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Evalution of Antidepressant Activity of *Picrorrhiza Kurroa* in Mice

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ABSTRACT:

Alcoholic extract of *Picrorrhiza kurroa* (PK extract) (15, 30 and 60 mg/kg, i.p.) was administered once daily for seven successive days to separate groups of young male Swiss albino mice. The immobility periods of control and treated mice were recorded in forced swim test (FST) and tail suspension test (TST). Effect of sulpiride (50 mg/kg, i.p.; a selective D_2 receptor antagonist), prazosin (3 mg/kg, i.p.; an α_1-adrenoceptor antagonist) and p-chlorophenylalanine (p- CPA, 100 mg/kg, i.p.; an inhibitor of serotonin synthesis) on antidepressant effect of PK extract in TST was also studied. The antidepressant effect of PK extract was compared to that of imipramine (10 mg/kg, i.p.) and fluoxetine (20 mg/kg, i.p.) administered for seven successive days. PK extract produced significant antidepressant effect at a dose of 15, 30 and 60 mg/kg administered for seven successive days, as indicated by reduction in the immobility times of mice in both FST and TST. PK extract in at a dose of 15 mg/kg did not show significant effect on locomotor activity of mice. The efficacy of PK extract was found to be comparable to that of imipramine and fluoxetine. Sulpiride and prazosin significantly attenuated the PK extract-induced antidepressant effect in TST. On the other hand, p-chlorophenylalanine did not reverse antidepressant-like effect of PK extract. This suggests that the antidepressant effect of PK extract seems to be mediated by an increase in brain norepinephrine and dopamine, but not by an increase in serotonin. The results of the present study indicate the involvement of adrenergic and dopaminergic systems in the antidepressant effect of alcoholic extract of *Picrorrhiza kurroa*.

KEYWORDS: Depression, forced swim test, tail suspension test.

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INTRODUCTION:

Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, specifically norepinephrine, serotonin and dopamine [1]. Reserpine, an antihypertensive drug that depletes neuronal storage granules of norepinephrine, serotonin and dopamine, causes clinically significant depression in 15% or more of patients [2].

Picrorrhiza kurroa has been used in indigenous system of medicine since a long time. This well – known drug is spoken as Dhanvantarigrasta, the plant eaten by Dhanwantari. According to Charka Samhita Picrorrhiza kurroa has antistress properties. This plant improves stamina and reduces incidence of gastric ulcers produced by restrain and chemical stress, liver damage and mortality induced by Carbon tetrachloride and have a calming effect. Psychosocial stress reduces neurogenesis in rodents, whereas chronic treatment with antidepressants increases neurogenesis and blocks the effects of stress. Picrorrhiza kurroa has been shown to potentiate the nerve growth factor (NGF) [3]. There is close clinical and biochemical resemblance between depressive symptoms and the response to stressful experiences, which led to the hypothesis that depression represents activation of the primary mediators of the stress response. As a result, dysregulation of the HPA axis has been implicated in depression and its treatment.

Clinical data indicates that a subset of patients with depressionexhibit hyperactivity of the HPA axis, which is normalized after successful antidepressant therapy [4-9]. Central serotonergic (5-HT) and noradrenergic (NA) neurons, which innervate the same regions of the brain, are known to play a crucial role in emotion and mood. These monoamine neurons have a great capacity to alter axonal morphology in response to repeated stress [10]. Therefore, the present study was undertaken (i) to investigate the effect of PK extract on depression in mice employing forced swim test (FST) and tail suspension test (TST) and (ii) to explore the possible underlying mechanisms of antidepressant-like activity of PK extract. Standard antidepressant drugs such as fluoxetine, a selective serotonin reuptake inhibitor, and imipramine, a tricyclic antidepressant were employed to standardize the animal models of depression and to compare the antidepressant efficacy of PK extract. (±) sulpiride (D_2-receptor antagonist), prazosin (α_1-adrenoceptor antagonist), and p-chlorophenylalanine (serotonin synthesis inhibitor) were used to evaluate the probable mechanisms of antidepressant effect of PK extract.

