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In Vivo Evaluation of the Antidepressant Activity of a Novel Polyherbal Formulation

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Abstract

The present study was undertaken to evaluate the anti-depressive activity of a polyherbal formulation (PF) after 28 days administration by using a rat forced swimming test (FST) and tail suspension test (TST). Animals were divided into six groups (n=6/group): group-1: The control rats received injections of 0.5% CMC solution only; group 2: FST+vehicle, groups 3: FST+SER (10 mg/kg, i.p.); group 4: FST+200 mg/kg; group 5: FST+400 mg/kg group 6; FST+800 mg/kg and drugs were administrated once daily for 28 days treatment. To assess the effect of PF on immobility activity through FST and TST were used to take as a measure of antidepressant activity. The probable mechanism of action of the anti-depressive effect of PF was also investigated by measuring the level of serotonin, dopamine, norepinephrine, MAO, GABA, homocysteine, IL-2 and IFN-gamma levels in the blood of the stress rats.PF significantly reduced the immobility time of rat in both the FST and TST. However, might explain the results. In addition, PF decreased the MAO, homocysteine, IL-2 and IFN-gamma level while it increased the levels of serotonin, dopamine, nor epinephrine and GABA in the blood. PF with 200 mg/kg treatment have shown the more significant improvement in stress rats. After 28 days administration, PF produced antidepressant-like effects. The mechanisms of action of anti-depressive effect of PF seemed to involve an increase of the monoamines level (serotonin, dopamine, norepinephrine, MAO, GABA) while decreasing the MAO, inflammatory marker and homocysteine in the stress of rats.

Keywords: Depression; Antidepressant; *Nyctanthes arbortristis; Hippophae salcifolia; Ocimum tenuiflorum; Reinwardtia indica;* Forced swimming test; Tail suspension test; Neurochemicals; Homocysteine; Inflammatory marker

Introduction

Depression is the most prominent and a highly incapacitating neuropsychiatric disease [1].World Health Organization (WHO) reported is the most burdensome diseases of society [2] and associated with miserable symptom and sign including mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy and poor concentration. It is also a multifaceted disease with heterogeneous pathology [3]. Stress is involved in the dysfunction of hypothalamic-pituitary-adrenal axis (HPA) and nervous system along with the discrepancy of neurochemicals, neurocytokine secretion and other biochemical [4-7]. Impairment of monoaminergic and neurotransmitter functions are also well-recognized factor in the etiology of depression [8,9].

Depression is also associated with a specific immunological state and imitative from the observation of so-called "sickness behaviour". Pro-inflammatory cytokines trigger sickness behaviour [10].Recent report have declared that increased pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF)-α and interferon (IFN)-γ, significantly, involved in the succession of depression [11,12]. In course of depression, stress-induced alteration in the cytokine system is linked to genetic abnormalities, mRNA expression, intracellular, serum or saliva cytokine levels and neurotransmitter concentrations [13-16]. However, depression may dysfunction the immune system of the body.

Bottiglieri et al. reported that the elevated homocysteine (hcy) or hyperhomocysteinemia (Hhcy) level plays a significant role in the onset of neurodegenerative disorders (Alzheimer's and Parkinson's disease) including several neuropsychiatric disorders (depression, schizophrenia and bipolar disorder). Hhcy indicated a failure of methylation of homocysteine to methionine due to a deficiency in the delivery of methyl groups from methyl folate [17]. Methionine is the immediate forerunner of S-adenosylmethionine (SAM), the methyl donor in innumerable methylation reactions in the synthesis of monoamines, neurotransmitters, nucleoproteins and membrane phospholipids. So, that the failure of one carbon metabolism in depression with folate deficiency associated with a high plasma homocysteine, was also accompanied by a significant fall in SAM [18-21].

New therapeutic products from the medicinal plant are used and progressed constantly, for the treatment of neuropsychiatric disorders. An increasing use of traditional medicine as antidepressant showed that traditional prescription drugs exhibited certain clinical efficacy,

enhanced efficacy, reduced dosages and side effects of modern medicines. In the ancient traditional system of medicine, several single and polyherbal formulations (PF) are mentioned for the treatment of psychiatric disorders [22]. PF generally provide synergistic effect and help to minimize the adverse effects of the major drugs [23].

