



## Research Article

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### BLIND SCREENING AND ACUTE TOXICITY STUDY ON DATE SEEDS COLLECTED FROM RIYADH REGION

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#### ABSTRACT

If a series of chemical substances, extract or new isolated compound available, with no pharmacological history, then blind screening provide clues to potential activity, at least, and preferably, indicates field of activity if exist. The chief purposes of the blind screening are to demonstrate whether the new group of substance is worthy for further attention or not. In the present study, we subjected aqueous extract of date seeds collected from Riyadh for acute toxicity and blind screening to reveal its toxicological and pharmacological data. We used OECD guidelines 425; Up and Down procedure for oral toxicity testing and behavioral profile using Irwin Score for blind screening to get raw data. The extract shown significant increase in alertness, grooming, and restlessness, and spontaneous activity, threshold for pain response, heart rate, urination and decrease in pupil size. The study revealed that date seeds are non toxic with minimum behavioral changes; may be a central nervous system stimulant; antinociceptive and muscarinic; may be a better prototype for further study.

**Keywords:** Irwin score, acute toxicity, date seeds, blind screening

#### INTRODUCTION

If a series of chemical substances available, either through isolation from a natural source or through synthesis, or extract, powdered or drugs as such in raw form, there may be no information on its pharmacological activity. Then blind screening ought to provide clues to potential activity, at least, and preferably, indicates field of activity if exist. The chief purposes of the screening are to demonstrate whether the new group of substance is worthy for further attention, and to indicate which among them have the most interesting pharmacological activity<sup>1</sup>. Acute toxicity study describes the adverse effects of drug that result from single exposure or multiple exposures in a short span of life usually less than 24 hours; gives information on median lethal dose LD<sub>50</sub> of drug on study<sup>2,3</sup>.

In the present study we subjected date seeds for acute toxicity study and for blind screening to determine the median lethal dose and to find the clues on potential pharmacological activity and to determine seeds are worthy for further attention.

**Plant Description:** Date palm belonging to the Angiosperms Monocotyledones, *Palmaceae* is a family of about 200 genera and 1500 species<sup>4</sup>. Phoenix is one of the genera which contain a dozen species, all native to the tropical or subtropical regions of Africa or Southern Asia, including *Phoenix dactylifera* L.<sup>5</sup>. Twelve species of the genus "Phoenix", along with their geographical distribution, were first listed by Chevalier<sup>6</sup>.

The fruit of the date palm is composed of a fleshy pericarp and seed<sup>7</sup>. As with the fruit, seed characteristics vary according to variety, environmental and growing conditions. A seed's weight could range from less than 0.5 g to about 4 g, in length from about 12 to 36 mm and in breadth from 6 to 13 mm. The seed is usually oblong, ventrally grooved, with a small embryo, and with a hard endosperm made of a cellulose deposit on the inside of the cell walls. The date seeds represent about 15% of the total weight of the date fruits<sup>8</sup>. The seeds of many fruits are used in complementary and alternative medicine to prevent or reduce stress and side effects diseases<sup>9</sup>. The date seed contain different chemical compounds such as saturated fatty acids as stearic and palmitic acid, unsaturated fatty acids such as linoleic and oleic acids which could inhibit the 5- $\alpha$  reductase enzyme, Zinc, Cadmium, Calcium and potassium<sup>10</sup>. Date seed contains 3.1–7.1% moisture, 2.3–6.4% protein, 5.0–13.2 fat, 0.9–1.8% ash and 22.5–80.2% dietary fiber. Also, seeds contain high levels of phenolics, antioxidants and dietary fiber<sup>11</sup>. The seed powder is also used in some traditional medicines and has been investigated for human potential health benefits<sup>12</sup>, also included in animal feed to enhance growth and plasma level of testosterone<sup>13</sup>. Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats<sup>14</sup>. The pollen grains of date palm have been used by Egyptians to improve fertility in women<sup>15</sup>. In addition, date seed extract shows an ability to restore the normal functional status of the poisoned liver and also

to protect against subsequent carbon tetrachloride hepatotoxicity on the liver in rats<sup>16</sup>. Dietary antioxidants are important in controlling and ameliorating the harmful effects of oxidative stress, high intake of fruits and vegetables with high antioxidant content contributes to reduced risk of oxidative stress-mediated diseases such as cardiovascular disease and cancer<sup>17, 18</sup>. Date seeds have been shown to contain significant amounts of antioxidants<sup>11, 19</sup>.

## MATERIALS AND METHODS

### Preparation of extract

The fresh dates were collected from farm, seeds were separated from pulp and shade dried and subjected to grinding and powdered. Coarse powder of seeds then subjected to maceration separation. The powdered materials were soaked in distilled water for 72 hours with occasional shaking. The extract obtained then subjected for evaporation and concentrated to dryness to get hard mass.

### Animals

Healthy young 8 to 12 weeks old Albino Wistar rats of laboratory strains of either sex weighing 200 -250g were employed for study. Females will be nulliparous and non-pregnant. Room temp was maintained at 22°C (±3 °C), Humidity were 50-60%, Lighting provided artificially- the sequence being 12 hours light and 12 hours dark, Placement of animal done Individually. The animals kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions and fasted ad libitum prior to dosing extract<sup>20</sup>. The research is approved by internal Institutional Animal Ethical Committee.