MATERIALS AND METHODS

Study design

The design of this experimental study was comparative and parallel group. Animals were divided into 24 groups and each group comprised of a minimum of five mice. PK extract was administered in three different doses (15, 30 and 60 mg/kg, i.p.) to different groups of mice. The dose selection of PK extract was based on the earlier study. The experimental protocol was approved by the Institutional Animals Ethics Committee before the start of the study.

Animals

Swiss male albino mice (3 months old), weighing around 25 g and procured from disease free animal house, National Institute of Toxicology, Pune (Maharashtra, India), were used in the present study. Animals had free access to food and water, and were maintained under standard laboratory conditions with a natural light and dark cycle. Food given to mice consisted of wheat flour kneaded with water and mixed with small amount of refined vegetable oil. The animals were acclimatized for at least 5 days before behavioural experiments. Experiments were carried out between 09:00 and 15:00 h.

Drugs and chemicals

Glycyrrhizin (Glycyrrhizic acid ammonium), (±) Sulpiride, Prazosin hydrochloride, DL-p-chlorophenylalanine, Imipramine hydrochloride (Sigma-Aldrich, St. Louis, USA), Fluoxetine hydrochloride (Ranbaxy Laboratories, Gurgaon, India), Glacial acetic acid (Central Drug House Pvt. Ltd., New Delhi, India), Sodium hydroxide pellets (Hi-Media, Mumbai, India) were used in the present study.

Vehicle

PK extract was dissolved in hot normal saline (60-62°C). Fluoxetine hydrochloride, imipramine hydrochloride and prazosin hydrochloride were dissolved separately in normal saline (0.9% sodium chloride). Sulpiride was dissolved in normal saline followed by addition of one drop of glacial acetic acid. P-chlorophenylalanine (p-CPA) was dissolved in minimum quantity of 0.1 N sodium hydroxide and pH was adjusted to 7 with 0.1 N hydrochloric acid. Volume of i.p. injection was 1 ml/100 g of mouse.

Laboratory models for testing antidepressant activity

Forced swim test (FST): Behaviour despair was proposed as a model to test for antidepressant activity by Porsolt et al [15, 16]. Mice were forced to swim individually in a glass jar (25 × 12 × 25 cm^3 sub) containing fresh water of 15 cm height and maintained at 25 ± 3°C. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The changes in immobility duration were studied after administering drugs in separate groups of animals. Each animal was used only once.

Tail suspension test (TST): The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al [17] as a facile means of evaluating potential antidepressants. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period [18]. Animal was considered to be immobile when it did not show any movement of body and hanged passively.

Study groups

Using FST: Group I (Control group): Normal saline (1 ml/100 g). Group II: Fluoxetine (20 mg/kg, i.p.). Group III: Imipramine (10 mg/kg, i.p.). Groups IV-VI: PK extracts (15, 30 and 60 mg/kg, respectively). In all these groups, respective drug treatment (i.p.) was given for seven successive days. After 60 min of the last dose, the immobility period was recorded.

Using TST: Group VII (control group for one day treatment): Normal saline (1 ml/100g). Groups VIII-X: PK extracts (15, 30 and 60 mg/kg, respectively). In all these groups, respective drug treatment (i.p.) was given only once. After 60 min of administration, the immobility period was recorded.

Group XI (control group for 7 days treatment): Normal saline (1 ml/100 g). Group XII: Fluoxetine (20 mg/kg, i.p.). Group XIII: Imipramine (10 mg/kg, i.p.). Groups XIV-XVI: PK extracts (15, 30 and 60 mg/kg, respectively). In all these groups, respective drug treatment (i.p.) was given for seven successive days. After 60 min of the last dose, the immobility period was recorded.

Group XVII: Normal saline was injected i.p. for seven successive days and then sulpiride (50 mg/kg, i.p.) was administered on the seventh day after 30 min of last injection

of normal saline. The animals were subjected to TST after 30 min of Sulpiride injection.

