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Desensitization of Mild Stress Triggered Responses in Mice by a *Brassica juncea* Leaf Extract and some Ubiquitous Secondary Plant Metabolites

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ABSTRACT

Background: Ayurvedic practitioners have known diverse health benefits of *Brassica juncea* since centuries. Its edible green leaves are enriched in vitamins and other health promoting phytochemicals with therapeutically interesting pharmacological properties. Available information on bioactivity profiles of hydro-alcoholic extracts of *Brassica juncea* leaves and numerous such phytochemicals, also encountered in other edible plants, suggest that they could have diverse stress response desensitising properties. Experiments designed to experimentally verify such possibilities are reported. **Method:** Effects of graded oral doses of a standardized *Brassica juncea* leaf extract and salicylic, para-hydroxybenzoic, sinapic and nicotinic acids administered for 11 consecutive days on daily handling and intermittent foot-shock stress triggered changes on body weights, core temperatures, stress induced transient hyperthermic responses, tail suspension test and pentobarbitone hypnosis in male mice were quantified. For comparison sake, diazepam or imipramine or metformin were used as reference drugs in different experiments. **Results:** Each test agent and reference drug tested had their own therapeutically interesting bioactivity profile in the bioassay and their observed effects became apparent or more pronounced after their repeated daily doses. These observations add further experimental evidences in support of the conviction that anti-stress or adaptogenic activities of drugs and phytochemicals in dictating their therapeutically interesting bioactivity profiles and that some ubiquitously present in phytochemical in almost all medicinally used herbal extracts can also contribute to their therapeutic efficacy. **Conclusion:** The bioassay system could be used for identifying bioactive constituents of almost all traditionally known adaptogenic and other herbs.

Key words: Foot-shock stress, body weight, hyperthermia, rectal temperature, immobility, pentobarbitone induced sleep

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INTRODUCTION

Brassica juncea is an edible oil producing species of mustard plant of agricultural and economic interest. Its green leaves, commonly known as, mustard green are consumed as vegetable and salad in India and many other countries around the globe. Like other vegetables and fruits, *Brassica juncea* leaves are not only rich sources of many vitamins, minerals and other nutrients essential for health maintenance but also of numerous other bioactive phytochemicals with diverse spectrums of therapeutically interesting pharmacological activities. Classical texts of Ayurveda, i.e., the oldest known traditional system of medicine still widely practiced in India and many Asiatic and other countries, mentions *Brassica juncea* leaves as healthy vegetable and medicinal properties of its oily seeds have been well documented in classical Ayurvedic texts (Manohar *et al*., 2009). Information now available on medicinal phytochemistry, pharmacology and toxicology of different parts of *Brassica juncea* and their bioactive constituents are in agreement with Ayurvedic recommendations of their nutritive and medicinal values (Kumar *et al*., 2011; Mann and Khanna, 2013; Kumar and Andy, 2012) and indicate that regular consumption of *Brassica juncea* leaves could be used for prevention of diabetes, cancer and diverse other modern life style associated chronic diseases.

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Numerous aromatic plant metabolites encountered in *Brassica juncea* leaves and other edible or medicinal plants are biosynthesized from carbohydrates via the so called "Shikimate pathway" (Herrmann and Weaver, 1999). Several amino acids and vitamins synthesized by this pathway are essential for proper maintenance of health and survival and many of them like salicylic and nicotinic acid etc., have since long been known to possess broad spectrums of therapeutically interesting bioactivities including their antihyperglycemic, lipid lowering, anxiolytic, antidepressant and diverse other brain function modulating activities. Using a more holistic pharmacological approach (Chatterjee and Kumar, 2012) based on Ayurvedic principles of health care and medicine (Raha, 2013; Patwardhan, 2013), a hydro alcoholic *Brassica juncea* leaf extract has been identified as a potential nutraceutical that could be useful for prevention and cure of diabetes associated mental health problems (Thakur *et al*., 2013a, b, 2014). Qualitatively, the observed bio-activity profile of the extract revealed during such efforts was similar or analogous to those of numerous other edible or traditionally known medicinal plants now often considered as herbal adaptogen (Wagner *et al*., 1994; Winston and Maimes, 2007; Panossian, 2013). These and numerous other observations made with several Ayurvedic and other adaptogenic herbs suggest that regular intake of sufficient quantities of some common phytochemicals encountered in such and almost all terrestrial plants can desensitize physiological responses to systemic stress and that diverse spectrums of therapeutically interesting bioactivity profiles of their diverse types of extracts are due to the presence of different concentrations of such ubiquitous phytochemicals in them. Results of the initial set of experiments conducted to experimentally verify such possibilities are described and discussed in this communication.

MATERIALS AND METHODS

Animals: Adult male albino mice (25±5 g) were obtained from Central Animal House of Institute of Medical Sciences, Banaras Hindu University, Varanasi (Registration No. 542/AB/CPCSEA). All animals were acclimatized to constant laboratory conditions atleast one week before starting the experiment. They were randomly selected and group-housed (six animals per cage) in polypropylene cages (28×19×12.5 cm) at an ambient temperature (25±1°C) and relative humidity (50±10%) with a 12:12 h light/dark cycle (light on at 06:00 and off at 18:00). Cages were provided with sawdust, free access to standard rodent diet and water were cleaned routinely. Principles of laboratory animal care (NIH publication 85-23, revised in 1985) guidelines were always followed and prior approval from the Central Animal Ethical Committee of the University was obtained (Dean/13-14/CAEC/344 and 346, dated July 27, 2013). After acclimatization, all mice of a given experimental group remained in the same cage and they were marked by same colour codes on Head (H), Body (B), Tail (T), Head Tail (HT), Body Tail (BT) and Colourless (C) for identifying them during the course of the experiments. All the experimental groups in a given set of experiment were tested in parallel (i.e., on the same days of the experiments) and handled, weighed and observed by two different observers and using the same lab equipments.

Drugs and chemicals: Sinapic acid (95% pure) was purchased from Sigma Aldrich, Bangalore. Nicotinic acid, p-hydroxybenzoic acid and Salicylic acid were purchased from Himedia Laboratories Pvt. Ltd., Mumbai. Carboxymethylcellulose (CMC; Central Drug House Pvt. Ltd., New Delhi), Pentobarbitone sodium (Loba Chemie Pvt. Ltd., Mumbai), Metformin (Ranbaxy Laboratories, New Delhi), Diazepam (Lupin Ltd., Jammu) and Imipramine (Sun Pharmaceutical Industries Ltd., Mumbai) were used in this study.

