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Anti-Stress Activity of Phloroglucinol: A Transient Metabolite of Some Plant Polyphenolics

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ABSTRACT

Background and Objectives: Phloroglucinols are secondary metabolites that occur naturally in certain plant species. Microorganisms such as brown algae or bacteria also produce phloroglucinols. Phloroglucinols has potential health effect and it is used as a treatment for gallstones, spasmodic pain and other related gastrointestinal disorders. The objective of the study was to evaluate phloroglucinol and its tri-methyl ether for anti-stress activity and its beneficial effect on stress-induced perturbations. **Method:** Stress-induced hyperthermia and pentobarbital sedation models were used for pilot study cum relative dose response study at single and repeated per-oral doses of 3-300 mg kg^{-1} day^{-1}. Further study was performed to evaluate phloroglucinol (1-16 mg kg^{-1} day^{-1}, p.o., for 7 days) for anti-stress activity using validated animal models of stress viz., foot-shock stress and cold-restraint stress. Stress-induced perturbations were evaluated in stressed rats by using sexual behaviour, elevated zero maze and behavioural despair tests. **Results:** The observations revealed that phloroglucinol and its tri-methyl ether protect mice against stress-induced hyperthermia only after its daily administrations. After fairly low oral doses, phloroglucinol inhibited not only stress-induced gastric ulcers but also the exaggerated anxiety and depression observed in stressed animals. In the same dose range, it also improved sexual behaviour and suppressed adrenal hypertrophy and spleen atrophy in stressed animals. **Conclusions:** These observations strongly suggest that plasma or urinary levels of phloroglucinol are not proper indicators of its therapeutic benefits and that its observed anti-stress activity could as well be due to its actions on the microbiota-gut-brain axis.

Key words: Phloroglucinol, stress behaviour, gut microbiota, gut-brain axis, adaptogenic

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INTRODUCTION

Phloroglucinol is a symmetrically tri-hydroxylated benzene derivative encountered in edible brown algae and some terrestrial plants as well. The phloroglucinol moiety is also the structural backbone of many polyphenolic phytochemicals consumed with food or considered as bioactive components of herbal remedies. Despite growing evidence of health benefits of dietary polyphenolics, their mechanisms of actions at the molecular level are poorly understood or often controversially discussed (Stevenson and Hurst, 2007; Singh *et al*., 2008; Soto-Vaca *et al*., 2012; Halliwell, 2007; Gomez-Pinilla and Nguyen, 2012). Many such controversies arise from the fact that blood levels of polyphenolics and their metabolites observed after their oral intake are much below the ones necessary to observe their therapeutically interesting bioactivities in cell cultures or other *in vitro* models (Schaffer and Halliwell, 2012). It is now becoming increasingly apparent though, that rapid metabolism of plant polyphenolics by gut microbiota plays an important role in dictating their pharmacological activity profiles and that phloroglucinol is a common transient metabolite of numerous edible polyphenolics commonly consumed with fruits, vegetables and drinks (Vissiennon *et al*., 2012; Van Duynhoven *et al*., 2011; Dall'Asta *et al*., 2012; Moco *et al*., 2012). Although after high oral doses (120 mg) of pure phloroglucinol it can be detected in circulation, its plasma half-life is very short

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(Kim *et al*., 2003). Thus, it is fairly rapidly metabolized not only in the gastrointestinal tract but also by other bodily organs.

However, several reports have consistently revealed diverse therapeutically interesting pharmacological activities of fairly low oral doses of phloroglucinol in experimental animals (Chang *et al*., 2012; Kwon *et al*., 2012) and symptomatic relief of gastric discomforts after moderate oral doses (50 mg) of phloroglucinol have also been observed in patients with irritable bowel syndrome (Jafri *et al*., 2006). Such therapeutic uses of phloroglucinol in combination with its tri-methyl ether (1,3,5-trimethoxybenzene) have also been known since long and during more recent years several clinical trials have continued to reconfirm their therapeutic uses for symptomatic treatments of pain and other functional disorders of the digestive tract, bile duct and urinary tract (Chassany *et al*., 2007; Dellabella *et al*., 2005; Chen *et al*., 2010).

