Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20150921010411

Original Research

ScopeMed

Long lasting preventive effects of piperlongumine and a *Piper longum* extract against stress triggered pathologies in mice

Vaishali Yadav[1], Shyam Sunder Chatterjee[2], Muhammed Majeed[3], Vikas Kumar[1]

[1]Department of Pharmaceutics, Neuropharmacology Research Laboratory, Indian Institute of Technology, Banaras Hindu University, Varanasi, India, [2] Head of the Pharmacological Research Laboratories, Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany, [3]Sami Labs Limited, Bengaluru, Karnataka, India

Address for Correspondence:
Vikas Kumar, Department of Pharmaceutics, Neuropharmacology Research Laboratory, Indian Institute of Technology, Banaras Hindu University, Varanasi - 221 005, India.
Phone: +91 542 6702742,
Fax: +91 542 2368428,
E-mail: vikas.phe@iitbhu.ac.in

Received: August 07, 2015
Accepted: September 07, 2015
Published: November 05, 2015

ABSTRACT

Aim: To compare doxycycline (DOX) such as oral efficacies of piperlongumine (PL) and a *Piper longum* fruits extract (PLE) as stress resistance inducers. **Materials and Methods:** Efficacies of oral pretreatments with 5 mg/kg PL or PLE or of 50 mg/kg DOX for 10 consecutive days against stress resistance were compared. Mice in treated groups were subjected to a stress induced hyperthermia on the 1st, 5th, 7th, and 10th day. Treated mice were then subjected to tail suspension test on the 11th day. Alteration in body weights, core temperatures, and gastric ulcers triggered by occasional exposures to foot shocks were determined. **Results:** DOX like long-lasting protective effects of PL and PLE against gradual alterations in body weights, basal temperatures and transient hyperthermic responses triggered by foot shocks during the post-treatment days were observed. Altered responses of stressed mice in tail suspension test observed 1 day after the last foot-shock exposures and gastric ulcers and other pathologies quantified 1 day after the test were also suppressed in PL or PLE or DOX pretreated groups. **Conclusion:** PL and crude PLE are DOX like long-acting desensitizers of stress triggered co-morbidities. Reported observations add further experimental evidences justifying traditionally known medicinal uses of *P. longum* and other plants of the Piperaceae family, and reveal that PL is also another very long acting and orally active inducer of stress resistance. Efforts to confirm stress preventive potentials of low dose plant-derived products enriched in PL or piperine like amide alkaloids in volunteers and patients can be warranted.

KEY WORDS: Foot-shock stress, hyperthermia, gastric ulcer, *Piper longum*, piperlongumine, piperine, prophylaxes

INTRODUCTION

Piper longum L. is a plant of the Piperaceae family, the roots and fruits of which are often used in Ayurvedic and other traditionally known systems of medicine for prevention and cure of chronic diseases commonly associated with or caused by mental health problems [1,2]. The plant is native in Indo-Malesian region and in Sri Lanka. In India, it is widely distributed in northeastern regions such as Assam, Arunachal Pradesh, Meghalaya, Manipur, and in some parts of the lower hills of West Bengal, Tamil Nadu and evergreen forests of Western Ghats [1]. Traditionally, the roots and fruits of *P. longum* are used as carminative, tonic to the liver, stomachic, emmenagogue, abortifacient, and aphrodisiac [1,2]. Like numerous other plants of this family, *P. longum* is also a rich source of structurally diverse amide-alkaloids with pungent taste and broad spectrums of therapeutically interesting bioactivities [3]. Piperlongumine (PL)is one such amide-alkaloid now attracting considerable attention of modern researchers interested in identifying novel therapeutic leads from secondary plant metabolites [4]. However, most *P. longum* extracts (PLE)now widely used in modernized versions of Ayurvedic formulations are still analytically standardized, or characterized, by their contents of another quantitatively major amide-alkaloid piperine [5,6].

Although piperine was first isolated from *P. nigrum* (black and white piper)[7], and it is also the quantitatively major pungent tasting amide alkaloid of *P. longum* fruits [2].

PL and piperine are structurally analogous molecules [Figure 1], and both of them possess therapeutically interesting anti-mitotic and antimicrobial activities [8,9]. It is now evident that gut microbiota play a crucial role in regulating physiological stress responses [10,11], and that appropriate doses and treatment regimen of antibiotics and other agents with modulating effects on gut microbial ecology can have health benefits [12,13]. Doxycycline (DOX)is one such extensively studied and clinically used antibiotic with stress and neurohormonal status regulating [14], and gastric ulcer protective, anticonvulsant, antidepressant, neuroprotective, and other therapeutically interesting bioactivities [15-18]. Therefore, efforts are now being made in our laboratories to compare DOX like stress response suppressing efficacies of PL a medicinally used PLE.