Plant material: *Brassica juncea* leaves were collected from a local agricultural area in Varanasi (UP, India) and were botanically authenticated by Prof. N.K. Dubey in Herbarium of Department of Botany, Faculty of Science, Banaras Hindu University as *Brassica juncea* (Linn.) species Czern and Coss family *Brassicaseae* (Voucher specimen number Dubey/24/April/2013).

Preparation of extract: The fresh green leaves of *Brassica juncea* were dried at room temperature. The 800 g of the powdered dried leaves were refluxed with 2 L of ethyl alcohol and water in the ratio of 90:10 for 3 h in a soxhlet extractor till solvent became colourless. The total filtrate was evaporated on a water bath and dried in vacuum dryer at 40°C (Thakur *et al*., 2013a). Yield of the extract was 11.2% by weight of the dried leaves.

Analytical characterization of extract: A well-validated HPLC method described elsewhere (Chandrasekaran *et al*., 2009) and commonly used for quantifying total flavonoids contents of plant extracts was chosen. Briefly, a Shimadzu LC 2010HT HPLC system equipped with a quaternary pump, UV detector, degasser

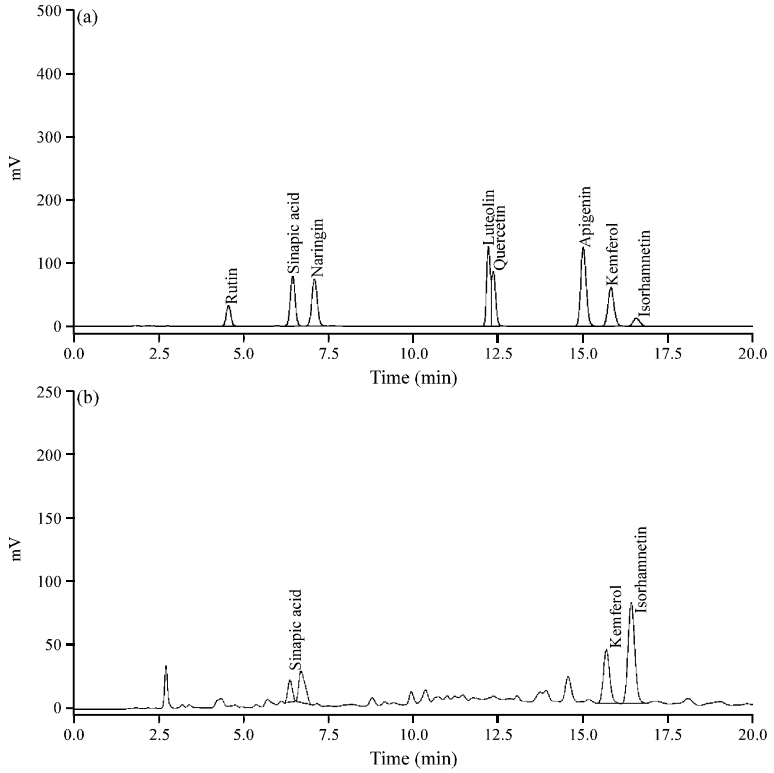

Fig. 1(a-b): HPLC chromatogram of (a) Flavonoids standard and (b) A hydrolyzed sample of the ethanolic *Brassica juncea* leaf extract

and an auto sampler with "Lab solution" software was used. Mobile phase was acetonitrile: phosphate buffer (pH 2.5-2.8). Column was C18-ODS (octadecyl silane) size = 5 μ, 250×4.6 mm, wavelength = 280 nm, flow rate = 1.5 mL min^{-1}, injection volume = 20 μL and run time = 40 min. The standard mixture was a mixture of flavonoids containing rutin, sinapic acid, narinjin, luteolin, quercetin, apigenin, keamferol and isorhamnetin. All reference standards used for HPLC analysis were purchased from Sigma, Bangalore, India. This analytical procedure was standardised as reported in studies with *Brassica juncea* leaves revealing their diverse neuronal function modulating efficacies in nondiabetic and diabetic animals (Thakur *et al*., 2013a, b; Thakur *et al*., 2014). HPLC chromatograms shown in Fig. 1 revealed that the *Brassica juncea* extract contain sinapic acid (0.034% w/w of its total phenolics), kaemferol (0.170% w/w) and isorhamnetin (0.367% w/w). No other flavonoids could be detected using the analytical procedure and other peaks detected in the chromatograms of hydrolysed samples of the extract cannot yet be assigned to specific phytochemicals known to be present in *Brassica juncea* leaves.

Animal grouping and drug administration: In each experiment, the effects of different daily oral doses of a given test agents were compared to those observed after daily such treatments with the vehicle used and with a reference drug. The doses of the test agents and those of the reference drugs used in a given experiment were as follows:

Experiment I: *Brassica juncea* extract (10, 30, 100 and 300 mg kg^{-1} day^{-1} P.O.) and the reference drug imipramine (15 mg kg^{-1} day^{-1} P.O.).

Experiment II: Sinapic acid (3, 10, 30 and 100 mg kg^{-1} day^{-1} P.O.) and the reference drug diazepam (5 mg kg^{-1} day^{-1} P.O.).

Experiment III: Nicotinic acid (1, 4 and 16 mg kg^{-1} day^{-1} P.O.) and the reference drug imipramine (15 mg kg^{-1} day^{-1} P.O.).

Experiment IV: Salicylic acid (30, 100 and 300 mg kg^{-1} day^{-1} P.O.) and the reference drug diazepam (5 mg kg^{-1} day^{-1} P.O.).

Experiment V: Para-hydroxybenzoic acid (30, 100 and 300 mg kg^{-1} day^{-1} P.O.) and the reference drug metformin (50 mg kg^{-1} day^{-1} P.O.).

All the test agents and reference drugs were administered as suspension in 0.3% CMC (vehicle) and each treated group consisted of 6 mice each. Since the effects of imipramine observed in the experiments conducted with *Brassica juncea* leaves extract and nicotinic acid were qualitatively similar, the data obtained for the two vehicles or imipramine treated groups were pooled for comparative statistical analysis. Due to similar reasons, the data for the two vehicles and diazepam treated groups in the two experiments conducted with sinapic acid and salicylic acid were also pooled.