In the present study, we were prepared the first time this polyherbal formulation (PF) with the following composition with their bioactive molecules *Nyctanthes arbortristis* (it possess serotonergic properties and its leaves have numerous bioactive chemicals, including oleanolic acid, nyctanthic acid and iridoid glycosides (arborsides A, B, C) with potential health benefits [24,25]). *H. salicifolia* is a nutrient effluent plant, having anti-oxidant properties and anti-inflammatory due to the presence of cerebroside, flavoind, folic acid, 1-O-hexadecanolenin and qurecetine [26]. *O. tenuiflorum* possesses cholinergic properties and contains eugenol, which has reported acetylcholinesterase inhibitory activity [27,28]. *R. indica* contains terpenoids, glycosides and saponins, which could potentially help in the management of hyperglycemia [29]. However, to date, the pharmacological effects and especially the antidepressant effect of this PF have not been established. Thus, we evaluated the antidepressant activity of this PF for Force Swim Test rats model (FST) to get a better understanding of this extract.

Although, animal stress models are widely used in the pre-clinical evaluation of antidepressants. The behavioral despair tests (forced swim and tail suspension test) were developed using rats for the development of antidepressant drugs [30,31]. Since then it has become one of the most widely used tests for antidepressant screening [32,33].

Moreover, we determined whether the alteration of biogenic amines [serotonin (5HT), dopamine (DA), monoamine oxidase (MAO), gamma-aminobutyric acid (GABA)] and homocysteine (Hcy) might predict the antidepressant properties of PF. We evaluated the effect of PF on the immune system by measuring alterations in serum inflammatory markers like interleukin-2 (IL-2), Interferon gamma (IFN-Y) which is involved in the rats FST model of depression.

Material and Methods

Animals

The experiments were performed on healthy albino Wistar rats (AW) (150-200 g) of both sexes procured from Animal Central House, Institute of Medical Science, Banaras Hindu University, Varanasi, India. The rats were kept in plastic cages with paddy husk as bedding in the animal house with a regulated ambient temperature of 23 ± 2°C, a relative humidity of 30–70% with a 12 h/12 h light dark cycle and had free access of food with water ad libitum. The experiments were carried out between 10.00 to 5.00 h and animals were allowed to acclimatize to the laboratory conditions for 7 days prior to dosing. The water used to be always double distilled. The animals were used only once for each experiment. The experimental studies were performed in accordance with the Guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee of Institute of Medical Science, Banaras Hindu University.

Plant collection and identification

Professor Dubey NK, Department of Botany, Faculty of Science, Banaras Hindu University (Varanasi, India) was identified and authenticated the leaves of *N. arbortristis*, fruits and leaves of *H. salicifolia*, whole plant of *O. tenuiflorum* and roots of *R. indica*. These plants were collected from forests of India and fruits and leaves of *H. salicifolia* were collected from Himachal Pradesh (Leh and Laddakh) respectively. The voucher specimens of the plants have been deposited in the herbarium for further reference.

Extraction of the plant material

The shed dried leaves of *N. arbortristis*, fruits and leaves of *H. salicifolia*, whole plant of *O. tenuiflorum*, seeds and roots of *R. indica* were used for the preparation of extracts. The shade dried and powdered plant materials (500-700 g) were extracted with ethanol (70%) by a cold extraction process. The extract was then concentrated in-vaccuo and the yield percent was calculated (Table 1). Extracts were stored at 4°C until use.

S. No.	Plant	Percentage yield
		70% ethanol
1	*Nyctanthes arbortristis*	0.91-2.11
2	*Ocimum tenuiflorum*	0.84-0.98
3	*Hippophae salicifolia*	1.22-1.27
4	*Reinwardtia indica*	0.98-2.03

Table 1: The concentration of the major phyto-constituents in the extracts depends on the percentage yield of the hydro alcoholic extracts of PF.

Drugs, reagents and drug administration

Sertraline hydrochloride (SER), a SSRI antidepressant, was gifted from Pharmaceutical Company, Badi, Punjab and was used as standard drug for antidepressant effect. PF has been prepared in this composition: *Nyctanthes arbortristis*-75 mg/kg, *Hippophae salicifolia*-50 mg/kg, *Ocimum tenuiflorum*-40 mg/kg and *Reinwardtia indica*-35 mg/kg and all drugs were dissolved in 0.5% Carboxyl Methyl cellulose (CMC). All the chemicals used were of analytical grade from standard companies.