Group XVIII: PK extract (30 mg/kg) was administered i.p. for seven successive days and then sulpiride (50 mg/kg, i.p.) was administered on the seventh day after 30 min of last injection of PK extract. The animals were subjected to TST after 30 min of sulpiride injection.

Group XIX: Normal saline was injected i.p. for seven successive days and then Prazosin (62.5 µg/kg, i.p.) was administered on the seventh day after 30 min of last injection of normal saline. The animals were subjected to TST after 30 min of Prazosin injection.

Group XX: PK extract (30 mg/kg) was administered i.p. for seven successive days and then Prazosin (3 mg/kg, i.p.) was administered on the seventh day after 30 min of last injection of Glycyrrhizin. The animals were subjected to TST after 30 min of Prazosin injection.

Group XXI: Normal saline was injected i.p. for three successive days to mice. From the fourth day to seventh day, p-CPA (100 mg/kg, i.p.) was administered after 30 min of injection of Normal saline. The animals were subjected to TST after 30 min of p-CPA injection on the seventh day.

Group XXII: PK extract (30 mg/kg) was administered i.p. for three successive days to mice. From the fourth day to seventh day, p-CPA (100 mg/kg, i.p.) was administered after 30 min of i.p. injection of PK extract. The animals were subjected to TST after 30 min of p-CPA injection on the seventh day.

Group XXIII: Fluoxetine (20 mg/kg) was injected i.p. for three successive days to mice. From the fourth day to seventh day, p-CPA (100 mg/kg, i.p.) was administered after 30 min of injection of Fluoxetine. The animals were subjected to TST after 30 min of p-CPA injection on the seventh day.

Group XXIV: Effect of PK extract (15, 30 and 60 mg/kg, i.p.) on locomotor function of mice was studied using a Photoactometer (INCO, Ambala, India) to rule out the increase in locomotor performance of mice. The difference in the locomotor activity scores was noted before and after the administration of PK extract.

Statistical analysis

All results are expressed as Mean ± SEM. All the groups were analysed using one-way ANOVA followed by Tukey's multiple comparison test. The locomotor activity scores were subjected to Student's paired *t* -test. $P < 0.05$ was considered significant.

RESULTS

Effect on immobility periods in FST and TST

Single dose (15, 30 and 60 mg/kg) of PK extract had significant effect on immobility periods of mice as compared to control in TST [Figure 2]. PK extract (15, 30 and 60 mg/kg) administered i.p. for seven successive days also had significant effect on immobility periods as compared to control in both FST and TST. Imipramine (15 mg/kg, i.p.) and Fluoxetine (20 mg/kg, i.p.) for seven successive days significantly reduced the immobility periods as compared to respective controls in both FST [Figure 1 and 2, Table 1 and 2] and TST [Figure 3 and 4 and Table 3 and 4].

Effect of combination of Glycyrrhizin with Sulpiride, Prazosin and p-CPA on immobility periods in TST

Sulpiride (50 mg/kg, i.p.) and Prazosin (3 mg/kg, i.p.) alone significantly increased the immobility period while p-CPA alone did not have significant effect as compared to control. Pre-treatment of animals with Sulpiride or Prazosin significantly blocked the decrease of immobility time produced by Glycyrrhizin. On the other hand, pre-treatment of mice with p-CPA (100 mg/kg, i.p.) did not significantly reverse the decrease in immobility period produced by Glycyrrhizin. However, pre-treatment of mice with p-CPA (100 mg/kg, i.p.) significantly reversed decrease of immobility period produced by Fluoxetine [Table 1].

Effect on locomotor activity

There was significant effect on locomotor activity of mice (426 ± 14.7) when treated with PK extract (15, 30 and 60 mg/kg, i.p.) for seven successive days as compared to control (before treatment) (434.2 ± 9.7) [Figure 5 and Table 5].