Foot-shock Stress Induced Hyperthermia (FSIH): On days 1, 5, 7 and 10 of a given experiment, individual mouse from a group was weighed and then placed in a black box (24×29×40 cm) with a grid floor for 1 min. Electric foot-shock through the grid floor (2 mA, 50 Hz of 2 msec duration) was delivered for induction of stress. Five consecutive foot-shocks of 2 mA at 10 sec interval were given starting at 10 sec. At the end of the minute, the animals were placed back in their home cage. Rectal temperature before application of foot-shock was recorded by a rectal probe. After 10 min of foot-shock stress, rectal temperature was recorded again (Zethof *et al*., 1994). The difference in the rectal temperature before and after application of foot-shocks was considered as Foot-shock Stress Induced Hyperthermia (FSIH).

Tail suspension test: On the 11th day of the experiments and after 60 min of drugs administrations, individual mouse was hung 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 (Steru *et al*., 1985). Before oral administrations, weights of animals and their rectal temperatures were recorded.

Pentobarbitone induced sleep test: On the 12th day of the experiments, no oral treatments were given and all animals were tested for prior treatment effects on sleep onset (loss of righting reflex) and duration of sleep induced by pentobarbitone (40 mg kg^{-1}, i.p.) induced hypnosis (Ojima *et al*., 1995). The rectal temperatures and body weights of animals were recorded before pentobarbitone challenges.

Statistical analysis: Mean±Standard Error of Mean (SEM) was calculated for the observed values in experimental groups. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. GraphPad Prim 5 (GraphPad Software, Inc. CA, USA) and Origin-Pro 8 (OrginLab Corporation, MA, USA) software were used for statistical analysis and drawing graphs. $p<0.05$ was considered to be statistically significant.

RESULTS

Body weights: Mean body weights of the control, reference drugs and different doses of test agents treated groups are summarised in the Fig. 2a-e. It is apparent from these figures that mean body weights of all 5 control groups used in different experiments using the same experimental procedure consistently decreased during the 12 observational days. The mean body weights of the control animals recorded on the first observational day of a given experiment were not always significantly different from those of the group recorded on subsequent days of the experiment. However, pooled data analysis (not shown) of all the 30 vehicle treated control animals used in the five independent experiments revealed that the mean differences of their body weights recorded on the first day were always significantly higher than those recorded on the 5th and subsequent experimental days and that such reductions increased in magnitude with increasing test days. Since, continuous reductions in body weights of vehicle treated mice were always observed in numerous other earlier experiments conducted and using analogous bioassay systems, they seem to be due to daily handling and intermittent foot-shocks administered during the course of the experiments.

Results summarised in Fig. 2 also revealed that the tested oral doses of the imipramine, diazepam and metformin afforded protections against such body weight losses and that the *Brassica juncea* extract as well as all the four aromatic acids tested had similar protective effects against daily handling and intermittent foot-shock induced losses of body weights observed in the vehicle treated control groups during the course of the experiments. However, in their tested daily dose ranges, no clear dose dependencies of this effect of any of the test agents were observed.

Basal core temperatures: Mean basal rectal temperatures of different groups recorded during the course of the experiment are summarised in the Fig. 3a-e. As expected from earlier observations made under similar experimental conditions, the mean basal rectal