### Acute oral toxicity study

Study performed according to the “Up and down” procedure for oral toxicity study; OECD guidelines 425. 2000mg/kg body weight of drug administered to the animals and observed for behavioral profile and for mortality for 24 hrs after dosing.<sup>20, 24</sup>.

### Blind Screening for pharmacological profile

Six numbers of rats fed with date seed extract at a dose 2000mg/kg body weight per oral were observed for General behavioral test according Irwin scale (Irwin. 1961). At each observation time, list of parameters are studied. These parameters are distributed as follows: behavioral profile (body position, locomotor activity, positional passivity, toe pinch, transfer arousal, touch escape, corneal, pinna, tail elevation etc); neurological profile (body tone, abdominal tone, limb tone, righting reflects, hypotonic gait, grip strength, tremors, twitches, and convulsions etc); autonomic profile (palpebral closure, piloerection, hypothermia, respiratory rate, heart rate and skin colour etc)<sup>1, 21</sup>.

### Statistics

The chi-square test was used for analyzing the results obtained in the Irwin score. Differences were considered significant when associated with a probability of 5% or less.

## RESULTS AND DISCUSSION

### Acute oral toxicity study

No mortality observed within 24 hours at the recommended dose 2000 mg/kg body weight per oral. The extract is found to be non toxic with minimum behavioral changes. This result indicates the absence of toxic effects.

### Blind Screening for pharmacological profile

The results are recorded in tabular form [Table 1], with the use of scores wherever applicable. The effects of the test substance on the animal are scored with the use on nine degrees, generally, that is, with the scale of 0 to 8. Scoring is recorded at the time of peak effect. The base score for normal signs or effects is 4; scores below are subnormal responses, those above 4 are supernormal. The base score for abnormal sign is 0, and 8 is the maximal score<sup>21, 22</sup>.

The drug has increased the alertness of the animal significantly (5.8\*). This indicated stimulation of central nervous system. Stimulants produce variety of different kinds of effects by enhancing the activity of central nervous system. The common effects are increased alertness, awareness, wakefulness etc. Normally the unaffected rats groom itself frequently. The animals grooming was excessive (5.5\*) and indicate the central stimulation or sympathetic stimulation. Restlessness, absent in unaffected rat, may indicate central stimulation, discomfort, visceral changes, or the approach of the convulsion. In the present study, the treated rats shown increase in restlessness (2.3\*). A central stimulation effect was also confirmed through its increase in spontaneous activity (5.6\*). The threshold for pain response also increased; revealed antinociceptive effect of test drug. The pain response measures analgesia, sedation and central depression. The pupil size compared with its size before injection of the test substance. Enlargement indicates parasympatholytic activity or sympathomimetic activity; low scores indicate possible muscarinic activity. The animals shown decrease in the pupil size (1.2\*) with significant increase in urination (3.2\*); may be muscarinic. The animal did not show any signs of excessive salivation. Excessive heart rate (6.2\*) revealed the parasympatholytic activity, but there are many other possibilities for this as the drug shown muscarinic activity through decrease in pupil size and increase in urination<sup>1, 21, 22, 23</sup>.

Table 1: Behavioral profile of animal during first 2h of observation

Behavioral Profile		Normal Score	Observed Score
Awareness	Alertness	4	5.8*
	Visual Placing	4	4
	Passivity	0	0
	Stereotypy	0	0
Mood	Grooming	4	5.5*
	Vocalization	0	0
	Restlessness	0	2.3*
	Aggression	0	0
	Fearfulness	0	0
Motor Activity	Reactivity	4	4
	Spontaneous activity	4	5.6*
	Touch Response	4	4
	Threshold for Pain Response	4	5.8*
CNS excitement	Startle Response	0	0
	Straubs Tail	0	0
	Tremor	0	0
	Twitches	0	0
	Convulsions	0	0
Posture	Body posture	4	4
	Limb position	4	4
Motor Incoordination	Staggering gait	0	0
	Abnormal gait	0	0
	Righting reflex	0	0
Muscle Tone	Limb tone	4	4
	Grip strength	4	4
	Body sag	0	0
	Body tone	4	4
	Abnormal tone	4	4
Reflexes	pinna	4	4
	Corneal	4	4
Autonomic	Writhing	0	0
	Pupil size	4	1.2*
	Palpebral opening	4	4
	Exophthalmus	0	0
	Urination	0	3.2*
	Salivation	0	0
	Piloerection	0	0
	Hypothermia	0	0
	Skin colour	4	4
	Heart rate	4	6.2*
Respiratory rate	4	4	
Miscellaneous	Lacrimation	0	0
Dead	Number Acute	0	0
	Number Delayed	0	0

n= 6, \* versus normal score. Differences were considered significant when associated with a probability of 5% or less.

## CONCLUSION

The study revealed that date seeds are non toxic with minimum behavioral changes; may be a central nervous system stimulant, antinociceptive and muscarinic. The date seeds can be subjected to the further neuro pharmacological study and for anti nociception with large number of animals using sophisticated instruments. Also the drug has to be subjected for sub acute and chronic toxicity testing to reveal its safety margins.

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