Phloroglucinol is catabolised by gut microbiota to acetate, butyrate and CO_2 (Chassany *et al*., 2007; Winter *et al*., 1989; Brune and Schink, 1992) and is known to possess antibacterial activities (Bowden and Ross, 1965). Thus, it can alter the gut microbiota ecology which is well recognized to be an integral part of human physiology (Neish, 2009; Clemente *et al*., 2012). Profound impact of the gut microbiota on brain and behaviour is now well recognized (Forsythe and Kunze, 2013; Cryan and Dinan, 2012; Thakur *et al*., 2014a) and it is now apparent also that gastric pain and other discomforts of bowel diseases are due to alterations of central sensitivity (Kennedy *et al*., 2012; Wu, 2012; Yunus, 2008; Rook *et al*., 2012). Therefore, it was hypothesized that clinically observed symptomatic relief in patients observed after therapeutically used doses (50 mg) of phloroglucinol could as well be due to its effect on the microbiota-gut-brain axis. The very first observations made during efforts to experimentally verify this possibility are described and discussed in this communication.

MATERIALS AND METHODS

Animals: Male and female adult Charles foster rats (150±10 g) and Wistar mice (20±5 g) of 3 to 4 months old age in equal ratio were used. They were acquired from the Central Animal facilities of the Institute of Medical Sciences, Banaras Hindu University, Varanasi (Registration Number: 542/AB/CPCSEA) and were randomly distributed into different experimental groups. The animals were housed in poly-acryl cages with stainless steel wire lids and maintained under standard laboratory conditions (temperature: 25±1°C, relative humidity: 50±5% and 12:12 h dark and light cycle). Unless stated otherwise they were maintained with standard pellet diet and water *ad libitum*. They were always acclimatized to laboratory conditions for at least one week before using them for the experiments. Principles of laboratory animal care guidelines were strictly followed. The experimental protocol was approved by the Central Animal Ethical Committee of the University (No. Dean/11- 12/CAEC/250 dated 03.09.2011).

Drug administration: Phloroglucinol and its tri-methyl ether (Sigma-Aldrich, St. Louis, MO), *Panax ginseng* extract (Zenith Nutrition, Bangalore, India) and diazepam (Ranbaxy Laboratories Ltd., Gurgaon, India) were used in this study. For oral administrations phloroglucinol tri-methyl ether was macerated with 2% Tween 80 (Sisco Research Lab., Mumbai, India) and then suspended in 0.2% aqueous agar (Central Drug House, New Delhi, India), whereas phloroglucinol was dissolved in distilled water. Control animals in all cases received the corresponding vehicle (10 mL kg^{-1}, p.o.,) and all tests were conducted 1 h after the last treatments. Except for dose finding experiments, oral 1, 4-16 mg kg^{-1} day^{-1} doses of phloroglucinol were administered once daily for seven consecutive days. All behavioural and other tests used in this study are routinely used in laboratories for identifying potential anti-stress agents and are described in earlier publication (Singh *et al*., 2012; Thakur *et al*., 2014b, c; Langstieh *et al*., 2014). For comparison sake a group orally treated with 100 mg kg^{-1} daily dose of the *Panax ginseng* extract was always tested in parallel.

Pilot study cum dose finding experiments: For these experiments phloroglucinol or its tri-methyl ether (3, 10, 30, 100 or 300 mg kg^{-1} day^{-1}) were administered orally for seven consecutive days and they were daily weighed and closely observed for behavioural abnormalities. On the 7th day, the treated rats were additionally subjected to the ring test for catalepsy (Pertwee, 1972). In a separate experiment on first and seventh day of the treatments, the mice were subjected to stress-induced hyperthermia test using the Foot-shock procedure (Zethof *et al*., 1994). These animals were treated again on the following day and one hour thereafter challenged

with pentobarbital (40 mg kg^{-1}, i.p.). Effects of treatments on pentobarbital-induced sleep were quantified using the method described elsewhere (Ojima *et al*., 1995). For comparison sake a separate group of mice treated once with diazepam (3 mg kg^{-1}, 1 h before the test) was run in parallel in pentobarbital-induced sleep test.

Foot-shock stress: The Foot-shock stress procedure described elsewhere was used (Armando *et al*., 1993). The rats were randomly assigned to the stressed control and drug treated stressed groups. Those assigned to the vehicle or drug treated groups were subjected to unpredictable Foot-shock (2 mA) for 1 h during 21 consecutive days. The duration of each shock and the interval between the shocks were randomly programmed between 3-5 and 10-100 sec, respectively (Bhattacharya and Muruganandam, 2003). Treatments were given 1 h before the stress sessions during the last seven days. One hour after the last treatments the rats were sacrificed by cervical dislocation and stomachs were removed. Ulcer index in the stomachs were evaluated according to the procedure described earlier (Nagaraju *et al*., 2012; Bhargava and Singh, 1981).