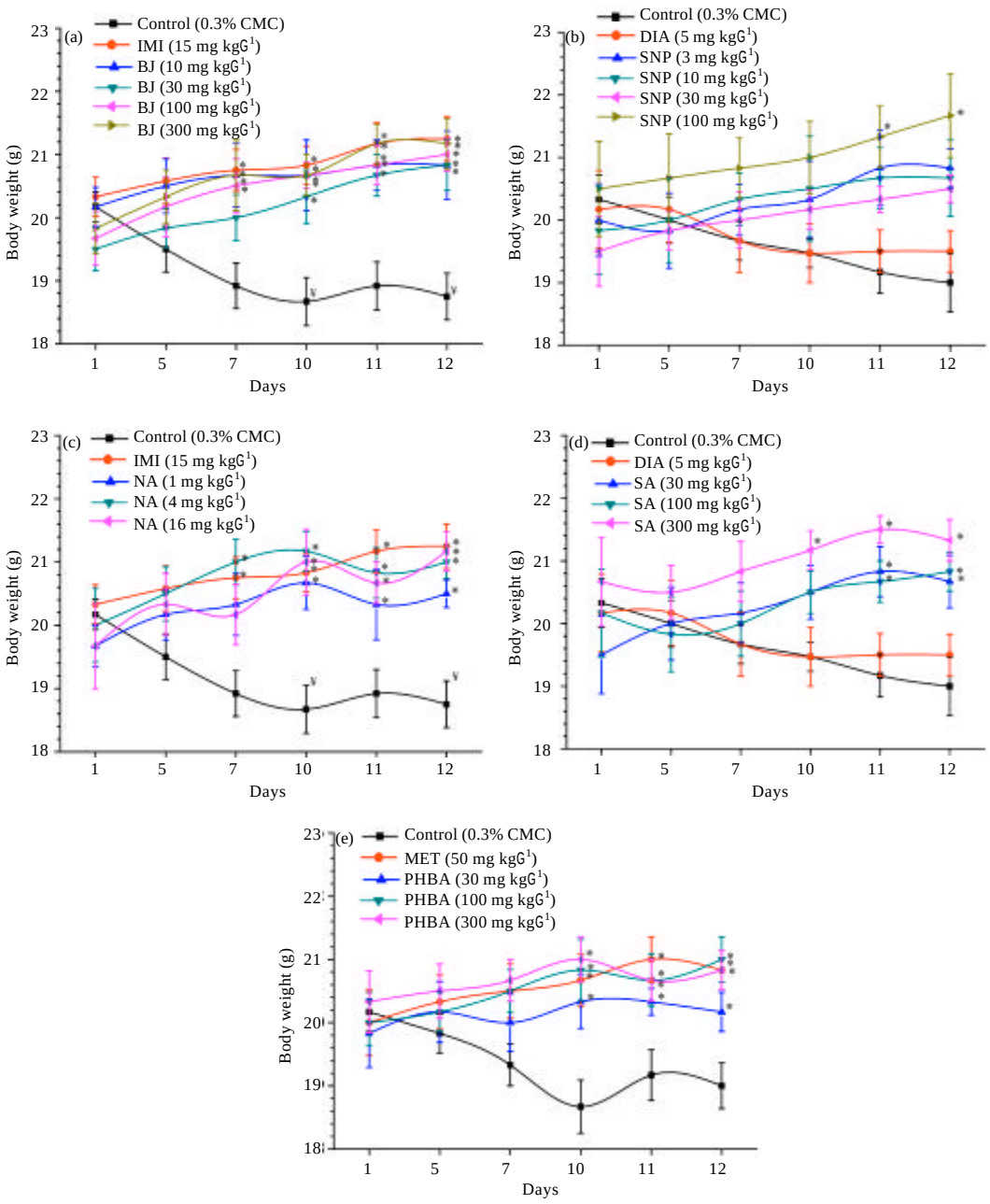

Fig. 2(a-e): Mean (±SEM) body weights of mice treated with (a) *Brassica juncea* extract (BJ), (b) Sinapic acid (SNP), (c) Nicotinic acid (NA), (d) Salicylic acid (SA) and (e) Para-hydroxybenzoic acid (PHBA). *p<0.05 vs. control values on the same day, ¥p<0.05 vs. day 1 values of the same group

temperatures of all vehicle treaded control groups increased slightly on the 5th and subsequent observational days and remained almost constantly elevated on the 10-12th days of the experiments. This daily handling and intermittent foot-shock induced elevation of core temperatures within physiological ranges was antagonised also by all test agents and standard drugs used. However, qualitatively as well as

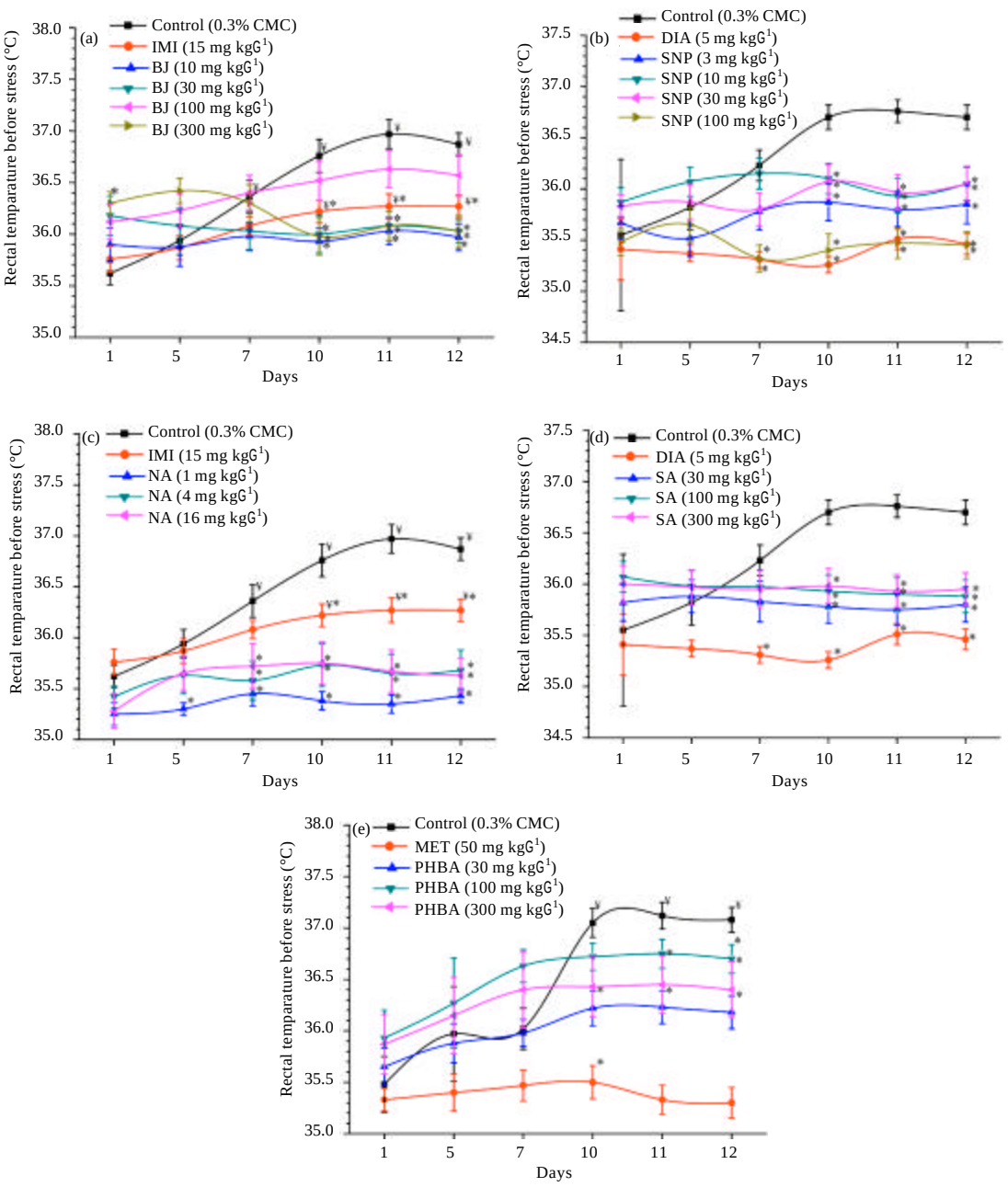

Fig. 3(a-e): Effect of daily handling and intermittent foot-shocks on basal rectal temperature of mice treated with (a) *Brassica juncea* extract (BJ), (b) Sinapic acid (SNP), (c) Nicotinic acid (NA), (d) Salicylic acid (SA) and (e) Para-hydroxybenzoic acid (PHBA). Data are represented as Mean±SEM. *p<0.05 vs. control values on the same day, ¥p<0.05 vs. day 1 values of the same group

quantitatively their effects differed considerably. On the first observational days, mean core temperatures of the 300 mg kg^{-1} *Brassica juncea* extract treated group only were significantly higher than the control group on that day. Though statistically not significant, mean basal temperatures of the three other lower doses of *Brassica juncea* extract treated mice were also dose dependently elevated on this experimental day.

