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Evaluation of *Japakusumadi Yoga* for safety profile - Acute Toxicity Profile

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ABSTRACT

Japakusumadi Yoga is an Ayurvedic formulation, indicated for contraception in Ayurveda. This study determines acute toxicity of '*Japakusumadi Yoga*' an oral formulation in wister albino rats. Single dose acute toxicity was assessed by employing OECD guidelines 425 using AOT software. Test formulations was administered to overnight fasted animals and 14 days observation of dosed (up down as per requirement) rats was done for general appearance, cage side behaviour including increased or decreasing motor activity, convulsions, straub's reaction, catatonia, muscle spasm, spasticity, ophisthotonus, hyperesthesia, muscle relaxation, anaesthesia, arching and rolling, lacrimation, salivation, diarrhoea, writhing movement, mode of respiration and changes in skin colour etc., with mortality and autopsy finding in case of dead animal. Based on the observation made and recorded it can be concluded that the test drug is without any toxic potential even at the dose of 2000 mg/kg in animals equivalent to 22.4g for human being.

Key words: *Japakusumadi Yoga, Mortality, Acute Oral Toxicity.*

INTRODUCTION

Overgrowth of population is one of the major rising problems in present era. The effective remedy to check the increase in population is to control the birth rate by adopting temporary or permanent method of contraception.[1] Oral steroidal contraceptive, a widely used temporary method of contraception has several adverse effect.[2] Hence there is a need for exploration of a safe, effective, easily available and cheaper contraceptive formulation preferable of plant origin.

Contraception is mentioned in Ayurvedic classics also.[3] *Japakusumadi Yoga* is a oral contraceptive formulation explained in Ayurvedic science.[4] The formulation should be put for clinical use only after safety profile of the drug is tested. Hence the formulation *Japakusumadi Yoga* was evaluated for the acute toxicity profile with reference to behavioural profile in wister albino rats.

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MATERIALS AND METHODS

Collection of samples

Medicine source

Japakusumadi Yoga consists of *Japakusuma, Purana Guda* and *Kanji*.

1.08 g. of *Japa Kusuma* freshly collected from the herb garden maintained in the campus of SDM College of Ayurveda, Kuthpady was ground into fine paste with 25 ml of *Kanji* and 4.32gms of *Guda*.

Animals

Colony bred wister albino rats of either sex weighing about 180-220 g. were used for evaluating acute oral

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

toxicity. The animals were procured from the animal house attached to the Pharmacology Laboratory, SDM Centre for Research in Ayurveda and Allied Science. Udyavara. They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature of 22 ± 3^{0}C and humidity of 50 to 70% and were acclimatized for 7 days before intitiation of the experiment. They were fed with rat pellets from Saidurga feeds, Bengaluru and tap water *ad libitum.* Food was provided throught the study period except on previous night of dosing. The animals were marked with saturated picric acid solution water for appropriate identification.

The experiment was carried out in conformity with guidelines of the Institutional Animal Ethical Committee (IAEC) after obtaining its permission. ETHICAL CLEARENCE NO- SDMCAU /IAEC/2010/11

Acute Oral Toxicity study

The trial drug *Japakusumadi Yoga* was screened for acute toxicity study. The study was performed according to OECD 425 guidelines.[5] Direct administration of the drug was done without any vehicle as the formulation was liquid. All the animals were dosed a constant dose volume (1 ml/ 100g body weight) and administered a single dose orally at one of the three dose levels as generated by AOT software. The different dose levels used were 175mg/kg, 550mg/kg, 2000mg/kg, 2000mg/kg and 2000mg/kg. The test formulation was administered through oral route at different dose levels to respective animal through oral feeding needle sleeved on to disposable syringe. The dose volume of the test drug was selected as per requirement.

The group number, animal number and sex of the animal were identified with the help of cage cards, as presented in table 1.

OBSERVATIONS

After dosing the animal was observed continuously for 4 hours. The careful cage side observation was done without disturbing the animal attention. The animal was individually exposed to open arena at the end of the every hours for recording the behavioural changes like increased or decreased motor activity, convulsions, straub's reaction, muscle spasm, catatonia, spasticity, ophisthotonus, hyperesthesia, muscle relaxation, anaesthesia, arching and rolling, lacrimation, salivation, diarrhoea, writhing, mode of respiration, changes in skin colour etc. exitus, CNS depression - hypo activity, passivity, relaxation, ataxia, narcosis, etc.

Mortality

All the animals were observed at ½, 1, 2, 3, 4, 24 h, 48 h after dosing and there after daily once for mortality during the entire period of the study.