Results of the very first experiments (under publication)have revealed that ten daily oral doses of 5 mg/kg/day PL or of a medicinally used PLEstandardizes on its piperine contents (1.75%)are high enough for observing their DOX like stress response suppressing effects after their 10 daily doses. Results of a further experiment conducted to verify their longer lasting preventive potentials against chronic mild stress triggered gastric ulcers and other pathologies will be described and discussed in this study.

MATERIALS AND METHODS

Animals

Adult male swiss albino mice (25 ± 5 g)were from Central Animal House of Institute of Medical Sciences, Banaras Hindu University (Registration Number: 542/AB/CPCSEA). They were acclimatized to laboratory conditions for 1 week before starting the experiment. Six animals were used in each group, and all experimental groups were housed in polypropylene cages (28 cm × 19 cm × 12.5 cm)with saw dust beddings and free access to standard rodent diet and tap water. They were maintained at 25°C ± 1°C ambient temperature and relative humidity of 50% ± 10% with 12:12 h light and dark cycle (light on at 06:00 and off at 18:00)and were acclimatized to the laboratory conditions for a week before performing the experiment. Principles and guidelines of laboratory animal care (NIH publication 85-23, revised in 1985)were always followed, and before start of the experiments an approval from Central Animal Ethical Committee of the University was obtained (Dean/2014/CAEC/729, dated August 07, 2014).

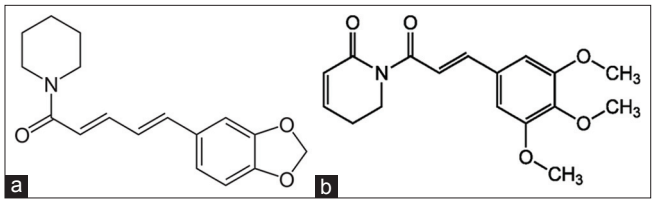

Figure 1: Structure of (a) Piperine and (b) Piperlongumine

Plant Extracts, Drugs and Chemicals

The methanolic extract of *P. longum* fruits analytically characterized to contain 1.75% piperine and almost pure PL (99.33%)isolated from *P. longum* roots used in this study and analytical data on them were generously supplied by Sami Labs Limited Bangalore, India. PLE is a methanolic extract of dried *P. longum* fruits, and purity of PL and piperine contents of the PLE sample used were established by high performance liquid chromatography equipped with ultraviolet/photodiode array detector and using acetonitrile and water as mobile phase.

DOX was acquired from Sigma Aldrich, Bengaluru, India; carboxymethyl cellulose (CMC)from Central Drug House, Delhi, India. All other chemicals and reagents used in this study were of highest purity commercially available in India.

Animal Grouping and Drug Administration

Six randomly selected mice groups were used in this study. Except for the animals of the one of the groups serving as reference (Group: REF), all others were subjected to foot shock stress triggered hyperthermia test. The REF groups and a control group (Group: CON-CMC)were not given any oral treatments. The other four groups were treated daily only on the first 10 days of the experiment either with 0.3% CMC (Group: CON + CMC), or 50 mg/kg/day DOX (Group: DOX), or 5 g/kg daily doses of PL (Group: PL)or PLE (Group: PLE), and on days 10, 15, 17, and 20 of the experiment all animals of these groups were subject to the foot shock stress triggered hyperthermia test describe later. For oral administrations, the test substances were suspended in 0.3% CMC, and oral application volumes were always 10 mg/kg/day, and basal core temperatures and body weights of all groups were recorded on the 1 h before the tests on all observational days. Further details of the experiment are graphically summarized in Figure 2.

Foot Shock Stress Induced Hyperthermia Test

This test was conducted by placing an individual mouse of a group in a black box (24 cm × 29 cm × 40 cm)with a grid floor for 1 min, when foot shocks through the grid floor (2 mA, 50 Hz of 2 ms duration)was delivered. Five consecutive foot shocks of 2 mA at 10 s intervals were given after the animals had stayed in the cage for 10 s. At the end of the minute, the animals were placed back in their home cages, and 10 min thereafter their rectal temperatures were recorded again by using a digital thermometer and a digital probe [19]. Calculated differences between this and the basal core temperature of a mouse recorded one hour before was used as an index for stress induced hyperthermic response of the animal. The animals of the reference group were not subjected to foot shock stress, but were also placed in the black box for a min, and their

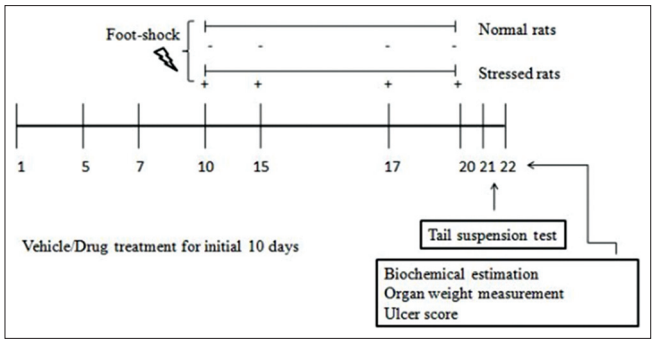

Figure 2: Summary of the experimental methods used

rectal temperatures were recorded again 10 min after they were returned to their home cage.