The effects of drug administration were examined on the total duration of immobility for the rats during the FST. Rats were administered with SER and PF, 60 min before the test swim session [34].SER was administered intraperitoneal in a volume equivalent to 2 ml/kg. PF were each given orally by gavages and the drug solutions were freshly prepared each morning. Doses were calculated as mg/kg of base and determined as described previously [35].

The 36 AW rats were equally and randomly assigned in to six groups- group-1: The control rats received injections of 0.5% CMC solution only; group 2: FST+vehicle, groups 3: FST+SER (10 mg/kg, i.p.); group 4: FST+200 mg/kg; group 5: FST+400 mg/kg group 6; FST +800 mg/kg.

Drugs were administrated once daily for 28 consecutive days to animals. Forced swimming was used to induce stress in rats and has shown the alterations in biogenic amines, neurocytokine and homocysteine, etc. The method for stress was similar to FST below [30-33]. All the groups of rat were subjected to a swimming test except group I.

Behaviour Despair Study

Immobility time measured by forced swim test (FST)

The FST is the most widely used pharmacological models for assessing antidepressant activity and was performed according to the method described by Porsolt with slight modifications [30,31]. FST was consisted of two parts; an initial training period of 15 min and an actual test for 5 min after 24 h. Rats were exposed to forced swimming stress daily for a duration of 5 min between 11.00 AM to12.00 AM till 28 days. During training rats were placed in the glass jar for the first time the rats were initially highly active, vigorously swimming in circles, and trying to climb the wall or diving to the bottom. After 2–3 min, their activity began to subside and was interspersed with phases of immobility or floating of increasing length. After 5–6 min, immobility reached a plateau where the rat remained immobile for approximately 80% of the time. After 15 min in the water, the rats were removed, wiped with a dry cloth and allowed to dry before being returned to their home cages. The glass jar was emptied and washed thoroughly after testing for each rat. The rats were again placed in the jar 24 h later, after initial administration of the SER and PF and their activity was recorded within 5 min. The time spent performing the behaviour alteration test was measured using a video camera.

Tail suspension test (TST)

Tail suspension induced the immobility was measured According to Steru et al. [38]. This is a simple, rapid and reliable method to screen antidepressants. In this method rat were suspended above the floor by adhesive tape placed approximately 1-2 cm from the tip of the tail and shows alternate agitation and immobility which is indicative of a state of depression. The remained immobile time of TST was quantified for 6 min. Rats were considered immobile only when they hung passively and completely motionless.

Blood collections

The animals were sacrificed by cervical dislocation immediately after FST. A total of 10 mL of blood was withdrawn immediately from orbital puncher from all groups, occurred during the light phase. Divided the blood sample in to two parts. One was collected in potassium (K2) ethylene diamine tetra acetic acid (EDTA)-coated tubes and other in plan tube. Blood sample of EDTA coated tubes was centrifuged (2000 rpm) at 4°C for 20 min and separated the plasma. It was collected and dispensed into 1.5 ml eppendorf tube and stored at –80°C until assay. For serum, blood samples were left to clot for 2 h prior to centrifugation for 15 min at 4000 rpm.

Quantification of serotonin, dopamine, norepinephrine, MAO, GABA, homocysteine, IL-2 and IFN-gamma. Serotonin and IFN gamma were estimated in serum using by available kit enzyme-linked immunosorbent assay (Cata. No. KA1894; Cata. No. NBP1-92680). Dopamine, norepinephrine, MAO, GABA, homocysteine and IL-2 in plasma were also quantified by ELISA (Cata. No. KA1887; Cata. No. KA1891; Cata. No. KA1242; Cata. No. FLMAO100-3, Cata. No. OKEH02564 and Cata. No. KA0986, respectively).Plasma samples were thawed on ice and further processed according to manufacturer's instructions). Except GABA ELISA kits (from Aviva Systems Biology) from Novus Biologicals a bioteche brands.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Data was analyzed using a statistical package (Statistical Package for the Social Sciences (SPSS). FST immobility time, monoamines, homocysteine and cytokine data were analyzed by one-way analysis of variance (ANOVA) with drug administration. If the P value is less than 0.05, the difference was considered statistically significant.