DISCUSSION

In the present study, PK extract (60 mg/kg) produced significant antidepressant-like effect in mice in both FST and TST. Both these models of depression are widely used to screen new antidepressant drugs [15-19]. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, monoamine oxidase (MAO) inhibitors and atypical [15, 17, 20]. In FST, mice are forced to swim in a restricted space from which they cannot escape, and are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair that can be reduced by several agents, which are therapeutically effective in human depression. The TST also induces a state of immobility in animals like that in FST. This immobility, referred as behavioural despair in animals, which is claimed to reproduce a condition similar to human depression [17, 21]. It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity [22].

The antidepressant effect of PK extract seems not to be associated with any motor effects, since it did not show significant change in locomotor function of mice as compared to control. This indicates that increased motor activity was not involved in the action seen in both FST and TST, and confirms the assumption that the antidepressant effect of PK extract is specific. The precise mechanisms by which PK extract produced antidepressant effect are not completely understood. However, according to our results, the antidepressant effect of PK extract was significantly reversed by the treatment of animals with Prazosin (an α_1-adrenoceptor antagonist) and Sulpiride (a selective dopamine

D_2- receptor antagonist) when tested in TST. This suggests that PK extract might produce antidepressant effect by interaction with α_1- adrenoceptors and dopamine D_2-receptors, thereby increasing the levels of norepinephrine and dopamine in brains of mice. However, p-CPA (a serotonin synthesis inhibitor) did not significantly attenuate the antidepressant effect of PK extract in TST, suggesting that antidepressant effect of PK extract is not mediated through serotonergic system. On the other hand, p-CPA significantly reversed the antidepressant effect of fluoxetine (a specific serotonin reuptake inhibitor) in TST, suggesting that fluoxetine has antidepressant effect through the serotonergic system.

Since PK extract has antistress activity [11], antidepressant effect of PK extract in mice might be through increase in the brain levels of monoamines like epinephrine and dopamine by inhibiting monoamine oxidase. Thus, it may be concluded that PK extract produced antidepressant effect in mice in both FST and TST, and this effect seems most likely to be mediated through an interaction with adrenergic and dopaminergic systems. The efficacy of the PK extract was comparable to that of Imipramine and Fluoxetine.

TABLE 1 Effect of combination of PK extract with sulpride, prazosin and p-CPA on immobility period in TST

Group	Treatment	Dose (kg^{-1})*	Immobility period mean (sec)± SEM
XI	Control (Normal Saline)	10	189±10.2
XV	PK extract for 7 days	15	150.7±12.2#
XVII	Normal saline + Sulpride	10 50	256±7.6#
XVIII	PK extract + Sulpride	30 50	198.7±7.7^
XIX	Normal saline + Prazosin	10 0.0625	219.9±5.4#
XX	PK extract for 7 days + Prazosin	30 0.0625	207.4±9.1^
XXI	Normal saline + p-CPA	10 100	204.9±5.9
XXII	PK extract for 7 days + p-CPA	30 100	146.5±6.4
XII	Fluoxetine for 7 days	20	72.0±9.3#
XXII	Fluoxetine + p-CPA	20 100	163.4±4.8@

n=5 in each group.
* the dose of normal saline ex expressed in mL, for other drugs it is in mg.
#P<0.05 as compared to normal saline (Dunnett's test)
^P<0.05 as compared to PK extract alone (Dunnett's test)
@P<0.05 as compared to fluoxetine alone (Dunnett's test)

Table 2 Effect of *Picrorrhiza kurroa* on Forced swim model. (Duration of immobility (sec) in the period of 5mins)

Group	Duration of immobility (Sec.)			
	1 hour	5 hour	12 hour	24 hour
Control	54.00± 1.22	56.00± 1.41	73.00± 2.72	67.20± 1.93
PK-1	24.40± 0.92***	34.00± 1.41***	74.00± 3.40	57.80± 1.65**
PK-2	17.00± 1.00***	29.00± 0.70***	66.00± 2.28	46.20± 1.74***
PK-3	11.60± 0.81***	24.00± 1.41***	33.80± 1.88***	35.40± 1.53***
Positive Control	21.80± 0.66***	28.00± 1.41***	36.80± 2.43***	35.60± 1.43***