Tail Suspension Test

The test procedure described elsewhere was used [20]. In short, an individual mouse of a group was hung by tail, 50 cm above the floor by an adhesive tapes placed 1 cm from the tip of the tail on a wire in an upside down posture. After initial vigorous movements, the mouse assumed an immobile posture and the period of immobility during a 5 min observation period was noted. All animals of all experimental groups were subjected to this test on day 21 of the experiment.

Plasma Glucose, Insulin and Cortisol Level, Organs Weights and Stomach Ulcer Scoring

Immediately after last temperature measurements on the 22nd day of the experiment, all animals were sacrificed by decapitation. Their blood samples from eye orbital puncture was collected in ethylenediaminetetraacetic acid coated tubes kept in ice and centrifuged at 1000 × g for 20 min at 4°C to separate plasma (Compufuge CPR-30 Plus, with Rotor No. 8; REMI, India). Plasma glucose levels were quantified by an enzymatic test kit (ERBA diagnostics Mannheim GmbH, Germany). Plasma insulin levels were quantified by using Enzyme-Linked Immunosorbent Assay (ELISA)test kit (Chemux BioSciences, Inc., USA), and plasma cortisol by using ELISA kit (DSI S.r.l., Italy). All biochemical estimations were done in a absorbance micro-plate reader (iMarkTM- Bio-Rad Laboratories, California, USA)according to instructions manual of respective enzyme test kits. Immediately after blood collections, adrenal glands, and spleen of the animals were dissected out and washed under slowly running tap water. After removing adhered water using filter papers and both the organs were weighed [21].

For stomach ulcer scoring, cardiac end of the stomach was dissected out and the contents were drained out. Thereafter, the stomach was cut and opened along with its greater curvature, and washed slowly under running tap water. After washing, stomachs were spread and fixed on a glass slide for scoring ulcers (under ×10 magnification). The ulcer index was evaluated according to their severity and scored as follows: 0 = normal colored stomach, 0.5 = red coloration, 1 = spot ulcers, 1.5 = hemorrhagic streaks, 2 = ulcer > 3 mm but < 5 mm, 3 = ulcers >5 mm [22].

Statistical Analysis

Means ± standard errors of means were calculated for the observed values in each experimental group. Statistical analysis was done by one-way Analysis of Variances (ANOVA)followed by Student Newman Keuls multiple comparison tests. When stated, two-way ANOVA followed by Bonferroni *post-hoc* test and *t*-test were performed. GraphPad Prism-5 (GraphPad Software, Inc. La Jolla, California, USA)and Origin-Pro 8 (OriginLab Corporation, Massachusetts, USA)software were used for statistical analysis and drawing graph. $P < 0.05$ were considered as statistically significant.

RESULTS

Body Weight and Basal Rectal Temperature

Mean body weights of all experimental groups increased slightly during first 10 days of the experiment [Figure 3a]. However, from the 15th experimental days onward, animals of both the control groups (CON + CMC and CON-CMC) continuously lost their body weights, which were not observed in the reference group [Figure 3b]. Mean body weights of the DOX, or PL of PLE treated groups remained almost constant until they were subjected to three foot shock stress sessions on the 10th, 15th and 17th day of the experiment, and thereafter the mean body weights of all these three groups continued to increase steadily.

Results summarized in the Figure 4a and b revealed that the mean basal core temperatures of the reference groups remained almost constant on all observational days, with a tendency to increase slightly during the course of the experiment. Until the 17th day observational day, basal core temperatures of all other groups also remained within the normal range of the mice colony used in the experiment (36.3-36.6°C), but also tented to increase continuously. Mean basal core temperatures of both the control groups (CON + CMC and CON-CMC)continued to increase further (but still remained within physiological range)till the last day of the experiment, whereas from the 17th day onward, mean basal core temperatures of the DOX, PL and PLE treated groups continued to decrease steadily toward the mean vales of all groups observed on the 1st day of the experiment.

