

**ANTIDEPRESSANT ACTIVITY OF THE UNANI FORMULATION:  
ITRIFAL KISHNEEZI**

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**Summary**

Depression is the most common of the affective disorders and is a major cause of disability and premature death worldwide. Unani system of medicine (Unaniopathy) originated in Greece, enriched by Persians and Arabs and now became an integral part of Alternative medicinal systems of India. Itrifal Kishneezi is a Unani medicine prescribed for gastric problems, head ache and used as a stimulant. In the present study the drug was tested for antidepressant activity in animal models viz., despair swim test, tail suspension test and apomorphine induced hypothermia in mice. IK decreased the immobility period in a dose dependent manner in both DST and TST and reversed hypothermia in mice.

**Key Words:** Itrifal Kishneezi, depression, apomorphine, immobility

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## Introduction

Depression is the most common of the affective disorders (disorders of mood not thought or cognition) and is a major cause of disability and premature death worldwide.<sup>[1]</sup> Unani system of medicine (Unaniopathy) originated in Greece based on the principles propounded by Galen, a Greek Practitioner and was called Galenic. After him many Arab and Persian scholars enriched the system and became Unani.<sup>[2]</sup> Now it has become a part of Indian traditional system of medicine.<sup>[3]</sup> Itrifal Kishneezi (IK) is used in unani for chronic catarrh, gastric problems- flatulence, indigestion, hyperacidity; head ache, eye pain and as a stimulant.<sup>[4-6]</sup> IK contains Terminalia chebula (Myrobalan) – black Myrobalan (unripe fruit-0.435g), yellow myrobalan (fresh ripe fruit-0.435g) and brown myrobalan (dried ripe fruits-0.435g); Terminalia belerica (0.435g); Coriandrum sativum (0.435g); clarified butter (ghee - 0.866g) and honey (6.953g).<sup>[7]</sup> There is no published scientific data available on this formulation for antidepressant activity. Our previous study showed that IK has significant antioxidant activity.<sup>[7]</sup>

Three experimental models were chosen for studying the antidepressant activity of IK viz., Despair swim test [DST], Tail suspension test [TST] and Apomorphine induced hypothermia in mice.<sup>[8]</sup> Both DST and TST were performed after acute single dose administration and chronic administration.

## Materials and Methods

### *Drugs:*

The formulations IK was obtained from M/s Hamdard (Wakf) Laboratories, Ghaziabad, Uttar Pradesh, India. Imipramine (Depsonil, S.G. Pharmaceuticals, India), Fluoxetine (Prodep, Sun Pharmaceuticals, India) were used in this study and 1% gum acacia was used as vehicle for all the drugs.

### *Animals:*

Three month old male Swiss albino mice (20-25g) were housed in groups of six under standard laboratory conditions (temperature-  $25\pm 1^{\circ}\text{C}$ , relative humidity- $55\pm 5\%$  and 12.00:12.00h dark:light cycle) with Amrut brand standard pellet diet and water ad libitum. The overnight fasted (water ad libitum) animals were transferred to the laboratory at least one hour before the beginning of the experiment. The experiments were performed during day (9:00-12:00h) and as per the guidelines of the Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Government of India. The Institutional Animal Ethics Committee approved the study protocol (IAEC/SUCP/01/2009).

### *Despair Swim Test in Mice [DST]<sup>[8-12]</sup>*

#### *Acute Study:*

In a pretest session male Swiss albino mice with food and water ad libitum were brought to the laboratory one hour before and were forced to swim in a cylindrical shaped container (height 50cm and 20cm diameter, containing water up to 15cm height maintained at  $25\pm 1^{\circ}\text{C}$ ).

After 15min they were removed from water and blow dried with a hair dryer (set in warm mode) and returned to their cages. The mice were divided into six groups and all the drugs were administered intraperitoneally. 23.5h after pretest, group I was given 1% gum acacia solution, group II – VI were given 30,100 and 300mg/kg of IK; 20mg/kg of Fluoxetine; and 30mg/kg of Imipramine respectively. After 30min each mouse was left in the container with water as above for 6min and in the last 4min, the following behaviors were recorded.

- i) Immobility – Floating in water without escape behavior.
- ii) Swimming – Active movements and circling in the water.
- iii) Climbing – Active movements of forelimbs on the wall of the container.

Water was changed after testing each animal as used water was shown to give behavioral alarm signals.<sup>[13]</sup> After the test the mice were blow dried and returned to their cages.

### ***Chronic Study:***

For understanding the chronic effects of the drug, the same procedure as above was followed except that for 13 days before the main test all the animals were given the respective drug doses once daily (food and water ad libitum) and 0.5h before the main test and observations were made same as above.