Results

Effects of the hydro-alcoholic extract of PF and Sertraline on immobility time in the rat FST

Force Swim stress (FSS) significantly increased the immobility period as compared to control rats. The clinically effective antidepressant drug Sertraline hydrochloride (10 mg/kg, ip) administered for 28 successive days significantly decreased the immobility period in stressed rats as compared to the stress controls. Whereas when compared to healthy control, it was non- significant. As like this the hydro-ethanolic extract of PF at 200, 400 and 800 mg/kg significantly reduced the duration of immobility ($F=92.75$, $P<0.001$), when orally administered for 28 days. We observed the maximal antidepressant action by PF was obtained at a dose of 200 mg/kg, resulting in 54.24% immobility reduction. PF, produced a marked reduction in immobility time and there was a significant effect of the PF administration for the immobility time in the FST as seen in Figure 1.

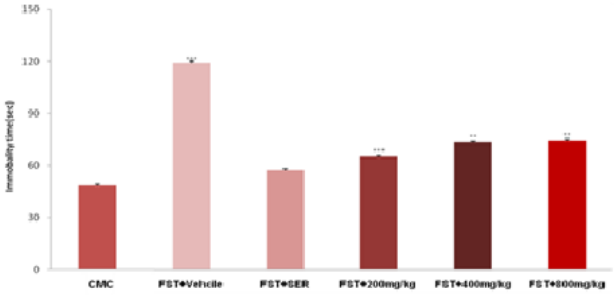

Figure 1: Effects of the hydro alcoholic extract of PF at 200, 400 and 800 mg/kg, Sertraline at 10 mg/kg on the immobility time in the stressed rats FST.

FS significantly increased the immobility period as compared to vehicle-treated unstressed rat. Sertraline (10 mg/kg, ip) administered for 28 successive days significantly ($P<0.001$) decreased the immobility period in stressed rats as compared to the unstressed and stressed controls. PF (200,400,800 mg/kg) orally administered for the same days to stressed rats and show highly significant effect on immobility period ($F=143.40$, $P<0.001$) the immobility period in stressed rat as compared to its stress and unstressed control (Figure 2).

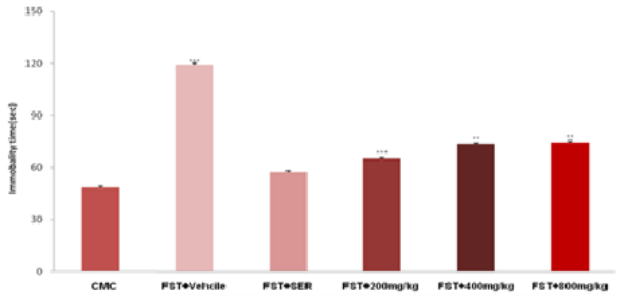

Figure 2: Effects of the hydro alcoholic extract of PF at 200, 400 and 800 mg/kg, Sertraline at 10 mg/kg on the immobility time in the stressed rats TST.

Effects of the hydro-alcoholic extract of PF and Sertraline in monoamine neurotransmitter, homocysteine level and inflammatory markers in the rat FST

Effects of the hydro-alcoholic extract of PF and Sertraline in serotonin (5-HT), dopamine (DA), mono-amineoxidase (MAO), gamma amino butyric acid (GABA) and norepinephrine (NE)

The effects of the hydro-alcoholic extract of PF and Sertraline after 28 repeated day treatments in the monoamine neurotransmitter levels in the rat FST were shown in Table 2. Significant decreased in serotonin (5-HT), dopamine (DA), gamma amino butyric acid (GABA) and norepinephrine (NE) levels in rats were observed after swim stress (5-HT: $F=32.54$, $P<0.01$; DA, $F=142.59$,$P<0.001$; GABA, $F=11.682$, $P<0.01$; NE, $F=54.46$, $P<0.001$) whereas increased monoamine oxidase (MAO) ($F=16.331$, $P<0.001$) compared to respective healthy control (Table 2).

Hydro-alcoholic extract of PF (200,400 and 800 mg/kg) produced a significant ($p<0.001$, $p<0.01$, $p<0.01$) increase in 5HT levels in rat as compared to its control. Sertraline did not significantly increase, but markedly elevated the 5HT in rat, when compared with its control. On the other hand, the hydro alcoholic extract was significantly attenuated swim stress induced increase in 5-HT level in rat. The maximal effect was obtained at 200 mg/kg which showed highly significant. PF was also attenuated the decrease in brain DA and GABA levels induced by swim stress, the maximal effect value being 200 mg/kg ($P<0.001$ and <0.01). The extract at 400 and 800 mg/kg reversed the decreased levels of the normal value. Sertraline was also significantly altered the DA levels (Table 2).