Mean ratios of body weight and basal core temperature of the reference group continued to increase steadily till the 21st observational day, i.e. until, they were subjected to tail suspension test for anti-depressants. However on the next day, this mean value of the group was almost equal to that calculated for the group on the 10th day of the experiment [Figure 5a and b]. During the ten oral treatment days, mean ratios of body weight and basal temperature of the reference and both control groups followed similar increasing trend [Figure 5a]. From the 10th day onward [Figure 5b], i.e., after the animals were first subjected to foot shock stress, this mean value steadily decreased in the both

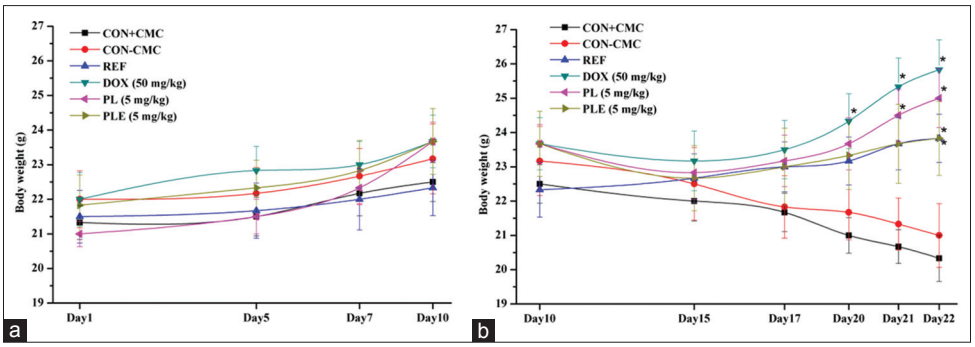

Figure 3: Effect of occasional stress on body weight of male mice treated with piperlongumine and Piper longum fruits extract on day 1-10 (a) and day 10-22 (b) of experiment. Abbreviations: PL: Piperlongumine, PLE: Piper longum fruits extract, DOX: Doxycycline, CMC: Carboxymethyl cellulose suspension and REF: Reference group. Values are mean ± standard error of mean (n = 6). * denotes statistically significant difference (two-way Analysis of Variance followed by Bonferroni post-hoc test) relative to CON + CMC group (*P < 0.05).

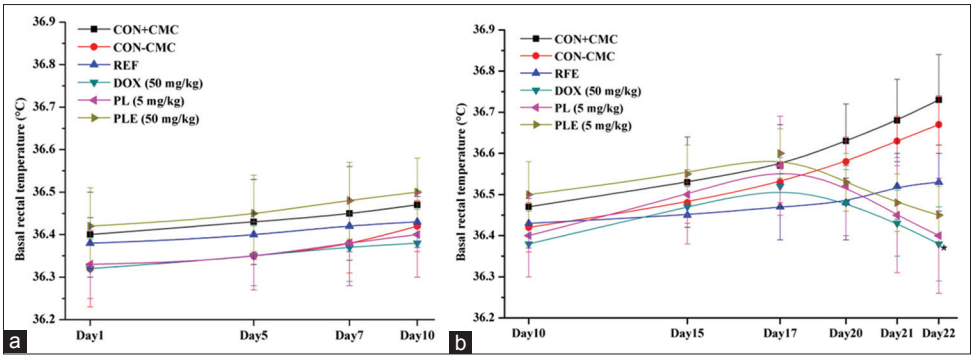

Figure 4: Effect of occasional stress on basal rectal temperatures of male mice treated with piperlongumine and Piper longum fruits extract on day 1-10 (a) and day 10-22 (b) of experiment. Values are mean ± standard error of mean (n = 6). *denotes statistically significant difference (two-way Analysis of Variance followed by Bonferroni post-hoc test) relative to CON + carboxymethyl cellulose group (*P < 0.05).

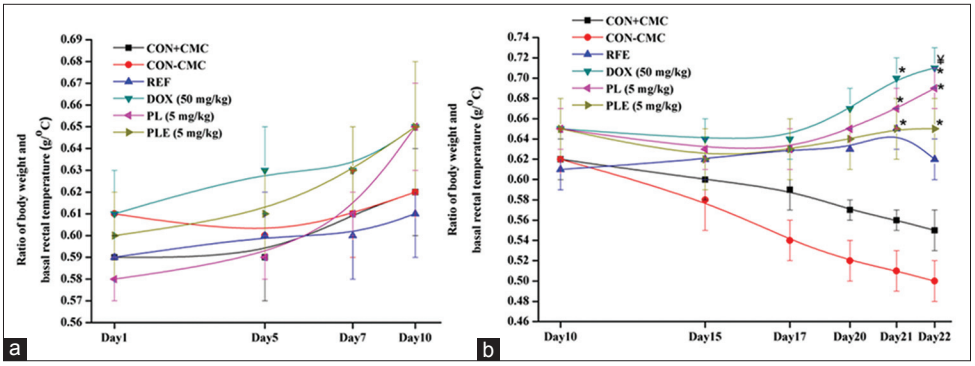

Figure 5: Effect of stress on ratio of body weight and basal rectal temperature of male mice treated with and Piper longum fruits extract on day 1-10 (a) and day 10-22 (b) of experiment. Values are mean ± standard error of mean (n = 6). *denotes statistically significant difference (two-way ANOVA followed by Bonferroni post-hoc test) relative to CON + carboxymethyl cellulose group (*P < 0.05). ¥denotes statistically significant difference (two-way ANOVA followed by Bonferroni post-hoc test) relative to reference (¥P < 0.05)