### ***Tail Suspension Test in Mice [TST]<sup>[8,14,15]</sup>***

#### ***Acute Study:***

Male mice were divided into five groups and were administered vehicle and test drug as mentioned in DST and Imipramine 30mg/kg intraperitoneally 0.5h prior to the test. A cord was tied between two burette stands at a height of 58cm and after 30min of administration of the drugs, each mouse was suspended from the cord by attaching its tail approximately 1cm from the tip of the tail with the help of an adhesive tape. Immobility of the mice in the last 4min of a 6min test period was observed. Mice were said to be immobile when they hung passively and were completely motionless.

#### ***Chronic Study:***

For understanding the chronic effects of the drugs, the same TST procedure as above was followed except that for 13 days before the main test all the animals were given the respective doses once daily (food and water ad libitum) and 0.5h before the main test and observations were made same as above.

### ***Apomorphine Induced Hypothermia in Mice<sup>[8,16-18]</sup>***

Male Swiss albino mice were divided into twelve groups and the experiment was carried out between 7.00-10.00am at a room temperature of 25±2°C. The colon temperatures of the mice were recorded using commercially available digital thermometer (DT-403, Hicks Thermometers, India), by dipping the thermometer in Vaseline and inserting approximately 0.5cm into the rectum of the mice by gently restraining the mouse with hands. Mice with body temperature between 37 and 38.4°C were included in the study. One hour after measuring the initial temperature, group I was given 1% gum acacia solution, group II – V

were given 30,100 and 300mg/kg of IK and 30mg/kg of Imipramine respectively. After 1hr, all the animals received apomorphine (16mg/kg s.c.). Measurement of colon temperatures in all the mice was repeated after another 1hr.

### Statistical Analysis:

The data obtained were analyzed using one-way ANOVA and Dunnett multiple comparison test using Graphpad InStat version 3 software.  $P < 0.05$  was considered statistically significant.

## Results

### Despair Swim Test in Mice

IK showed dose dependant decrease in immobility, but dose variation of BC did not change the immobility period. IK increased both climbing and swimming duration, but Fluoxetine and Imipramine increased the duration of swimming and climbing respectively. Chronic administration of all the drugs including standards decreased the immobility period. Results are shown in Table 1.

Table 1. Despair Swim Test in Mice after Acute and Chronic Administration of IK

Treatment	Despair Swim Test (Duration in sec) Mean $\pm$ SD					
	After Acute Administration			After Chronic Administration		
	Immobility	Climbing	Swimming	Immobility	Climbing	Swimming
Control	192 $\pm$ 12.3	23.7 $\pm$ 6.4	24.3 $\pm$ 7.2	177.7 $\pm$ 10.1	34.5 $\pm$ 4.6	27.8 $\pm$ 8.7
IK 30mg/kg	69.3 $\pm$ 10.5**	108.2 $\pm$ 8.9**	62.5 $\pm$ 10.5**	46 $\pm$ 9.1**	120.7 $\pm$ 9.4**	73.3 $\pm$ 7.4**
IK 100mg/kg	50.8 $\pm$ 9.6**	116.3 $\pm$ 7.7**	72.8 $\pm$ 6.7**	31 $\pm$ 10**	134.3 $\pm$ 13.1**	74.7 $\pm$ 10.3**
IK 300mg/kg	23 $\pm$ 6**	139.2 $\pm$ 8.7**	77.8 $\pm$ 6.4**	11 $\pm$ 4**	152.8 $\pm$ 11**	76.2 $\pm$ 10.1**
Fluoxetine 20mg/kg	12.3 $\pm$ 4**	24 $\pm$ 5.1†	203.7 $\pm$ 8.6**	8.3 $\pm$ 3.3**	22.1 $\pm$ 4.1†	204.5 $\pm$ 9.4**
Imipramine 30mg/kg	40.5 $\pm$ 5.8**	149.7 $\pm$ 13.7**	49.8 $\pm$ 10.1**	17 $\pm$ 6.8**	170.3 $\pm$ 7.2**	52.7 $\pm$ 17**
ANOVA F-statistic	156.44	92.584	107.44	187.05	134.27	88.436

ANOVA,  $P < 0.0001$ , Dunnett multiple comparison test † $P > 0.05$  (Not significant), \*\* $P < 0.01$  (Highly significant), IK- Itrifal Kishneezi.

### Tail Suspension Test in Mice

Similar to DST, IK displayed dose dependant decrease in the duration of immobility. All the drugs on chronic administration significantly reduced the duration of immobility after chronic administration. Table 2 shows the results of TST.

