[19] In the present study also, CS significantly inhibited the male sexual response indices, documenting an increase in latencies in licking female genitalia with a decrease in the number of mounting and intromission. PTE and WSE reversed these changes. This effect has been reported earlier with WSE using the same paradigm of CS in rats.[26] Stress has been found to profoundly inhibit reproductive functions by affecting various components of the hypothalamic-pituitary-adrenal (HPA) axis. Tuber root of *P tuberosa* has often been mentioned as aphrodisiacs that promote normal sexual function.[27] The present study also indicated that *P. tuberosa* not only influence normal male sexual behavior but also normalize stress-induced male sexual dysfunction.

Activation of HPA axis during stress is a well-known phenomenon, that increases plasma corticosterone in rodents and cortisol in human. It can be utilized as markers of stress and its intensity.[28,29] Similarly, production of gastro-duodenal ulcerations appears to be an inevitable consequence of stress and the intensity of the disease depends upon the duration of stressful situation and appears to involve stress-induced autonomic and neuroendocrine system activation.[15] CS induced ulceration in stomach, hypertrophy of spleen and adrenal glands and enhanced level of plasma corticosteroids. PTE and WS treatment inhibited CS

Table-4: Role of PTE and WSE on some physical and chemical parameters in foot-shock induced chronic stress in rats

	Ulcer Score	Adrenal gland (mg/100g)	Spleen (mg/100g)	Plasma Corticosteroids (µg/dl)
Control	-	18.05±1.06	0.315±0.04	30.15±1.20
CS	20.16±2.15	27.95±2.54(a)***	0.496±0.05(a)***	72.38±2.74(a)***
CS+PTE (50 mg/kg)	14.50±1.98(b)	24.18±2.03(b)	0.426±0.03(b)	64.52±2.40(b)
CS+PTE (100 mg/kg)	13.40±1.12(b)*	23.79±2.14(b)*	0.407±0.04(b)	58.29±2.27(b)*
CS+PTE (200 mg/kg)	11.70±1.44(b)***	21.44±2.01(b)**	0.388±0.06(b)*	42.18±2.10(b)**
CS+PTE (400 mg/kg)	9.15±1.35(b)***	20.82±1.98(b)**	0.371±0.04**	39.46±2.08(b)***
CS+WSE (100 mg/kg)	7.25±1.42(b)***	19.35±1.08(b)***	0.340±0.04***	36.85±1.98(b)***

Results were Mean±SEM with n=10 in each group. All the groups were compared statistically by ANOVA followed by Newman-Keul's multiple comparison tests using statistical software (spssV11.5). *P<0.05, **P<0.01 and ***P<0.001 and (a) and (b) means when compared to control and CS group correspondingly. CS= Chronic foot shock stress (2 mA) daily 1 h for 14 daysPTE= 70.0% hydroethanolic *P. tuberosa* tuberous root extract; WSE= 70.0% hydroethanolic *W. somnifera* rhizome extract

induced elevation of plasma corticosterone level significantly. Further, it was noted that PTE and WSE has the capability to protect CS induced gastroduodenal ulcerations significantly and dose dependently. Weights of adrenal glands and spleen were increased significantly in CS group, compared to unstressed control group. PTE treatment negated the hypertrophy of both the glands significantly. All these findings clearly indicated that PTE and WSE posses potential anti-stress activity.

The present study supports the contention because chronic foot-shock stress of unpredictable and inescapable nature were found to induce a wide range of physiological malfunctions, rise of anxiety level, fall of male sexual indices, and behavioral alterations: all of which inhibited by WSE, a known adaptogen and PTE, a putative adaptogen belonging to the class of Ayurvedic medicine. In this context the study demands more focus on regulation of HPA axis which give us the anxiolytic, antistress and neuroprotective properties of PTE.

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