Cold restraint stress: Rats were deprived of food but not of water, for 18 h before subjecting them to cold immobilization stress according to the procedure described elsewhere (Krishnamurthy *et al*., 2011). They were immobilized by strapping the fore and hind limbs on a wooden plank and then kept at a room temperature of 4-6°C for 2 h. Thereafter, they were sacrificed and their stomach ulcer index evaluated by the procedure described above. In addition, weights of the glandular portion of stomachs were calculated by subtracting the weight of the whole stomach minus rumen and expressed as mg/100 g body weight of animals.

Sexual activity in Foot-shock stressed rats: Sexual activity of vehicle treated unstressed rats were compared those of Foot-shock stressed ones treated with vehicle or phloroglucinol or *Panax ginseng*. For such purposes, treated male rats were placed in a dimly lighted room for 10 min with two oestrinized female rats. Total numbers of mounts were counted during this period as described earlier (Morishita *et al*., 1993). Immediately thereafter, they were sacrificed and their adrenal gland and spleen were removed and weighed (Bhattacharya *et al*., 2000).

Antidepressant and anxiolytic-like effects in stressed rats: The 21 days stress procedure described earlier was used (Willner, 1984). In all behavioural tests, a vehicle treated normal rats were always tested in parallel. Treatments were always given 1 h before the stress sessions during the last seven days of the stress period. The behavioural despair test for antidepressants described by Willner (1984) and the elevated zero-maze and novelty induced suppressed feeding tests for anxiolytics were used (Shepherd *et al*., 1994). Details of the well standardized versions of these tests have been published somewhere (Kumar *et al*., 2000). All observations were made by blind and well versed observers.

Statistical analysis: All values were expressed as Mean±Standard Error Mean (SEM), n = 6. Statistical significance between control and treatment groups was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. GraphPad Prism (version 5) software was used for all statistical analysis. A p-value less than 0.05 were considered as statistically significant.

RESULTS

Pilot experiments: Even the highest doses (300 mg kg^{-1} day^{-1}; p.o., for seven days) of phloroglucinol and its tri-methyl ether were well tolerated by the animals and body weight changes of drug treated groups during the treatment period were not different form the vehicle treated controls. No cataleptogenic effects of the phloroglucinols were observed in the rat ring test used (results not shown). Although no effects of phloroglucinol and its tri-methyl ether treatments in the mice stress-induced hyperthermia test were observed after their single oral doses, on the seventh day of the treatments their significant and dose dependant antagonistic effects were apparent in this test ($p<0.05$) compared to control mice (Table 1 and 2). It is apparent from the results that even 3 mg kg^{-1} day^{-1} doses of the two phloroglucinols tested were highly effective in suppressing the stress- induced hyperthermia after seven daily administrations. However, even after their highest dose tested (300 mg kg^{-1}) they did not completely antagonized the stress-induced hyperthermia.

Analogous was also the case in the pentobarbital sleep test. Significant decrease in onset of sleep and increase in duration of sleep were observed ($p<0.05$) by increasing daily doses of both the phloroglucinol and its

Table 1: Effect of phloroglucinol on stress-induced hyperthermia after single dose and repeated (for 7 days) administration in mice

Treatment groups	Dose (mg kg^{-1}, p.o)	Increase in rectal temperature (°C) Day 1	Day 7
Control	-	0.75±0.09	0.82±0.09
Phloroglucinol	3	0.78±0.15	0.47±0.10*
Phloroglucinol	10	0.72±0.23	0.43±0.11*
Phloroglucinol	30	0.73±0.18	0.37±0.08*
Phloroglucinol	100	0.72±0.07	0.35±0.08*
Phloroglucinol	300	0.77±0.16	0.35±0.05*

*=p<0.05 vs. Control

Table 2: Effect of phloroglucinoltrimethyl ether on stress-induced hyperthermia after single dose and repeated (for 7 days) administration in mice

Treatment groups	Dose (mg kg^{-1}, p.o)	Increase in rectal temperature (°C) Day 1	Day 7
Control	-	0.70±0.18	0.78±0.11
Phloroglucinol trimethyl ether	3	0.72±0.11	0.43±0.07*
Phloroglucinol trimethyl ether	10	0.65±0.16	0.42±0.11*
Phloroglucinol trimethyl ether	30	0.75±0.09	0.30±0.04*
Phloroglucinol trimethyl ether	100	0.73±0.16	0.28±0.07*
Phloroglucinol trimethyl ether	300	0.77±0.20	0.25±0.03*