Values are expressed in Mean ± SEM

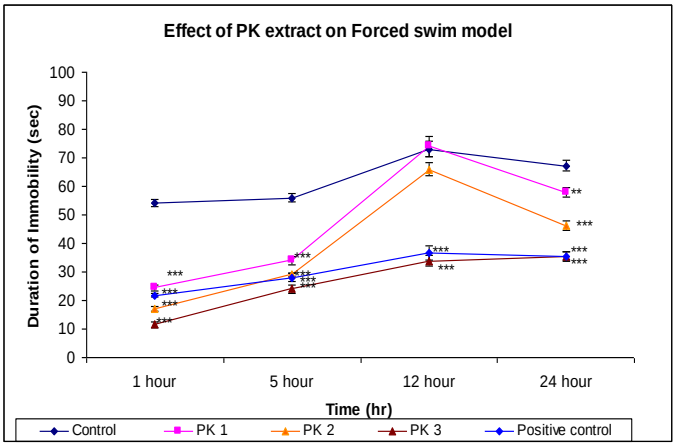


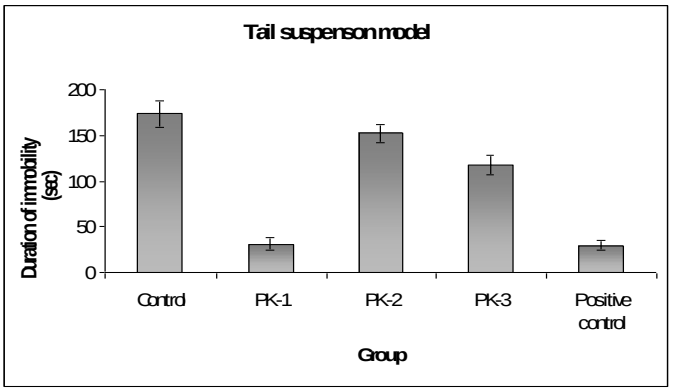
Figure 1. Effect of *Picrorrhiza kurroa* on Forced swim model
*** = p < 0.001, ** = p < 0.01, * = p < 0.05
ANOVA followed by Tukey's Multiple Comparison Test

Table 3 Effect of *PK extract* on tail suspension model (Duration of immobility (sec) in the period of 5mins).

Sr. No.	Group	Duration of immobility (Sec.)
1	Control	173.80±14.32
2	PK-1	31.20±6.93***
3	PK-2	152.00±9.83***
4	PK-3	117.60±11.25***
5	Positive Control	29.40±5.70***

Values are expressed in Mean ± SEM

Figure 2 Effect of *Picrorrhiza kurroa* on tail suspension model



*** = p < 0.001
ANOVA followed by Tukey's Multiple Comparison Test

Table 4 Effect of *Picrorrhiza kurroa* on Forced swim chronic model (Duration of immobility (sec) in the period of 5mins).

Group	Duration of immobility (Sec.)			
	1 hour	5 hour	12 hour	24 hour
Control	100.60± 3.26	163.00± 5.38	185.00± 4.78	163.20± 5.05
PK-1	70.00± 3.536***	101.00± 6.768***	107.20± 4.532***	96.40± 3.982***
PK-2	79.60± 2.15***	131.00± 5.94*	107.60± 4.52***	105.40± 4.97***
PK-3	30.80± 2.41***	101.00± 6.76***	99.40± 5.92***	67.20± 3.30***
Positive Control	60± 2.15***	150± 8.96**	101.8± 4.81***	82.2± 5.23***