Similarly, swim stress markedly reduced NE levels ($F=54.46$, $P<0.001$) whereas we were observed PF to elicit an increase NE levels. The maximal effect value being 200 mg/kg, which return the swim stress-induced decrease in NE levels to the normal value. However, Sertraline failed to significantly change the swim stress-induced decrease in NE levels ($P=0.06$) (Table 2).

As we know the forced swim stress increased MAO levels in the plasma whereas Administration of the PF (200 mg/kg) enhanced the reduction in the level of MAO level at 28th days, as compared to compared to respective FST ($P<0.01$). On the other hand, Sertraline significantly decreased in MAO levels ($P<0.001$) (Table 2).

Effect of PF and Sertraline in the level of homocysteine (Hcy)

Force stress resulted in significant ($p<0.001$) increase in Hcy level as compared to vehicle-treated unstressed rat. PF (200, 400 and 800 mg/kg) and Sertraline (10 mg/kg) per se administered for 28 consecutive days significantly reduced Hcy level in rat ($p<0.001$, $p<0.01$, $p<0.01$and $p<0.01$; $p<0.001$, respectively) in the rat whose stressed by force swims as compared to control rats. Chronic treatment with PF (200,400 and 800 mg/kg) and Sertraline (10 mg/kg) markedly reduced the level of both inflammatory markers (Table 2).

Sertraline failed to significantly decrease IL2 levels ($p<0.36$) whereas PF (200, 400 and 800 mg/kg) per se produced a significant ($p<0.001$, $p<0.05$ and $p<0.05$, respectively) decrease in the IL2 levels in stressed rats as compared to respective control (Table 2).

IFN-Y levels were increased significantly ($p<0.001$) after chronically stressed rats as compared to control rats. Chronic treatment with PF (200, 400 and 800 mg/kg) and Sertraline (10 mg/kg) per se produced a significant ($p<0.001$) reduction in IFN-Y levels in the stressed rat as compared to the respective controls. However, PF was showed a greatly significant decrease IFN-Y level in rat as compared to their respective control groups (Table 2).

Group	Monoamine neurotransmitter levels (pg/ml)				
	5HT	DA	NE	MAO	GABA
CMC	952.5 ± 3.8	1277.9 ± 6.2	389.6 ± 6.0	4.2 ± 0.09	0.3 ± 0.02
FST+Vehcile	566.1 ± 6.8***	946 ± 18.3**	262.1 ± 9.8***	6.7 ± 0.38***	0.2 ± 0.01**
FST+SER	852.9 ± 50.2	1259 ± 8.0***	363.9 ± 1.3	3.7 ± 0.15***	0.4 ± 0.04
FST+200 mg/kg	841.8 ± 17.0***	1214.1 ± 17.1***	362.9 ± 4.4**	5.2 ± 0.19**	0.4 ± 0.01
FST+400 mg/kg	710.2 ± 22.49*	1100.5 ± 1.3*	317.6 ± 2.9*	5 ± 0.38*	0.4 ± 0.01
FST+800 mg/kg	687.8 ± 14.2*	1027 ± 5.3*	326.7 ± 6.1*	5.5 ± 0.1*	0.3 ± 0.004

Table 2A

Group	Pro-inflammatory marker (pg/ml)	
	IL-2	IFN-Y
CMC	0.2 ± 0.01	15.7 ± 1.0
FST+Vehcile	1.6 ± 0.2*	29.9 ± 0.2**
FST+SER	0.3 ± 0.01*	19.5 ± 0.4**
FST+200 mg/kg	0.3 ± 0.01*	17.2 ± 1.5***
FST+400 mg/kg	0.3 ± 0.02*	23.8 ± 0.2*
FST+800 mg/kg	0.4 ± 0.02*	21.8 ± 0.3*

Table 2B

Group	Homocysteine (μ mol/)
CMC	3.9 ± 0.2
FST+Vehcile	9.1 ± 0.2***
FST+SER	5.3 ± 0.1*
FST+200 mg/kg	5.6 ± 0.2***
FST+400 mg/kg	6.4 ± 0.2**
FST+800 mg/kg	6.4 ± 0.1*

Table 2C, **Table 2 (A,B and C):** Effects of the hydro alcoholic extract of PF at 200, 400 and 800 mg/kg, Sertraline at 10 mg/ on monoamine neurotransmitter levels (Table 2A), pro-inflammatory (Table 2B)and homocysteine (Table 2C) in the rat FST.