*=p<0.05 vs. Control

Table 3: Effect of phloroglucinol and diazepam on potentiation of pentobarbital-induced sleeping time in mice

Treatment groups	Dose (mg kg^{-1}, p.o)	Onset of sleep (% relative value)	Duration of sleep (% relative value)
Control	-	100.00	100.00
Phloroglucinol	3	60.94*	141.93*
Phloroglucinol	10	48.73*	172.78*
Phloroglucinol	30	42.88*	205.87*
Phloroglucinol	100	38.53*	210.28*
Phloroglucinol	300	37.71*	214.69*
Diazepam	3	24.96*	243.60*

*=p<0.05 vs. Control

tri-methyl ether tested in pentobarbital-induced rats compared to control rats and their maximal effects were observed again after 30 mg kg^{-1} daily doses (Table 3 and 4). As in stress-induced hyperthermia test, their lowest doses tested (3 mg kg^{-1} day^{-1}) were also effective in this test.

Antiulcer activity: Significant dose dependant beneficial effects ($p<0.05$) of phloroglucinol against stress-induced ulcers (significant decrease in number of ulcer and ulcer index) were observed in chronically Foot-shock stressed rats (Table 5) and such was also the case in animals subjected to shorter duration stress in the cold restrained stress model (Table 6) compared to respective stress control rats. Increased weights of the glandular portions of the stomach in the stressed animals observed in both the tests were also significantly lower ($p<0.05$) in all phloroglucinol treated groups compared to respective stress control rats (Fig. 1). Quantitatively, the observed effects of 100 mg kg^{-1} day^{-1} doses of the herbal adaptogen *Panax ginseng* were higher than the highest dose of phloroglucinol tested. It must be noted though that even 1 mg kg^{-1} day^{-1} daily dose of phloroglucinol had significant beneficial effects against the pathological consequences of both acute and chronic stress.

Stress-induced behavioural perturbations: As compared to the non-stressed control animals, all the behavioural parameters quantified in this study were significantly ($p<0.05$) altered in the stressed animals and all these parameters in stressed rats were significantly improved ($p<0.05$) in the phloroglucinol and *Panax ginseng* treated groups compared to stress control rats. Results summarized in Fig. 2 reveal that the efficacy of the highest tested phloroglucinol dose

Table 4: Effect of phloroglucinol trimethyl ether and diazepam on potentiation of pentobarbital-induced sleeping time in mice

Treatment groups	Dose (mg kg^{-1}, p.o.)	Onset of sleep (% relative value)	Duration of sleep (% relative value)
Control	-	100.00	100.00
Phloroglucinol trimethyl ether	3	51.01*	145.91*
Phloroglucinol trimethyl ether	10	38.95*	171.51*
Phloroglucinol trimethyl ether	30	35.98*	195.35*
Phloroglucinol trimethyl ether	100	33.84*	197.34*
Phloroglucinol trimethyl ether	300	32.20*	205.28*
Diazepam	3	23.30*	241.04*

*=p<0.05 vs. Control

Table 5: Effect of phloroglucinol on Foot-shock chronic stress-induced gastric ulceration in rats

Treatment groups	Dose (mg kg^{-1}, p.o.)	Ulcer incidence (%)	Number of ulcers (N)	Severity of ulcers (severity score)	Ulcer index
Control+Stress	-	100	9.67±0.76	3.00±0.26	22.67±0.95
Phloroglucinol+Stress	1	83.00*	7.50±0.34*	2.83±0.31	18.63±0.56*
Phloroglucinol+Stress	4	67.00*	4.83±0.48*	2.33±0.21	13.87±0.60*
Phloroglucinol+Stress	16	33.00*	3.00±0.26*	2.17±0.17	8.47±0.17*
Panax ginseng+Stress	100	50.00*	2.50±0.22*	1.83±0.17*	9.33±0.33*

*=p<0.05 vs. Control+Stress. N = Number

Table 6: Effect of phloroglucinol on cold restraint stress-induced gastric ulceration in rats