Table 2. Tail Suspension Test in Mice

Treatment	Duration of Immobility in TST (sec) Mean ± SD	
	Acute	Chronic
Control	175.5 ± 30.9	165.3 ± 32.4
IK 30mg/kg	106.5 ± 17.6**	72.8 ± 12**
IK 100mg/kg	78.5 ± 11.3**	44.2 ± 9.9**
IK 300mg/kg	46.7 ± 11.0**	31 ± 10.9**
Imipramine 30mg/kg	54.2 ± 15.8**	25.2 ± 14.5**
ANOVA F-statistic	30.479	34.717

ANOVA,  $P < 0.0001$ , Dunnett multiple comparison test  
 \*\* $P < 0.01$  (Highly significant), IK- Itrifal Kishneezi.

### Apomorphine Induced Hypothermia

Apomorphine produced hypothermia in mice which was reversed by IK and Imipramine. IK antagonized apomorphine action at 30mg/kg itself, difference in rectal temperature before and after apomorphine in presence of the drugs and in control were recorded as shown in Table 3.

Table 3. Apomorphine Induced Hypothermia in Mice

Treatment	Temperature (°C) Mean ± SD		
	Before Apomorphine	After Apomorphine	Difference
Control	38 ± 0.3	34.1 ± 0.4	3.9 ± 0.6
IK 30mg/kg	37.6 ± 0.4	36 ± 0.8	1.6 ± 0.6**
IK 100mg/kg	37.9 ± 0.4	37.8 ± 0.4	0.1 ± 0.2**
IK 300mg/kg	37.7 ± 0.4	37.6 ± 0.4	0.1 ± 0.1**
Imipramine 30mg/kg	37.6 ± 0.5	37.6 ± 0.4	0.1 ± 0.3**

ANOVA,  $P < 0.0001$ , Dunnett multiple comparison test F-static = 132.95  
 \*\* $P < 0.01$  (Highly significant); IK- Itrifal Kishneezi.

### Discussion

Despair swim test (DST) proposed by Porsolt et al<sup>[19,20]</sup> suggests that when rodents are forced to swim in a restricted and inescapable space show characteristic alternating immobility and escape-directed behavior that includes swimming and climbing. The immobility signifies behavioral despair resembling a state of depression reduced by several antidepressants effective in human depression. Drugs that potentiate central dopaminergic and  $\alpha$ -adrenergic systems reduce the immobility time in rodents.<sup>[21]</sup> Recent studies suggest that antidepressants that selectively inhibit norepinephrine uptake and those that inhibit serotonin reuptake

reduce immobility time, but the former selectively increases climbing without affecting swimming and the later increases swimming instead of climbing. This modified Porsolt method was adopted in the present study.<sup>[9-11]</sup>

Tail suspension test [TST] suggested by Steru et al<sup>[8,14]</sup> in mice is a simple, economic, reliable and rapid method to evaluate antidepressant activity. Mice suspended by tail show alternate agitation and immobility. The immobility indicates a state of depression and is reduced by number of antidepressants. A slight modification of the study suggested by Steru et al was adopted in this study.<sup>[15]</sup>

Apomorphine produces hypothermia which is antagonized by antidepressants. It is a dopaminergic agonist that has high affinity for D<sub>4</sub> receptors and moderate affinity for D<sub>2</sub>, D<sub>3</sub>, D<sub>5</sub> and adrenergic  $\alpha$ 1D,  $\alpha$ 2B,  $\alpha$ 2C receptors; and low affinity for D<sub>1</sub> receptors.<sup>[22]</sup> Dopaminergic and serotonergic mechanisms in the brain mediate the effects of apomorphine on body temperature.<sup>[23-28]</sup> Direct action of apomorphine on D<sub>1</sub> and D<sub>2</sub> receptors in thermoregulatory centre in hypothalamus has been demonstrated.<sup>[25,26]</sup> D<sub>1</sub> and D<sub>2</sub> synergistic involvement has been shown in these studies. There is also evidence for independent D<sub>1</sub> and D<sub>2</sub> action in thermoregulation.<sup>[25,27]</sup> However, as mentioned, involvement of serotonin receptors in thermoregulation is also documented.<sup>[28]</sup> It is suggested that low dose apomorphine (up to 2mg/kg) induced hypothermia is antagonized by neuroleptics and high doses (over 10mg/kg) by antidepressants.<sup>[29]</sup>

### Conclusions

The present study confirms the antidepressant activity of Itrifal Kishneezi and further studies are essential to isolate the chemical moiety responsible for the antidepressant activity of these drugs. Studies involving estimation of central monoamine concentration with varying doses of these drugs would help in establishing their mechanism of action.

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