Discussion

The present study showed that administration of the hydro-alcoholic extract of PF could normalize behavioral, monoamines, homocysteine and neurocytokine alterations induced by swimming stress. These findings confirmed antidepressant-like effects of the hydro-alcoholic extract of PF through neurochemicals, homocysteine and neurocytokine mechanisms.

Ancient medicine is widely held to beneficial but generally neither the active principles nor their molecular targets are well defined [39], therefore, an understanding of the active component(s) and the mechanism(s) of action can make such medicines more acceptable. The present polyherbal formulation (PF) was prepared by following composition-*Nyctanthes arbortristis*, *Hippophae salicifolia*, *Ocimum tenuiflorum* and *Reinwardtia indica*.

Nyctanthes arbortristis (family Oleaceae), is used as an medicinal plant owing to its numerous medicinal properties. In Ayurveda, its leaves used for the improvement of memory, treatment of nervous system disorders, hypnotic, tranquilizing and local anaesthetic activities. It is possessing anti-depressant, anti-convulsive activity, improvement of memory and CNS depressant action [24,25,40]. Due to contain of several bioactive molecules like mannitol, glucose, essential oil, carotene, β-amyrin, β-sitosterol, hentriacontane, benzoic acid, triterpenoid (oleanolic acid, nyctanthic acid, friedeline, lupeol tannic acid, ascorbic acid, methyl salicylate, an amorphous glycoside) and iridoid glycosides (arborsides A, B, C) [24,25].

Hippophae salicifolia (family Elaeagnaceae) also known as seabukthron (SBT), is used in traditional Chinese medicine since the Tang Dynasty, going back more than 1000 years. This plant is a deciduous and medicinal tree with great nutritional value. Its berries have several bioactive such as cerebroside, oleanolic acid, ursolic acid, 19-alpha-hydroxyursolic acid, dulcioic acid, 5-hydroxymethyl-2-furancarbox-aldehyde, cirsiumaldehyde, octacosanoic acid, palmitic acid and 1-O-hexadecanolenin with a wide range of pharmacological activities including antioxidant, immuno-modulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective, and tissue repair properties [26]. The berries have preventive effects against cardiovascular diseases, mucosa injuries, skin problems, cancer and altered immune system function [41-43].

Ocimum tenuiflorum (family Lamiacea) in Ayurveda has been used for wide range of pharmacological activity such as adaptogenic, anti-asthmatic, anti-oxidant, COX-2 inhibitor and anti-inflammatory because of presence of core chemical constituents like eugenol, oleanolic acid, ursolic acid, rosmarinic acid, etc., Eugenol, (4-9%) Carvacrol, Linalool, β-Caryophyllene 8%, β –elemene , 11%, Germacrene D 2%, β–bisabolene 13-20%, Methyl chavicol 3-19%, 1-8 cineol 9-33%, α-bisabolene, α-terpineol. Due to the presence of high concentration of eugenol, it is used as anti-analgesic, anti-hyperlipidemic and showed cardioprotective effects, promotes immune system function and involved in cell repairing [27,28,44,45].

Reinwardtia indica (basanti) belongs to the Linaceae family and is found in the Himalayan range [29], traditionally used in the treatment of paralysis, wounds, cuts, boils and carbuncle [46]. The hydro-alcoholic extract of the leaves has anti-bacterial and anti-oxidant (nitric oxide radical scavenging activities) properties *in vitro* [47].

Numerous studies have shown force swims induced depression model can be used for evaluating the potential antidepressants by employing behavioural tests like FST and TST. FST and TST are commonly used behavioural despair models in rodents to predict antidepressant potential by measuring the decrease in immobility periods and both are valuable for probing the mechanism of depression [30,31,38]. Induction of depression using force swim stress is considered as the most valid animal model of depressive behavior observed in humans after a long-term exposure to multiple stressor [30,31,38].

In the present study, rat that was exposed to stress, exhibited greater immobility periods in the FST and TST as compared to healthy control animals, thus showed depression-like behaviour. Sub-chronic treatment with SER (10 mg/kg, ip) or PF (200, 400, 800 mg/kg, oral) produced a significant decrease in immobility periods of stress rat in the FST and TST, indicating significant antidepressant-like activity. However, in the forced swim stress AW rat, the hydro-alcoholic extract of PF produced a significant reduction in immobility time when orally administered for 28 consecutive days. In addition, the compounds enhancing mobility activity may give rise to a false positive effect in the stress rat. Thus, antidepressant-like activity of PF in stressed rats is specific. PF caused a reduction in the immobility time in FST and TST (Figures 1 and 2, respectively,). The results presented here show, to our knowledge for the first time, that PF given orally is effective in producing significant antidepressant-like activity, when assessed in the FST and TST. In both tests, anti-depressants can also be distinguished from stimulants, because stimulants cause marked motor stimulation, in contrast to antidepressants, which do not [1,32].