Treatment groups	Dose (mg kg^{-1}, p.o.)	Ulcer incidence (%)	Number of ulcers (N)	Severity of ulcers (severity score)	Ulcer index
Control+Stress	-	83.00	6.33±0.21	2.83±0.17	17.47±0.17
Phloroglucinol+Stress	1	67.00	5.33±0.21*	2.17±0.17*	14.20±0.22*
Phloroglucinol+Stress	4	50.00	4.00±0.37*	1.83±0.17*	10.83±0.31*
Phloroglucinol+Stress	16	50.00	3.00±0.26*	1.83±0.17*	9.830±0.31*
Panax ginseng+Stress	100	67.00	2.17±0.17*	1.67±0.21*	10.53±0.17*

*=p<0.05 vs. Control+Stress. N: Number

Table 7: Chronic stress-induced changes in adrenal gland and spleen weights in rats

Treatment groups	Dose (mg kg^{-1}, p.o.)	Weight of adrenal gland (mg/100 g)	Weight of spleen (mg/100 g)
Control	-	19.00±0.73	225.33±1.36
Control+Stress	-	32.17±0.54*	173.50±1.61*
Phloroglucinol+Stress	1	30.83±0.31	183.33±1.20#
Phloroglucinol+Stress	4	28.00±0.37#	186.17±0.95#
Phloroglucinol+Stress	16	27.17±0.31#	189.00±0.37#
Panax ginseng+Stress	100	25.50±0.43#	213.50±0.85#

*=p<0.05 vs. Control; #p<0.05 vs. Control+Stress

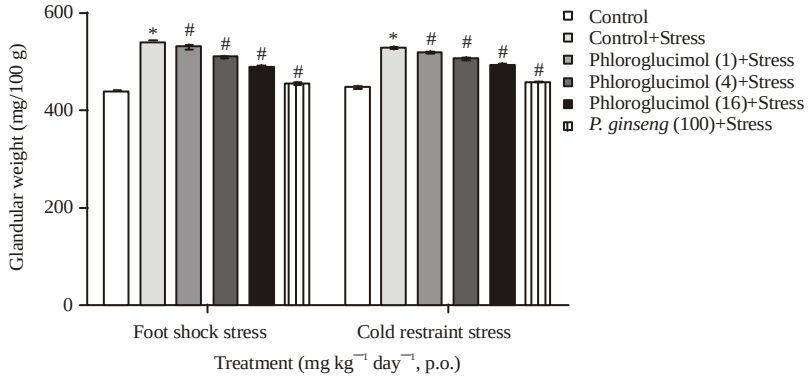

Fig. 1: Effect of phloroglucinol on Foot-shock and cold restraint stress-induced changes in the glandular weight of the stomach in stressed rats. *=p<0.05 versus Control; #=p<0.05 versus Control+Stress

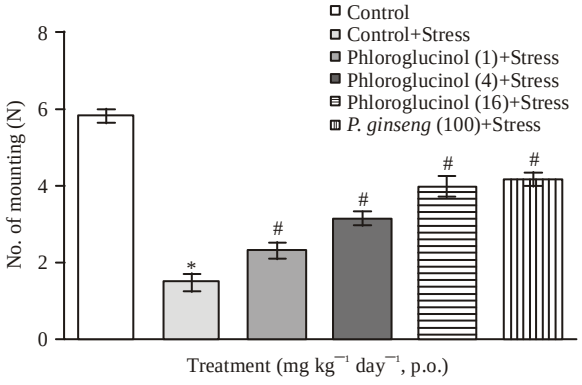

Fig. 2: Effect of phloroglucinol on sexual behaviour in stressed male rats. *=p<0.05 versus Control, #=p<0.05 versus Control+Stress

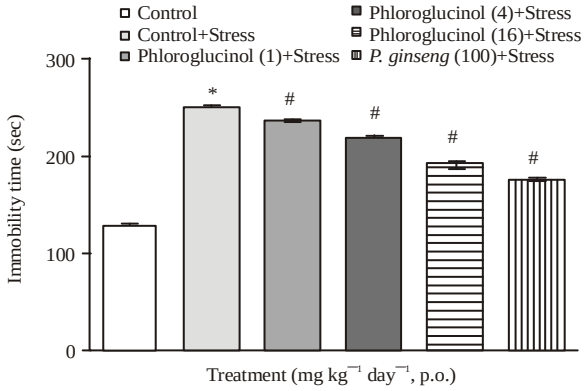

Fig. 3: Effect of phloroglucinol on behavioural despair test in stressed rats. *=p<0.05 versus Control; #=p<0.05 versus Control+Stress