Somewhat analogous but statistically insignificant, higher basal temperatures were also observed for sinapic, salicylic and para hydroxybenzoic acids. On the other hand, mean basal temperatures of the nicotinic acid treated groups were lower than the control group on this day. No statistically significant effects on basal temperatures of the tested imipramine, diazepam and metformin were observed on the first day of the experiments.

Unlike in the control groups, basal core temperatures of the diazepam and metformin treated groups remained almost constant on all observational days. Such antagonistic effects of the tested imipramine dose against daily handling and intermittent foot-shocks were quantitatively lower than the other two reference drugs used in this study and in other similar experiments. Except for the 100 mg kg^{-1} day^{-1} *Brassica juncea* extract treated group, mean basal temperatures of all other groups treated with the extract were significantly lower than those of the control group on the 10-12th days of the experiment and were similar to that of the control group recorded on day 1 (Fig. 3a). No elevations of mean basal temperatures of all the three doses of salicylic acid and nicotinic acid treated groups was seen instead it remained almost constant on all experimental days (Fig. 3c, d). Daily handling and intermittent foot-shock triggered elevation of basal temperatures were also inhibited by sinapic and para-hydroxybenzoic acids but the observed effects were not strictly dependant on their daily oral doses (Fig. 3b, e). Such minor discrepancies could as well be due to inability of 6 animals in a group to accurately detect only slight elevation of rectal temperatures caused by the experimental procedure used.

Foot-Shock Stress Induced Hyperthermia (FSIH): Mean FSIH of all control groups increased somewhat during the course of the experiments but were always within 0.7-0.9°C. The tested imipramine dose had no effects on this response on any of the experimental days whereas, antagonistic effects of the tested doses of both diazepam and metformin increased with the increasing numbers of treatment days. No statistically significant effects of any of the tested *Brassica juncea* extract doses on FSIH were observed on the 1st and 5th days of the experiment. On the 7th and 10th observational days, slight but significant inhibitory effects of the extract on this response were observed. The dose effect relationships of the extract on FSIH on both these days were not very steep and were parallel to each other. Except for the statistically significant effects of 30 and 100 mg kg^{-1} daily doses of sinapic acid observed on day 5, all other effects of this acid on FSIH were qualitatively analogous to that observed for BJ (Fig. 4a, b).

Unlike *Brassica juncea* extract and other ubiquitous secondary plant metabolites tested, nicotinic acid dose dependently inhibited FSIH even after its single oral doses. However, its efficacy also increased somewhat with the number of treatment days. On days 7 and 10, the mean FSIH observed even after the lowest nicotinic acid dose tested (1 mg kg^{-1} day^{-1}) were significantly lower than those of the controls and such efficacies of all its tested daily oral doses (1, 4 and 16 mg kg^{-1} day^{-1}) observed on these two days were almost equal (Fig. 4c). Statistically significant effects of the highest tested doses (300 mg kg^{-1} day^{-1}) of salicylic and para-hydroxybenzoic acids were also observed after their single oral doses and efficacies of all their tested doses (30, 100 and 300 mg kg^{-1} day^{-1}) on FSIH increased also on the subsequent test days (Fig. 4d, e).

Tail suspension test: As expected, mean immobility period of imipramine treated groups were significantly lower in this test often used for detecting antidepressant like effects of test agents. However, slight but statistically significant reductions in immobility period were observed after tested doses of diazepam and metformin as well. All tested doses of *Brassica juncea* extract and those of the organic acids also significantly lowered the immobility period of mice after their 11 daily doses. These effects of all test agents increased somewhat with their increasing doses (Fig. 5a-e). Hereupon the efficacies of the highest nicotinic acid dose tested (16 mg kg^{-1} day^{-1}) and that of sinapic acid (300 mg kg^{-1} day^{-1}) were almost equal to or somewhat more than that of imipramine. Quantitatively, the efficacy of 30 mg kg^{-1} day^{-1} daily doses of *Brassica juncea* extract was equal to that of 15 mg kg^{-1} day^{-1} imipramine and those of 100 and 300 mg kg^{-1} day^{-1} doses of the extract were higher than that of the reference antidepressant drug.

Pentobarbitone induced sleep test: Except for imipramine, all test agents and reference drugs either shortened the sleep onset time and prolonged the duration of sleep induced by pentobarbitone or had significant effects on both of them (Fig. 6a-e). Since this test was conducted 24 h of the last administered doses of the test agents, their observed effects seem to be due to their longer lasting physiological effects after their repeated daily oral doses.