the control group, whereupon the decrease rate of the CMC treated one was less steeper than the other one not receiving any oral treatments. This ratio of the DOX or PLE treated groups decreased somewhat or remained almost constant till 17th day of the experiment. There after they continued to increase until the last day of the experiment. This slightly elevated energy balance toward higher growth rates observed in the DOX treated group during the 10 treatment days was quite analogous to those of the PLE or PL treated groups, and the protective effects of DOX against stress triggered alterations in growth rate were also somewhat higher than those of PL or PLE.

Foot Shock Stress Induced Transient Hyperthermia

The magnitude of transient hyperthermic response in the REF group observed on day 10th (i.e. the last treatment day) and subsequent observational days remained almost constant and within normal physiological range. It is apparent from the

Figure 6 that there was constant elevation in stress induced hyperthermic response in both the CON + CMC and CON-CMC groups, whereas the magnitude of this response in the groups treated with PL, PLE, or DOX tended to decrease on the 15th and subsequent observational days. Quantitatively, these preventive effects of ten 5 mg/kg/day PLE or PL oral doses were almost identical to that of similar treatments with 50th mg/kg/day DOX.

Tail Suspension Test

Mean immobility period of both the control groups (CON + CMC and CON-CMC)were almost identical and higher than that of the reference group not subjected to foot shock stress. These mean values of the PLE, PL, or DOX treated groups were statistically significantly lower than that of the reference group, whereupon that of the DOX treated one was the lowest. Although the mean value of the PLE pretreated group was numerically slightly higher than the PL pretreated one, these two values were not statistically significantly different from each other. The results are summarized in Figure 7.

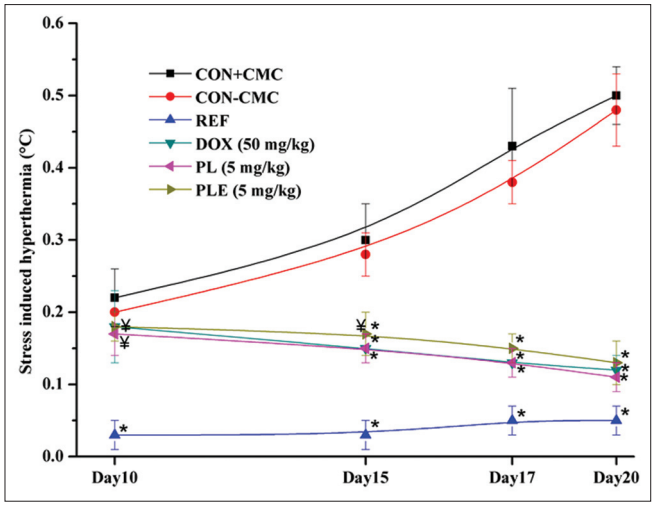

Figure 6: Stress induced hyperthermia of male mice treated with piperlongumine and Piper longum fruits extract. Values are mean ± standard error of mean (n = 6). *denotes statistically significant difference (two-way Analysis of Variance [ANOVA] followed by Bonferroni post-hoc test) relative to CON + carboxymethyl cellulose group (*P < 0.05). ¥denotes statistically significant difference (two-way ANOVA followed by Bonferroni post-hoc test) relative to Reference (¥P < 0.05)

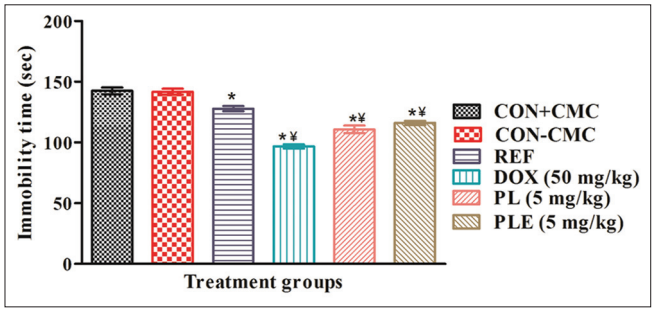

Figure 7: Effect of piperlongumine and Piper longum fruits extract on tail suspension test in male mice. Abbreviations: Values are mean ± standard error of mean (n = 6). * denotes statistically significant difference (One-way Analysis of Variance [ANOVA] followed by Student's t-test) relative to CON + carboxymethyl cellulose group (*P < 0.05). ¥denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to REF (¥P < 0.05)

Plasma Glucose, Insulin, and Cortisol Levels

Mean plasma glucose and cortisol levels of both the control groups were significantly higher than those of the reference group [Table 1]. These values of the PLE, PL, or DOX treated ones were significantly lower than that of the CMC treated stressed control group, but were also higher than those of the unstressed reference group. Although mean plasma insulin levels of the reference and the three drugs treated groups were higher than both the control groups, there were no statistically significant differences between these and the mean values of the control groups.