(16 mg kg^{-1} day^{-1}) in the sexual behaviour test was quantitatively similar to that of the herbal adaptogen *Panax ginseng*. However, the efficacy of phloroglucinol in compensating stress-induced alterations in the weights of adrenal gland and spleen (Table 7) were somewhat lower than that of *Panax ginseng*. Such were also the cases in behavioural despair test for antidepressants (Fig. 3) and in the novelty induced suppressed feeding (Fig. 4)

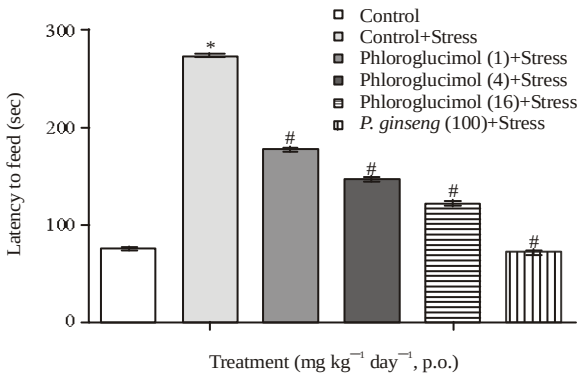

Fig. 4: Effect of phloroglucinol on novelty induced suppressed feeding latency test in stressed rats. *=p<0.05 vs. Control; #=p<0.05 versus Control+Stress

Table 8: Effect of phloroglucinol on elevated zero maze test in stressed rats

Treatment groups	Dose (mg kg^{-1}, p.o.)	Time spent in open arms (sec)	Entries in open arms (N)	Head dips (N)	Stretched attend postures (N)
Control	-	65.50±1.70	5.33±0.33	8.17±0.17	3.17±0.17
Control+Stress	-	22.50±0.76*	2.00±0.26*	4.00±0.26*	2.00±0.26*
Phloroglucinol+Stress	1	32.67±0.71#	3.33±0.21#	5.67±0.33#	2.50±0.22
Phloroglucinol+Stress	4	41.83±0.48#	4.00±0.26#	6.67±0.33#	3.17±0.17#
Phloroglucinol+Stress	16	52.17±0.87#	7.50±0.43#	11.50±0.43#	4.17±0.17#
Panax ginseng+Stress	100	77.17±0.54#	8.17±0.31#	12.67±0.49#	3.67±0.21#

*=p<0.05 vs. Control, #=p<0.05 vs. Control+Stress. N: Number

and elevated zero maze test (Table 8) for anxiolytics. In all these tests significant (p<0.05) effects of the lowest tested dose of phloroglucinol (1 mg kg^{-1} day^{-1}) was observed and its observed effects in all of them were always strictly dependant on its daily dose.

DISCUSSION

Although for therapeutic purposes phloroglucinol tri-methyl ether is often co-administered with phloroglucinol as yet little attention has been paid to its psychotherapeutic potentials. However, it has been reported to be a urinary biomarker for epigallocatechin consumption (Loke *et al*., 2009) and that it is an effective (more potent than penicillin) bactericidal agent (Bowden and Ross, 1965). Therefore, the dose finding experiments were conducted to compare the effects of phloroglucinol and its tri-methyl ether in a battery of rodent models commonly used in laboratories for identifying potential adaptogenic potentials of traditionally known medicinal plants (Chatterjee and Kumar, 2012). Results of these experiments revealed that both of them suppressed stress-triggered hyperthermia after their repeated daily administrations only. Although their maximal possible effects were observed after their 30 mg kg^{-1} day^{-1} doses, the calculated ED_{50} value of phloroglucinol (17.22 mg kg^{-1} day^{-1}) against the stress response was somewhat higher than that of its tri-methyl ether (8.88 mg kg^{-1} day^{-1}). This could be due to more rapid catabolism of phloroglucinol by gut microbiota or due to higher bactericidal efficacy of its tri-methyl ether. It must be noted though, that the dose response relationships of both of them in potentiating pentobarbital sleep were almost identical and that again in this test their maximal efficacies were also observed after their 30 mg kg^{-1} daily doses.