Values are expressed in Mean ± SEM

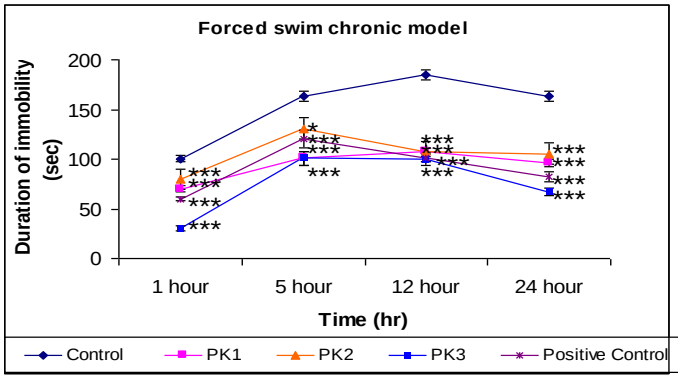


Figure 3 Effect of *Picrorrhiza kurroa* on Forced swim chronic model

*** = p < 0.001, ** = p < 0.01, * = p < 0.05
ANOVA followed by Tukey's Multiple Comparison Test

Table 5 Effect of *Picrorrhiza kurroa* on Tail suspension chronic model (Duration of immobility (Sec.) in the period of 5mins).

Sr. No.	Group	Duration of immobility(Sec.)
1	Control	145.00±5.70
2	PK1	110.00±7.07***
3	PK2	115.00±4.66**
4	PK3	100.00±3.30***
5	Positive Control	85.00±4.40***

Values are expressed in Mean ± SEM

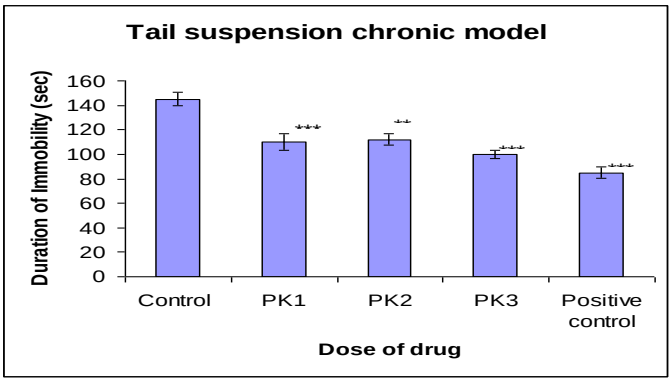


Figure 4 Effect of *Picrorrhiza kurroa* on Tail suspension chronic mode

*** = p < 0.001, ** = p < 0.01, * = p < 0.05
ANOVA followed by Tukey's Multiple Comparison Test

Table 6 Effect of Picrorrhiza kurroa on locomotor activity (Count in nos. by the actophotometer for duration of 10 mins).

Sr. No.	Group	Before Count	After Count
1	PK-1	447.00 ±25.30	438.60 ±33.14
2	PK-2	446.40 ±13.98	678.00 ±39.3**
3	PK-3	461.20 ±13.65	861.40±40.22***
4	Caffeine	434.40 ±28.48	691.20 ±93.21**

Values are expressed in Mean ± SEM

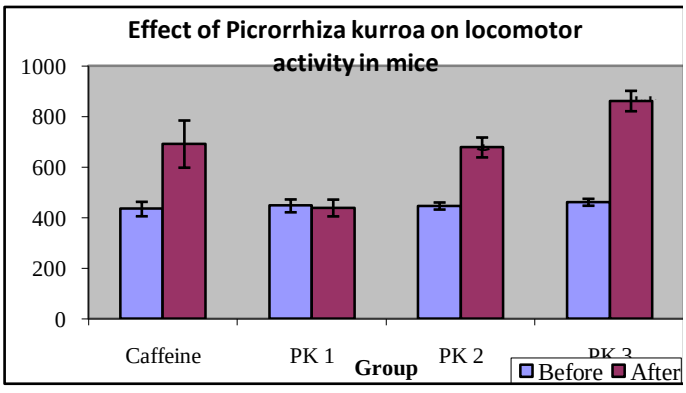


Figure 5 Effect of Picrorrhiza kurroa on locomotor activity

*** = p < 0.001, ** = p < 0.01
ANOVA followed by Tukey's Multiple Comparison Test

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