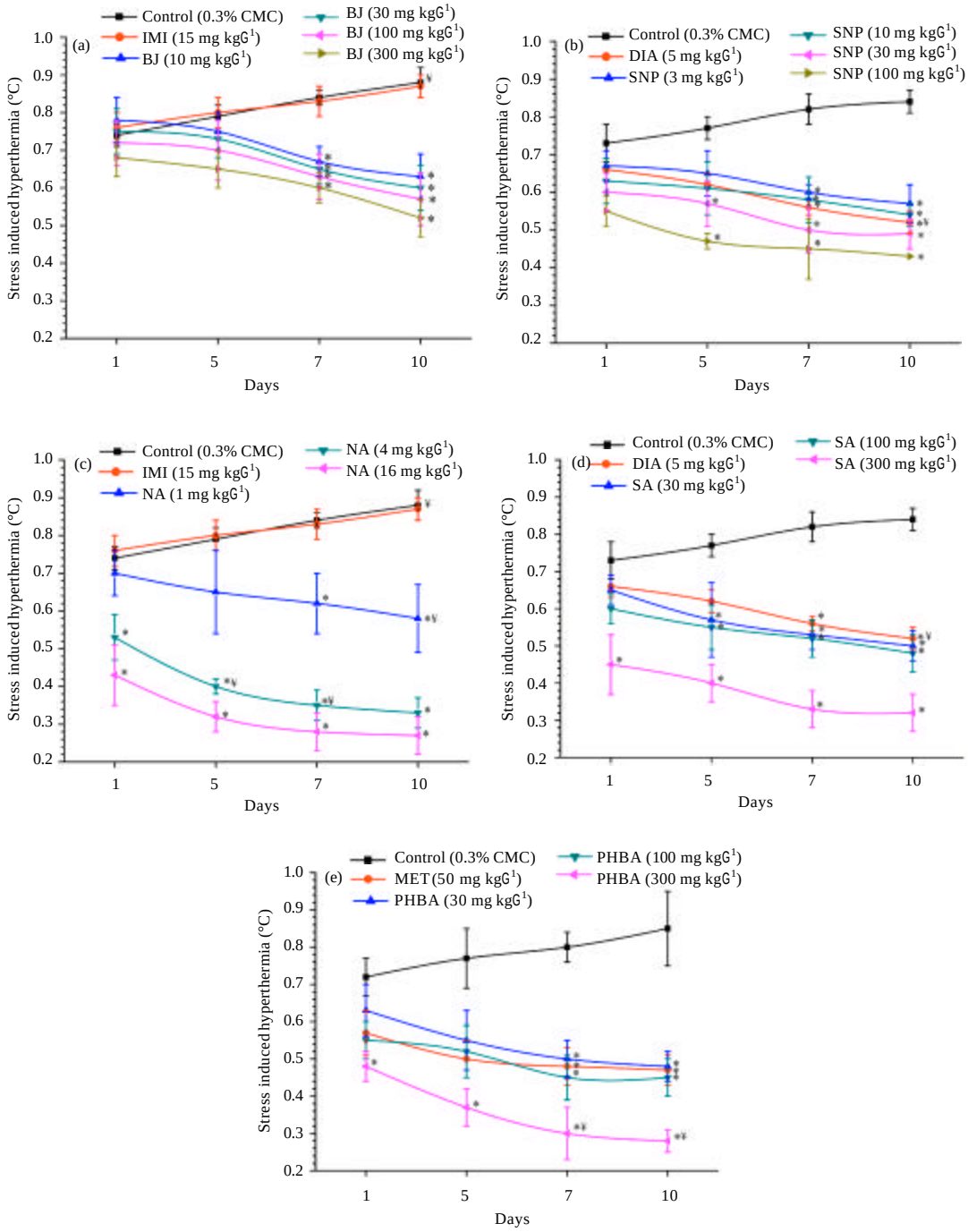

Fig. 4(a-e): Effect of foot-shock stress triggered transient hyperthermia in mice treated with (a) *Brassica juncea* extract (BJ), (b) Sinapic acid (SNP), (c) Nicotinic acid (NA), (d) Salicylic acid (SA) and (e) Para-hydroxybenzoic acid (PHBA). Data are represented as Mean±SEM. $^{\star}p<0.05$ vs. control values on the same day, $^{¥}p<0.05$ vs. day 1 values of the same group

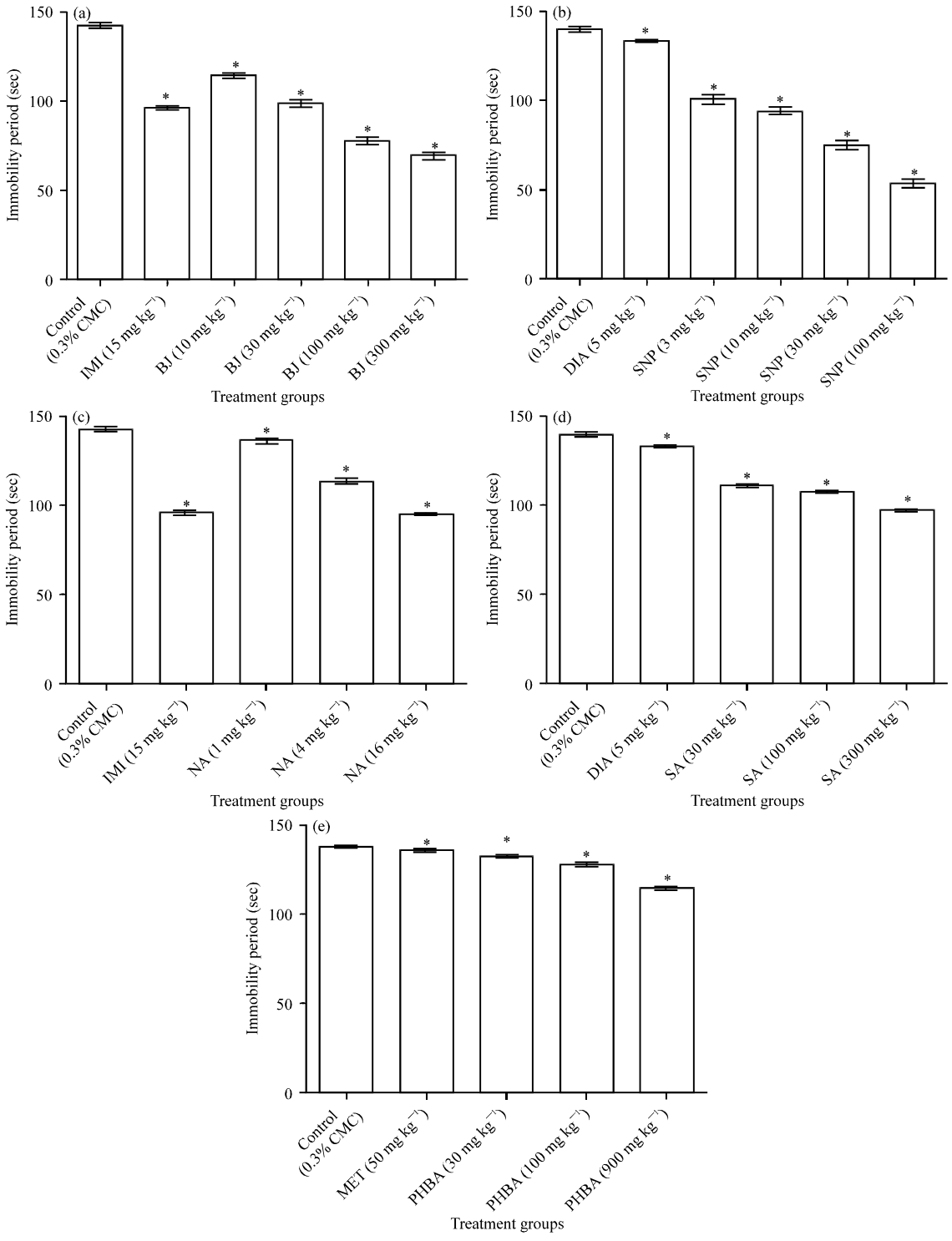

Fig. 5(a-e): Effects of 11 daily oral doses on immobility period of mice treated with (a) *Brassica juncea* extract (BJ), (b) Sinapic acid (SNP), (c) Nicotinic acid (NA), (d) Salicylic acid (SA) and (e) Para-hydroxybenzoic acid (PHBA) observed in tail suspension test conducted one hour after their last doses. Data are represented as Mean±SEM, *$p<0.05$ vs. control values