Organ Weights and Gastric Ulcers

These results are summarized in Table 2. As compared to the corresponding mean values of the reference group, the absolute as well as the relative mean weights of the adrenal glands of the both the control groups were significantly higher, whereas those of the spleen significantly lower [Table 2]. Such adrenal gland hypertrophy and spleen hypotrophy observed in control groups were less pronounced in all drugs treated groups. Gastric ulcers observed in both the stressed control groups were not observed in the reference or the DOX pretreated groups, and the mean ulcer index of the PL or PLE treated groups were much lower than the CMC treated control group (ca. 90% protection).

DISCUSSION

The bioassay procedure used in the experiment is a slightly modified version of the one now often used in our laboratories for estimating pharmacologically interesting doses ranges of stress response suppressing herbal extracts and their bioactive constituents [23-25]. Using this and analogous bioassays in our laboratories and elsewhere, it has often been observed that pharmacological observations made after acute doses of plant

Table 1: Effect of PL and PLE on plasma glucose level in male mice plasma cortisol level and plasma insulin level in male mice

Treatment groups	Glucose	Insulin	Cortisol
CON+CMC	113.08±2.07¥	10.82±2.56	105.09±2.36¥
CON-CMC	110.88±0.93¥	9.52±2.28	104.00±3.24¥
REF	85.68±2.04*	16.95±1.68	83.49±1.76*
DOX (50 mg/kg)	92.41±2.01*¥	12.78±1.63	94.95±1.84*¥
PL (5 mg/kg)	97.74±1.57*¥	14.03±1.25	102.70±1.66¥
PLE (5 mg/kg)	103.24±1.61*¥	16.21±1.34	102.77±1.91¥

Values are mean±SEM (n=6). *Denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to CON+CMC group (*P<0.05). ¥denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to REF group (¥P<0.05), REF: Reference, ANOVA: Analysis of Variance, CMC: Carboxymethyl cellulose, SEM: Standard error of mean, PLE: *Piper longum* fruits extract, PL: Piperlongumine, DOX: Doxycycline

Table 2: Effect of PL and PLE on the weights of adrenal glands, spleen and gastric ulceration index in mice

Treatment groups	Absolute organ weight (mg)		Relative organ weight (mg/g of body weight)		Mean ulcer index	% inhibition
	Adrenal glands	Spleen	Adrenal glands	Spleen		
CON+CMC	23.67±0.99$^{¥}$	64.17±1.17$^{¥}$	1.16±0.03$^{¥}$	3.16±0.67$^{¥}$	2.33±0.21$^{¥}$	-
CON-CMC	22.17±0.70$^{¥}$	62.67±0.92$^{¥}$	1.06±0.04$^{¥}$	2.98±0.25$^{¥}$	2.50±0.22$^{¥}$	-
REF	13.83±0.48*	143.33±1.14*	0.58±0.05*	6.01±0.62*	0.00±0.00*	100
DOX (50 mg/kg)	15.50±0.43*	144.50±1.34*	0.60±0.03*	5.59±0.34*	0.00±0.00*	100
PL (5 mg/kg)	17.33±0.42*$^{¥}$	125.17±1.01*$^{¥}$	0.69±0.04*	5.01±0.46*	0.17±0.11*	92.7
PLE (5 mg/kg)	19.17±0.31*$^{¥}$	112.00±1.29*$^{¥}$	0.80±0.03*$^{¥}$	4.70±0.16	0.25±0.11*	89.27

Values are mean±SEM (n=6). *Denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to CON+CMC group (*P<0.05). $^{¥}$denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to REF group ($^{¥}P$<0.05), REF: Reference, ANOVA: Analysis of Variance, CMC: Carboxymethyl cellulose, SEM: Standard error of mean, PLE: *Piper longum* fruits extract, PL: Piperlongumine, DOX: Doxycycline

extracts and their bioactive constituents are not very predictive of their medicinal values traditionally known to the scholars and practitioners of traditionally known systems of medicine [26,27]. However, the therapeutically important question concerning their treatment regimen and durations of actions still remains unanswered. Since like aspirin and numerous other covalently binding drugs [28], PL, piperine and other α, β-unsaturated alkyl amides bind covalently to their biological targets [29], we speculated that durations of actions of PLE and pure PL should be longer than predictable from their biological half-lives. However, the results of the reported experiment revealed that 1 h after their 10 daily oral doses neither of them had any significant effects in the stress induced hyperthermia test and also had no significant effects on body weight gains or on basal core temperatures of the animals during the treatments. However, several days after pretreatments, both of them afforded protections against body weights losses and slight elevation in basal core temperature triggered by repeated exposures to foot shock stress, as well as stress induced transient hyperthermia. These observations strongly suggest that both PLE and PL are DOX like very long acting stress resistance inducers with growth promoting effects in stressed animals only. Although these observations could also indicate that PL is somewhat more effective than PLE as growth promoter or stress response desensitizers, further dose response studies with PL, PLE and other types of PLE will be necessary to reconfirm this possibility.