A moment ago clinically employed antidepressants exert their effects predominantly on monoaminergic system [1,3], cytokine [52-55] and homocysteine [21,56-58] although it is unlikely that pharmacological manipulation of a neurotransmitter in relative isolation would produce changes sufficient to remedy severe neurochemicals dysfunction and other biochemical [1-3]. Numerous evidence from anatomical, electrophysiological and pharmacological studies that the interactions between neurotransmitter and other biochemical systems are imperative in depression [32].

Monoamine neurotransmitters, including 5-HT, NE, MAO, GABA and DA play important roles in depression and in mediating behavioural effects of antidepressant drugs. Abnormal monoamine levels in the FSS rat may be relevant to the depressed state. Force swims stress produced the reductions in 5-HT, NE, GABA and DA whereas also increased MAO in AW rat. Of great interest were the results showing that the hydro-alcoholic extract of PF clearly elevated the decreased monoamine neurotransmitter levels and enhanced the MAO induced by swim stress. As shown in Tables 2, PF (200, 400 and 800 mg/kg, p.o) increased the level of 5-HT, NE, GABA and DA in stressed rats, whereas 200 mg/kg, p.o. PF increased the level of 5-HT, NE, GABA and DA with decreased the MAO. These results indicated that the effect of PF on depression may be mediated via the increase in monoamines levels in rats. These findings suggested that antidepressant-like effects of the hydro alcoholic extract of PF were mediated through the modulation of serotonergic, GABAergic system and nor-epinephrinegic neurotransmission system, as well as dopaminergic neurotransmission.

Evidences from both clinical and preclinical studies support that there may be an impairment of inflammatory immune function in depression [59,60].The most frequently occurring cytokine abnormality in depressed subjects is hyperactivity of the Hypothalamic-Pituitary-Adrenal (HPA) axis characterized by glucocorticoids. Stress leads to habituation of the HPA axis response and thus impairs the delayed-type hypersensitivity' (DTH) response [61,62].

Several types of psychological stressors can induce an alteration in neurotransmitter including disturbed functioning of the adaptive immune system, as well as T and B lymphocytes and innate immune cells, particularly natural killer (NK) cells and macrophages. As a result, stress stimuli lead to a variety of changes in the function, shape, signalling and proliferative capacity of neuronal cells. It can also cause decreases in neurogenesis, compressed the hippocampus and impaired the structural neuronal plasticity [63-66].

The 'cytokine hypothesis of depression' demonstrated that psychosocial stressors and internal stressors raised secretion of pro-inflammatory cytokines are supposed to increase the risk of developing depression. More than a little evidence supported the elevations of the pro-inflammatory cytokines such as interleukin (IL)-1, IL-2, IL-6, interferon-g (INF-g) and tumor necrosis factor-a (TNF-a), play a vital role in the progression of depression [67-69].

Molecular mechanisms may contribute to cytokine involvement in the progression of depression. Various stressors raised pro-inflammatory cytokines which stimulate the activity of indolamine-2,3-dioxygenase (IDO) is an enzyme metabolizing tryptophan (act as precursor of serotonin and melatonin) to kynurenine. IDO stimulation increased synthesis of the potent neurotoxic metabolites kynurenine and quinolinic acid and ultimately, reduced synthesis of neurotransmitters [70]. During mechanism raised cytokines trigger up-regulation of serotonin reuptake transporters by increasing gene expression and potentially leading to more rapid reuptake of serotonin from the synaptic cleft [71]. However, cytokine may contribute to dysregulation of the hypothalamo-pituitary-adrenocortical (HPA) axis in depression [72,73] by a state dependent effect on the secretion of adrenocorticotropic hormone (ACTH),cortisol and microglial cells. As result cytokine induced serotonergic dysfunctions by regulating serotonin transporter activity [13,14,70].

Various reports have supported that action of antidepressants may involve U-turn the effects of stress through direct regulation of synaptic architecture, dendritic morphology and survival of neurons and its treatments exert positive actions on the cellular processes. These studies have focused on the hippocampus, and future work will be required to determine the influence of antidepressants on cell survival in prefrontal cortex and other brain regions [75].