Table 1: Identification of animals

SN	Identification of animals	Desired dose (according to AOT)	Body weight (grams)	Calculated dose (ml) Assuming presence of 1g drug material in 10 ml
1	Head	175mg/kg	198	1.98
2	Neck	550mg/kg	204	2.04
3	Back	2000mg/kg	260	2.60
4	Base of the tail	2000mg/kg	243	2.43
5	No mark	2000mg/kg	221	2.21

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

Table 2(a): Signs and Symptoms during Gross Behavioral Study of Group: 175mg

Signs & Symptoms		Basal	30min	1h	2h	3h	4h	24h	48h
General impression		N	N	N	N	N	N	N	N
Increased motor activity		-	-	-	-	-	-	-	-
Convulsion: Tonic		-	-	-	-	-	-	-	-
Clonic		-	-	-	-	-	-	-	-
Straubs reaction		-	-	-	-	-	-	-	-
Muscle spasm		-	-	-	-	-	-	-	-
Catatonia		-	-	-	-	-	-	-	-
Opisthotonus		-	-	-	-	-	-	-	-
Hyperaesthesia		-	-	-	-	-	-	-	-
Decreased motor activity		-	-	-	-	-	-	-	-
Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-	-	-
	Watery	-	-	-	-	-	-	-	-
Respiration	Stimulation	-	-	-	-	-	-	-	-
	Depression	-	-	-	-	-	-	-	-
	Failure	-	-	-	-	-	-	-	-
Skin colour	Blanching	-	-	-	-	-	-	-	-
	Cyanosis	-	-	-	-	-	-	-	-
	Vasodilatation	-	-	-	-	-	-	-	-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N
Auditory response		N	N	N	N	N	N	N	N
mucus membrane		N	N	N	N	N	N	N	N

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

Piloerection	-	-	-	-	-	-	-	-

Table 2(b):

Time interval	Rat No. 1	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Table 3(a): Signs and Symptoms during Gross Behavioral Study in Group: 550mg/kg

Signs & Symptoms	Basal	30min	1h	2h	3h	4h	24h	48h
General impression	N	N	Active	Active	Active	Active	N	N
Increased motor activity	-	-	-	-	-	-	-	-
Convulsion:Tonic	-	-	-	-	-	-	-	-
Clonic	-	-	-	-	-	-	-	-
Straubs reaction	-	-	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-	-	-
Catatonia	-	-	-	-	-	-	-	-
Opisthotonus	-	-	-	-	-	-	-	-
Hyperaesthesia	-	-	-	-	-	-	-	-
Decreased motor activity	-	-	-	-	-	-	-	-
Muscle relaxation	-	-	-	-	-	-	-	-
Anaesthesia	-	-	-	-	-	-	-	-
Arching and rolling	-	-	-	-	-	-	-	-

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-	-	-
	Watery	-	-	-	-	-	-	-	-
Respiration	Stimulation	-	-	-	-	-	-	-	-
	Depression	-	-	-	-	-	-	-	-
	Failure	-	-	-	-	-	-	-	-
Skin colour	Blanching	-	-	-	-	-	-	-	-
	Cyanosis	-	-	-	-	-	-	-	-
	Vasodilatation	-	-	-	-	-	-	-	-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N
Auditory response		N	N	N	N	N	N	N	N
Mucus membrane		N	N	N	N	N	N	N	N
Piloerection		-	-	-	-	-	-	-	-

Table 3(b)

Time interval	Rat No. 1	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Table 4(a): Signs and Symptoms during Gross Behavioral Study in Group: 2000mg/kg

Signs & Symptoms		Basal	30min	1h	2h	3h	4h	24h	48h
General impression		N	N	Active	Active	Active	Active	N	N
Increased motor activity		-	-	-	-	-	-	-	-
Convulsion: Tonic		-	-	-	-	-	-	-	-
Clonic		-	-	-	-	-	-	-	-
Straubs reaction		-	-	-	-	-	-	-	-
Muscle spasm		-	-	-	-	-	-	-	-
Catatonia		-	-	-	-	-	-	-	-
Opisthotonus		-	-	-	-	-	-	-	-
Hyperaesthesia		-	-	-	-	-	-	-	-
Decreased motor activity		-	-	-	-	-	-	-	-
Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-	-	-
	Watery	-	-	-	-	-	-	-	-
Respiration	Stimulation	-	-	-	-	-	-	-	-
	Depression	-	-	-	-	-	-	-	-
	Failure	-	-	-	-	-	-	-	-
Skin colour	Blanching	-	-	-	-	-	-	-	-
	Cyanosis	-	-	-	-	-	-	-	-
	Vasodilatation	-	-	-	-	-	-	-	-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

Auditory response	N	N	N	N	N	N	N	N
mucus membrane	N	N	N	N	N	N	N	N
Piloerection	-	-	-	-	-	-	-	-

Table 4(b)

Time interval	Rat No. 1	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Table 5(a): Signs and Symptoms during Gross Behavioral Study in Group: 2000mg/kg

Signs & Symptoms	Basal	30min	1h	2h	3h	4h	24h	48h
General impression	N	N	Active	Active	Active	Active	N	N
Increased motor activity	-	-	-	-	-	-	-	-
Convulsion: Tonic	-	-	-	-	-	-	-	-
Clonic	-	-	-	-	-	-	-	-
Straubs reaction	-	-	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-	-	-
Catatonia	-	-	-	-	-	-	-	-
Opisthotonus	-	-	-	-	-	-	-	-
Hyperaesthesia	-	-	-	-	-	-	-	-
Decreased motor activity	-	-	-	-	-	-	-	-