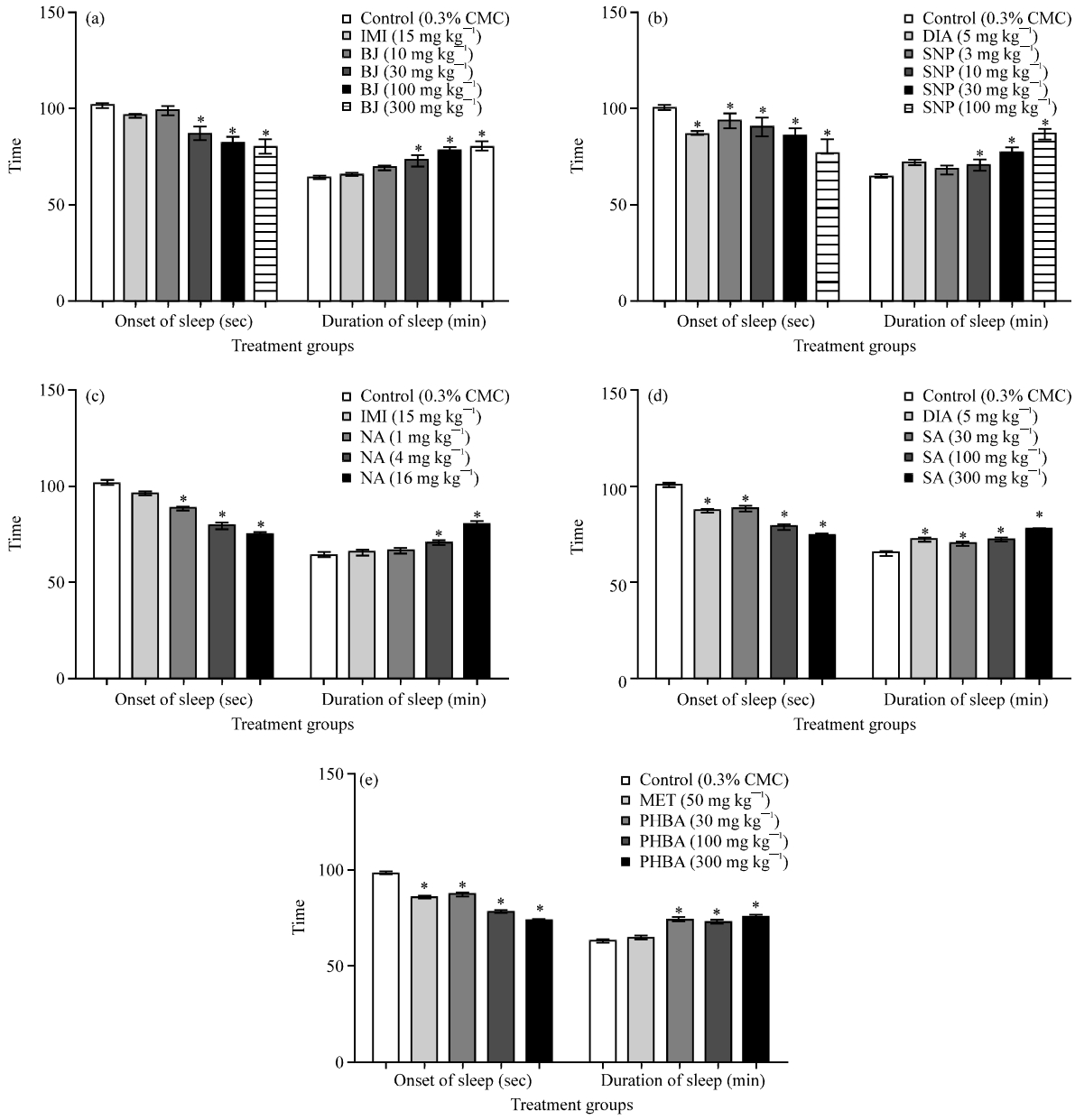

Fig. 6(a-e): Effect of 11 daily oral doses on onset and duration of sleep in mice treated with (a) *Brassica juncea* extract (BJ), (b) Sinapic acid (SNP), (c) Nicotinic acid (NA), (d) Salicylic acid (SA) and (e) Para-hydroxybenzoic acid (PHBA) and challenged with pentobarbitone on the 12th day of the experiments. Data are represented as Mean±SEM, ⋆p<0.05 vs. control values

DISCUSSION

It is now well recognized that disorders of the stress response regulating systems eventually leads to diabesity and other metabolic disorders associated mental health problems and that appropriate modulations of psychological and physiological stress responses could as well be used be for prevention and cure of such and numerous other lifestyle disorders (Chrousos, 2009; Kassi *et al*., 2011; Guarner-Lans *et al*., 2011; Bystritsky *et al*., 2013). One such stress response easily quantifiable in all vertebrates is alterations in their body temperatures by environmental or metabolic stress. Therefore, stress triggered hyperthermic responses are now often used for detecting acute dose effects of psychoactive agents

potentially useful for treatment of exaggerated anticipatory anxiety (Bouwknecht *et al*., 2007; Van Der Heyden *et al*., 1997; Vinkers *et al*., 2010) or for overall estimation of stress responses in experimental and other animals as well (Careau *et al*., 2012). Based on earlier observations, that repeated daily oral doses of *Brassica juncea* extracts are necessary for quantifying their antidiabetic and anxiolytic and other brain function modulating effects (Thakur *et al*., 2013a, b, 2014), the bioassay system used in this communication was conceived to estimate their pharmacologically interesting dose ranges that could be used for identifying its bioactive constituents and mechanisms involved in their such efficacies. Since many ubiquitous small molecular weight aromatic acids biosynthesised by many medicinal and edible plants have been reported to possess bioactivity profiles analogous to that observed for *Brassica juncea* and other edible or medicinal plants studied, four such aromatic acids commonly consumed with fruits and vegetables were also screened in this bioassay.