Taken together, we may speculate that the effects of the hydro alcoholic extract of PF may produce on inflammation and pro-inflammatory cytokines may contribute to its amelioration on the serotonergic dysfunctions and the impaired feedback inhibition of the HPA axis. Finally contribute to its maintenance of hippocampal morphologic and functional plasticity. In the present study, our findings have shown that the antidepressant-like effects of PF are associated with lowering the level of IL-2 and IFN gamma. Thus, the subtle network and the precise role of each component warrant further explorations. In the present study, our finding has shown PF, acts as an herbal antidepressant, exerts extensive anti-inflammatory activities including the inhibitory activity on IL-2 and IFN gamma. PF with these doses 200, 400 and 800 mg/kg have shown anti-inflammatory indicating that this effect was concomitance with decreases the level of pro-inflammatory marker (IL-2 and IFN gamma). Therefore, we may speculate that the anti-inflammatory effect of these hydro-alcoholic extract is possibly related to their antidepressant-like effect. Our finding suggested that the PF could be beneficial in stress related psychiatric disorders associated with an over activity of the HPA axis system.

As various report hcy is a pro-inflammatory or non-proteogenic sulfur containing redox active endogenous amino acid, derived from dietary methionine through demethylation and can be re-methylated to methionine (precursor of S-adenosylmethionine (SAM)) via the re-methylation or by the action of metabolic enzymes and cofactors [56]. The synthesis of methionine from homocysteine requires a transfer of CH3 groups from methyl folate, and also vitamin B12 as a cofactor. As available report methionine is in turn the instant precursor of SAM. The methylation reactions are essential for the synthesis of monoamines, neurotransmitters, proteins, nucleoproteins, and membrane phospholipids [18].

Various studies have shown higher plasma hcy is a sensitive marker of functional deficiency of either folic acid or vitamin B12. Many types of stress may impair the metabolism of hcy which caused HHcy that raised the neurotoxic effects in the pathogenesis of depression [75]. Previously reported that stress inhibited synaptic transmission at the neuromuscular junction, targeting primarily the motor nerve terminals [76]. However, Hhcy is indicates a failure of methylation of hcy to methionine due to a shortage of supply of methyl groups from methyl folate or, more rarely in depression. Folate deficiency associated with the significant fall in SAM and effects on mood [75,77].

In short, PF have major effective constituents such as nyctanthic, oleanolic acid, elagic acid, flavonoids, folic acid, quercein, tocopherols, ursolic acid, α–terpinene,eugenol, rosmarinic and saphonin [40-48]. Thus, PF have shown their action on various targets involved with depression like monoamine content, pro-inflammatory markers, behavioural pattern and oxidative marker, etc. We hypothesized that PF could be effective in the management of depression or disease caused by imbalance of neurotransmitter, inflammatory and oxidative stress. Our present study can be summarized by following finding– (1) the force swim stress procedure caused depressive-like behaviour in treatment rats, as observed by different behaviour despaired test like FST and TST, (2) significant reduction in depressive-like behaviours was evident in the stressed rats treated with PF and SER (3) forced swim test procedures imbalance the neurotransmitter along with oxidative damage by increasing hcy in the treated rats and (4) PF and SER treatments exerted protective effects against forced swim stress and increase the level of neurotransmitter and decrease the hcy and inflammatory marker in the rat. However, PF with 200 mg/kg treatment have shown the significant improvement and reduced the level of hcy. In our present study, PF may take up the re-methylation reaction for the synthesis of methionine and it is involved in enhancing the concentration of neurotransmitter in stressed rat through the augment the synaptic transmission at the neuro-junction and motor nerve terminals.

Conclusion

In conclusion, our data indicated that low-dose of PF had an antidepressant activity effect on force swim stress induced depression model rats. The behavioral indicators improved compared with model group. We found that 5HT, DA, GABA and NE level in serum and plasma, respectively, were significantly increased whereas MAO were decrease as compared with the model group by ELISA. Thus, we confirmed that PF has antidepressant activity, its mechanism may be related to the decreased levels of pro-inflammatory cytokines (IL-2 and IFN gamma) activity, regulating the function of the HPA axis and inhibiting glucocorticoid receptor expression. The hydro-alcoholic extract of PF suppressed the level of plasma hcy in stressed rat. Lowering effect of PF may restart the re-methylation and increased the concentration of neurotransmitter.

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