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-	-	-
	Watery	-	-	-	-	-	-	-	-
Respiration	Stimulation	-	-	-	-	-	-	-	-
	Depression	-	-	-	-	-	-	-	-
	Failure	-	-	-	-	-	-	-	-
Skin colour	Blanching	-	-	-	-	-	-	-	-
	Cyanosis	-	-	-	-	-	-	-	-
	Vasodilatation	-	-	-	-	-	-	-	-
Grip strength		N	N	N	N	N	N	N	N
Visual placing response		N	N	N	N	N	N	N	N
Tail pinch response		N	N	N	N	N	N	N	N
Auditory response		N	N	N	N	N	N	N	N
Mucus Membrane		N	N	N	N	N	N	N	N
Piloerection		-	-	-	-	-	-	-	-

Table 5(b)

Time interval	Rat No. 1	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Table 6(a): Signs and Symptoms during Gross Behavioral Study in Group: 2000mg/kg

Signs & Symptoms		**Basal**	**30min**	**1h**	**2h**	**3h**	**4h**	**24h**	**48h**
General impression		N	N	Active	Active	Active	Active	N	N
Increased motor activity		-	-	-	-	-	-	-	-
Convulsion:Tonic		-	-	-	-	-	-	-	-
Clonic		-	-	-	-	-	-	-	-
Straubs reaction		-	-	-	-	-	-	-	-
Muscle spasm		-	-	-	-	-	-	-	-
Catatonia		-	-	-	-	-	-	-	-
Opisthotonus		-	-	-	-	-	-	-	-
Hyperaesthesia		-	-	-	-	-	-	-	-
Decreased motor activity		-	-	-	-	-	-	-	-
Muscle relaxation		-	-	-	-	-	-	-	-
Anaesthesia		-	-	-	-	-	-	-	-
Arching and rolling		-	-	-	-	-	-	-	-
Lacrimation		-	-	-	-	-	-	-	-
Diarrhoea		-	-	-	-	-	-	-	-
Writhing		-	-	-	-	-	-	-	-
Salivation	Viscid	-	-	-	-	-	-	-	-
	Watery	-	-	-	-	-	-	-	-
Respiration	Stimulation	-	-	-	-	-	-	-	-
	Depression	-	-	-	-	-	-	-	-
	Failure	-	-	-	-	-	-	-	-
Skin colour	Blanching	-	-	-	-	-	-	-	-
	Cyanosis	-	-	-	-	-	-	-	-
	Vasodilatation	-	-	-	-	-	-	-	-

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

Grip strength	N	N	N	N	N	N	N	N
Visual placing response	N	N	N	N	N	N	N	N
Tail pinch response	N	N	N	N	N	N	N	N
Auditory response	N	N	N	N	N	N	N	N
Mucus Membrane	N	N	N	N	N	N	N	N
Piloerection	-	-	-	-	-	-	-	-

Table 6(b)

Time interval	Rat No. 1	Time after drug administration	Exitus	CNS Depression					ANS		CNS Stimulation						Analgesia	Others
				Hypo activity	Passivity	Relaxation	Ataxia	Narcosis	Ptosis	Exophthalmoses	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsion	Straub tail		
B			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
1h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
2h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
3h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
4h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
24h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N
48h			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

RESULT

Physical and behavioural examination

There were no physical and behavioural changes in all the treated animals on day one at ½, 1, 2, 3, 4 hours intervals after dosing and there after once daily for 14 consecutive days. Thus the data obtained from the study on single dose administration of *Japa Kusumadi Yoga* oral administration with up to 14 days of observation period did not result in any physical and behavioural changes. The different parameters observed before and after the study are depicted in table 2a, 3a, 4a, 5a, 6a.

Mortality

In the present study no mortality was observed in treated rats and no toxic effect was observed throught the 14 days observation period after dosing.

DISCUSSION

Experimental standardization of the drug should better be done before the clinical usage because it gives good idea about the drug activity, dosage, merits, demerits and toxicity of the drug. The acute toxicity profile is one of the parameter to evaluate the toxicity of the drug. In the present study, acute

ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2017

toxicity profile of *Japakusumadi Yoga* has been evaluated. The study revealed that there was no mortality, toxic effects and any physical and behavioural changes in the treated animals.

CONCLUSION

The acute oral toxicity study of *Japakusumadi* conducted as OECD guidelines reveals the non toxic nature of the test formulation. From the observations recorded in acute toxicity, the oral administration of the test drug did not produce any mortality up to the dose of 2000mg/kg which is equivalent to 22.4g total dose for a human being weighing 70 kg man. At the dose level studied the drug also did not produce any physical and behavioural changes. Thus it could be concluded that the test drug is without any toxic potential even at the dose of 2000mg/kg in animals equivalent to 22.4g for human being.

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