It was interesting to note though that in the tail suspension test for antidepressants conducted 11 days after the pretreatments both PLE and PL had DOX such as effects in stressed mice, and that protective effects of their 10 fairly low doses (5 mg/kg/day)against stress triggered adrenal gland hypertrophy, spleen hypotrophy, gastric ulcers, as well as plasma glucose, and cortisol levels persisted 12 days after their last oral dose. These observations strongly suggest that their observed stress resistance promoting effects are most probably due to their very longer lasting effects on glucose and cortisol homeostasis, and that like DOX both of them are desensitizers of stress triggered physiological responses regulating not only body weights and core temperatures, but also the functions of the central nervous system involved in thermoregulation and depressive state of male mice.

Stress affects food intake in a bidirectional way in both animals and human, and depending on stress intensity and environmental factors stress triggered responses can induce body weight changes accompanying metabolic disorders and co-morbid mental health problems [30-33]. Abnormal body weight gains or losses are the most apparent symptoms of mal- or over-nutrition triggered health problems, and abnormal thermoregulation is a common symptom of almost all systemic inflammatory diseases [34]. Medicinal uses of *P. longum* fruits and roots for prevention and cure of such diseases have since long been known to the scholars and practitioners of traditionally known systems of medicine and for such purposes regular intake of their relatively low oral doses are recommended. Our observations not only justify such medicinal uses of the plant, but also strongly suggest that traditionally known medicinal uses of numerous plant derived products enriched in PL and structurally analogous alkyl amides is mainly due to their ability to promote resistance against chronic unavoidable stress.

CONCLUSION

Appropriate uses of the stress biomarkers quantified in this study are easily quantifiable ones not only for estimating pharmacologically interesting dose ranges of adaptogenic herbs and their bioactive constituents, but also for estimating their durations of actions. PL is another such bioactive secondary plant metabolite of the Piperaceae family.

REFERENCES

1. Manoj P, Soniya EV, Banerjee NS, Ravichandran P. Recent studies on well-known spice, *Piper longum* Linn. Nat Prod Radiance 2004;3:222-7.
2. Kumar S, Kamboj J, Suman, Sharma S. Overview for various aspects of the health benefits of *Piper longum* Linn. fruit. J Acupunct Meridian Stud 2011;4:134-40.
3. Boonen J, Bronselaer A, Nielandt J, Veryser L, De Tré G, De Spiegeleer B. Alkamid database: Chemistry, occurrence and functionality of plant N-alkylamides. J Ethnopharmacol 2012;142:563-90.
4. Bezerra DP, Castro FO, Alves AP, Pessoa C, Moraes MO, Silveira ER, *et al*. *In vivo* growth-inhibition of Sarcoma 180 by piplartine and piperine, two alkaloid amides from Piper. Braz J Med Biol Res 2006;39:801-7.
5. Hamrapurkar PD, Jadhav K, Zine S. Quantitative estimation of piperine in *Piper nigrum* and *Piper longum* using high performance thin layer chromatography. J Appl Pharm Sci 2011;1:117-20.
6. Qu H, Lv M, Xu H. Piperine: Bioactivities and structural modifications. Mini Rev Med Chem 2015;15:145-56.
7. Szallasi A. Piperine: Researchers discover new flavor in an ancient spice. Trends Pharmacol Sci 2005;26:437-9.