Stress-induced hyperthermia test is often used for identifying benzodiazepines like anxiolytic agents well known for their pentobarbital sleep potentiating effects (Zethof *et al*., 1994; Groenink *et al*., 2009). Therefore, the observed effects of phloroglucinol and its tri-methyl ether in the dose finding experiments could as well be due to their modulating effects on the benzodiazepine site of GABA receptors. However, it must be noted that the pentobarbital sleep test was conducted in animals stressed twice before the experiments. Foot-shock stressed mice are more sensitive to pentobarbital (Carmody, 1983) and that induction of pentobarbital metabolizing enzymes is another stress-triggered effect in rodents (Driever and Bousquet, 1965). Therefore, it could as well be that the observed effects of the phloroglucinols in the pentobarbital sleep test are due to their inhibitory effects on metabolizing enzymes.

Since phloroglucinol is the smallest possible aromatic metabolite of many polyphenolic components

of herbal adaptogens, further efforts were made to pharmacologically better define its activity profile in stressed animals. Reported results clearly reveal that fairly low daily oral daily doses of phloroglucinol affords protection against gastric ulcers induced either by a shorter period of cold restraint stress or by repeated daily exposures to Foot-shock stress for 21 consecutive days. In both the test significant protective effects of phloroglucinol were observed even after its lowest dose tested (1 mg kg^{-1} day^{-1}). However, even after its highest tested dose (16 mg kg^{-1} day^{-1}) only partial protections against ulcers were observed. Since in cold restraint stress model the efficacy of the 4 mg kg^{-1} day^{-1} doses was marginally higher than that of 16 mg kg^{-1} day^{-1}, it appears that even very high phloroglucinol doses cannot afford completely protection against stress-induced ulcers and other pathologies. This inference is in agreement with the observed ceiling effect of phloroglucinol in the stress-induced hyperthermia test. During the course of these studies, a report revealing tumour angiogenesis suppressing effect of 21 days treatments with 0.94 and 9.4 mg kg^{-1} day^{-1} oral doses of phloroglucinol has appeared (Kwon *et al*., 2012). In this report, the efficacies of both the phloroglucinol doses tested were almost identical and the epithelial progenitor cells were identified as the cells involved in the observed effects of phloroglucinol. Interestingly, beneficial effects of dietary flavanols on angiogenic progenitor and endothelial cells have also been implicated in their health benefits (Heiss *et al*., 2010). Since angiogenesis plays a pivotal role in gastric ulcer repair (Takahashi *et al*., 1997), the observed ulcer protective effects of phloroglucinol might be due to its actions on epithelial progenitor cells of the gastrointestinal tract. If such would indeed be the case, phloroglucinol itself could be used as a cheaper dietary immune modulator for prevention of stress mediated organ pathologies.

The ultimate goal of this study was to experimentally verify the possibility that phloroglucinol is a bioactive transient metabolite of some plant polyphenolics involved in their reported beneficial effects on mental health (Vauzour, 2012). Brain is the key organ responding to stress (McEwen and Gianaros, 2011; Zoladz and Diamond, 2008) and disorders of stress system are also the root causes of most mental health problems (Adler *et al*., 1984; Chrousos, 2009). Exaggerated anxiety and depression and sexual behaviour are major stress-induced psychopathologies and we have consistently observed that these abnormalities can be quantified in the rodent Foot-shock paradigm used in this study. It is apparent from the results of behavioural test presented in this communication that fairly low daily oral doses of phloroglucinol effectively prevent all stress-induced behavioural abnormalities quantified. Moreover, stress-induced adrenal hypertrophy as well as spleen atrophy was also significantly less pronounced in the phloroglucinol treated groups. These observations reconfirm that the observed behavioural effects of phloroglucinol are due to its anti stress activity and that most probably its site(s) of action(s) is not the brain tissue. Thus, it seems reasonable to suggest that phloroglucinol is most probably a psychoactive transient metabolite of some flavonoids and that most probably the gut-brain axis is also involved in their mode(s) of action(s).

CONCLUSION

Phloroglucinol and its tri-methyl ether suppressed stress-induced hyperthermia strongly suggest that the tri-oxygenated benzene moiety could as well be a common pharmacophore of numerous plant flavonoids and that their phenolic functions are not essential for their adaptogenic potentials. Since both of them posses antibacterial activities and it is now apparent that gut microbiota plays an important role in pathogenesis of environmental stress triggered depression, they could be useful experimental tools for identifying novel pharmacological targets and drug leads useful for combating diverse stress-triggered pathological conditions commonly associated with co-morbidities of depressive disorders. Attempts to experimentally verify such possibilities now are made in laboratories.

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