Observations reported in this communication reconfirm good reproducibility of the bioassay and its potential uses for therapeutically interesting stress response desensitising effects not only of diazepam or imipramine like psychoactive drugs but also those of the antidiabetic drug metformin. This biguanide derivative is currently the drug of first choice widely recommended for treatment of type-2 diabetes and also for prevention of diabesity associated co-morbidities. Although, as yet many questions concerning its modes and sites of actions still remain open, during more recent years a few reports dealing with its neuronal function modulating potentials have appeared (Pintana *et al*., 2012; Adedeji *et al*., 2014). It has been also reported that its metabolic effects could as well be due to its regulating effects on gut microbial ecology (Shin *et al*., 2013; Burcelin, 2013). Therefore, it was used as a reference drug in the experiment conducted to estimate pharmacologically interesting dose range of para-hydroxybenzoic acid in the bioassay used. This acid with bactericidal activities is widely used for food preservation purposes and is also encountered in many edible and other plants (Khadem and Marles, 2010) including some adaptogenic herbs (Zhou *et al*., 2007). Results of this experiments clearly revealed that repeated daily oral doses of even 30 mg kg^{-1} day^{-1} of this acid possess stress response desensitizing and other bioactivities analogous to that of 50 mg kg^{-1} daily oral doses of the standard antidiabetic drug. However, even after the highest tested oral dose (300 mg kg^{-1} day^{-1}), it did not completely inhibit the foot-shock stress triggered hyperthermic response.

Sinapic acid is one of the quantitatively major oxygenated aromatic acids encountered in *Brassica juncea* leaves and it has been reported to possess numerous therapeutically interesting bioactivities (Niciforovic and Abramovic, 2014). Some of them like its antidiabetic, anxiolytic and other psychopharmacological properties are quite analogous to those observed for *Brassica juncea* leaf extracts. Therefore, activity profile of such an analytically standardised extract was compared with that of sinapic acid in the bioassay. Although, qualitatively, the observed activity profile of the pure acid in the bioassay was identical to that of the tested extract, quantitatively the efficacy of the extract cannot be explained by its analytically quantified total sinapic acid contents only. Therefore, it is apparent that other stress response desensitizing components are also present in the tested extract.

Although, the contents of nicotinic and salicylic acids in the extract have not yet been quantified, both of them have been reported to be secondary metabolites of *Brassica juncea* and numerous other edible and medicinal plants. Although, both these acids are some of the pharmacologically better studied secondary plant metabolites and both of them have been reported to possess metabolic as well as mental function alerting properties in patients and experimental animals, as yet little attention has been paid to their stress response modifying potentials. However, both of them have been reported to possess ulcerogenic potentials and the crucial roles of metabolic as well as mental stress in ulcer genesis are well recognized. These observations clearly revealed that even their lowest daily oral doses tested (1 mg kg^{-1} day^{-1} of nicotinic acid and 30 mg kg^{-1} day^{-1} of salicylic acid) both of them are effective in desensitizing the animals against daily handling and intermittent foot-shock triggered physiological stress responses and that their such efficacies do not disappear even after their several fold higher daily oral doses (16 mg kg^{-1} day^{-1} for nicotinic acid and 300 mg kg^{-1} day^{-1} of salicylic acid).

The classical Ayurvedic concepts that, appropriate choices of food and drinks is essential for proper maintenance of mental health and that there can be no health without mental health, are now well accepted by most modern scholars, researchers and medical practitioners well trained in modern medical sciences. However, many questions concerning the type of foods and drinks and their appropriate quantities necessary for prevention and cure of diverse lifestyle disorders cannot yet be answered with certainty. This is mainly because most modern nutritional researchers still continue to concentrate their efforts on the potential health benefits of a few bioactive phytochemicals only and that too mainly to better understand their sites and modes of actions potentially involved in diverse well known bioactivities of each of them. Hereupon, little attention is paid to the facts that even several ubiquitous

phytochemicals always consumed with any food or drink could also have regulating roles on homeostatic processes necessary for survival and that complex biological interactions between diverse food components dictate their ultimate health effects.

Such are not only the cases for edible and structurally simple phytochemicals ubiquitously encountered in all plants but also for numerous other structurally and functionally complex secondary plant metabolites encountered in diverse traditionally known medicinal plants. Critical analysis of information now available on medicinal phytochemistry and pharmacology of numerous such phytochemicals strongly suggest though, that their modulatory effects on diverse homeostatic processes are involved in their clinically observed health benefits and also in their therapeutically interesting bioactivity profiles observed in experimental animals. Observations reported in this communication clearly reveal that even some phytochemicals ubiquitously present in numerous edible and medicinal plants are fairly effective in desensitizing homeostatic stress responses and that the dose response relationships for such diverse effects of a given phytochemical are not identical. Since, it can be expected that different quantities of such stress response desensitizing molecules can be present in any plant extract, they could as well be some functionally common bioactive constituents of many plant extract. That such, indeed is the case also indicated by numerous reports revealing analogous or similar bioactivity profiles of different plant extracts on metabolic and behavioural functions.

Current understanding on aetiology, pathogenesis and progression of life style associated disorders, taken together with the observations reported in this communication strongly suggest that the described bioassay system could a versatile and more holistic one not only for better pharmacological standardization of medicinally used herbal extracts but also for identifying their bioactive constituents using appropriate bioactivity guided fractionation procedures. Moreover, this bioassay could as well be used for estimating the pharmacologically interesting dose ranges of edible and other plant extracts and for better understanding of the pharmacological interactions between their bioactive and other constituents (by appropriate uses of their mixtures as test agents). Therefore, efforts are now being made to experimentally verify such possibility using *Brassica juncea* and other Ayurvedic medicinal plant extracts not yet well recognized for their adaptogenic potentials. Such efforts could not only enable more rational medicinal uses of such plants but also for better understanding of Ayurvedic biology according to the postmodern concepts of systems biology dealing with thermoregulation, metabolism and body weight regulating physiological processes.

CONCLUSION

The bio-assay system used in this study is suitable for estimating pharmacologically interesting dose range and dosing regimen of plant extracts and their bioactive constituents with adaptogenic potentials. Structurally diverse and often ubiquitously encountered secondary plant metabolites of the Shikimic acid pathway can contribute to therapeutically interesting bio-activities of numerous medicinally used plant extracts with broad bioactivity profiles.

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