8. Reddy SP, Jamil K, Madhusudhan P, Anjani G, Das B. Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*. Pharm Biol 2001;39:236-8.
9. Bezerra DP, Pessoa C, de Moraes MO, Saker-Neto N, Silveira ER, Costa-Lotufo LV. Overview of the therapeutic potential of piplartine (*Piper longumine*). Eur J Pharm Sci 2013;48:453-63.
10. Dinan TG, Cryan JF. Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology. Psychoneuroendocrinology 2012;37:1369-78.
11. Thakur AK, Shakya A, Husain GM, Emerald M, Kumar V. Gut microbiota and mental health: Current and future perspectives. J Pharmacol Clin Toxicol 2014a;2:1016.
12. Arnold DL, Jackson RW, Waterfield NR, Mansfield JW. Evolution of microbial virulence: The benefits of stress. Trends Genet 2007;23:293-300.
13. Dicks LM, Botes M. Probiotic lactic acid bacteria in the gastro-intestinal tract: Health benefits, safety and mode of action. Benef Microbes 2010;1:11-29.
14. Wang Y, Kasper LH. The role of microbiome in central nervous system disorders. Brain Behav Immun 2014;38:1-12.
15. Singh LP, Mishra A, Saha D, Swarnakar S. Doxycycline blocks gastric ulcer by regulating matrix metalloproteinase-2 activity and oxidative stress. World J Gastroenterol 2011;17:3310-21.
16. Mello BS, Monte AS, McIntyre RS, Soczynska JK, Custódio CS, Cordeiro RC, *et al.* Effects of doxycycline on depressive-like behavior in mice after lipopolysaccharide (LPS)administration. J Psychiatr Res 2013;47:1521-9.
17. Cho Y, Son HJ, Kim EM, Choi JH, Kim ST, Ji IJ, *et al.* Doxycycline is neuroprotective against nigral dopaminergic degeneration by a dual mechanism involving MMP-3. Neurotox Res 2009;16:361-71.
18. Wang DD, Englot DJ, Garcia PA, Lawton MT, Young WL. Minocycline- and tetracycline-class antibiotics are protective against partial seizures *in vivo*. Epilepsy Behav 2012;24:314-8.
19. Zethof TJ, Van der Heyden JA, Tolboom JT, Olivier B. Stress-induced hyperthermia as a putative anxiety model. Eur J Pharmacol 1995;294:125-35.
20. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology (Berl)1985;85:367-70.
21. Salman TM, Alagbonsi IA, Biliaminu SA, Ayandele OA, Oladejo OK, Adeosun OA. Blood glucose-lowering effect of *Telfairia occidentalis:* A preliminary study on the underlying mechanism and responses. Biokemistri 2013;25:133-9.
22. Govindani H, Dey A, Deb L, Rout SP, Parial SD, Jain, A. Protective role of methanolic and aqueous extracts of *Cucurbita moschata* Linn. fruits in inflammation and drug induced gastric ulcer in wister rats. Int J Pharm Tech Res 2012;4:1758-65.
23. Langstieh AJ, Verma P, Thakur AK, Chatterjee SS, Kumar V. Desensitisation of mild stress triggered responses in mice by a *Brasssica juncea* leaf extracts and some ubiquitous secondary plant metabolites. Pharmacologia 2014;5:326-38.
24. Thakur AK, Chatterjee SS, Kumar V. Adaptogenic potential of andrographolide: An active principle of the king of bitters (*Andrographis paniculata*). J Tradit Complement Med 2014;5:42-50.
25. Thakur AK, Soni UK, Rai G, Chatterjee SS, Kumar V. Protective effects of Andrographis paniculata extract and pure andrographolide against chronic stress-triggered pathologies in rats. Cell Mol Neurobiol 2014;34:1111-21.
26. Kumar V, Chatterjee SS. Single and repeated dose effects of phytochemicals in rodent behavioural models. EC Pharm Sci 2014;1:16-8.
27. Lee J, Jo DG, Park D, Chung HY, Mattson MP. Adaptive cellular stress pathways as therapeutic targets of dietary phytochemicals: Focus on the nervous system. Pharmacol Rev 2014;66:815-68.
28. Singh J, Petter RC, Baillie TA, Whitty A. The resurgence of covalent drugs. Nat Rev Drug Discov 2011;10:307-17.
29. Sun LD, Wang F, Dai F, Wang YH, Lin D, Zhou B. Development and mechanism investigation of a new piperlongumine derivative as a potent anti-inflammatory agent. Biochem Pharmacol 2015;95:156-69.
30. Bazhan N, Zelena D. Food-intake regulation during stress by the hypothalamo-pituitary-adrenal axis. Brain Res Bull 2013;95:46-53.
31. Sinha R, Jastreboff AM. Stress as a common risk factor for obesity and addiction. Biol Psychiatry 2013;73:827-35.
32. Whitebird RR, Kreitzer MJ, Vazquez-Benitez GX, Enstad CJ, Stuck LH, O'Connor P. Improving mental health and diabetes management with mindfulness-based stress reduction. J Altern Complement Med 2014;20:A58.
33. Pandit AU, Bailey SC, Curtis LM, Seligman HK, Davis TC, Parker RM, *et al.* Disease-related distress, self-care and clinical outcomes among low-income patients with diabetes. J Epidemiol Community Health 2014;68:557-64.
34. Romanovsky AA. Do fever and anapyrexia exist? Analysis of set point-based definitions. Am J Physiol Regul Integr Comp Physiol 2004;287:R992-5.

Source of Support: Nil, Conflict of Interest: None declared.