INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

REPEATED DOSE 28-DAY ORAL TOXICITY STUDY OF RAW ARECA NUT EXTRACT IN RATS

Lohith T.S, Shridhar N.B, Dilip S.M, Pattar Jayashree* and Suhasini K.
Department of Pharmacology and Toxicology, Veterinary College, KVAFSU, Hebbal, Bangalore, India
*Corresponding Author Email: jayashri.vet@gmail.com

Article Received on: 13/03/13 Revised on: 07/04/13 Approved for publication: 14/05/13

DOI: 10.7897/2230-8407.04550
IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

ABSTRACT
The aqueous extract of raw areca nut extract was evaluated for the repeated dose 28-day oral toxicity effect on biochemical, haematological, feed consumption, body weight gain and histopathological parameters in rats. In repeated dose 28-day oral toxicity study, the doses administered orally were distilled water (control), 100, 250 and 500 mg/kg of raw areca nut extract for a period of 28 days. There was a significant increase ($P<0.05$) in AST, ALT and Cr of both male and female rats during the study period, but haematological parameters such as TEC, TLC, PCV and Hb concentration did not show any significant changes. Significant decrease ($P<0.05$) in feed consumption and body weight gain was also noticed. The histopathological changes of liver and kidney in rats indicate hepatotoxicity and mild degree of nephrotoxicity. Satellite high dose group administered with 500 mg/kg of raw areca nut extract showed normal in biochemical and haematological values, and architecture of the internal organs. This indicates that normal recovery of the animals after the treatment has been stopped.
Keywords: Areca nut, Biochemical, Haematological, Hepatotoxicity, Nephrotoxicity

INTRODUCTION

Areca nut is the seed of the fruit of the oriental palm *Areca catechu*. It is an important agricultural product in many regions of the world. Raw areca extract is the one obtained from boiling the betel nut, *Areca catachu* in water overnight and it is used for colouring the betel nuts for better marketing. Areca extract is stored in open containers for drying in open field. Cattle will have an easy access for drinking it and will have severe toxic reactions in coastal belt of Karnataka during the harvesting seasons of betel nut. The toxicity study of the raw areca nut extract was undertaken to elucidate biochemical, haematological, feed consumption, body weight gain and histopathological changes in rats.

MATERIALS AND METHODS

The raw areca nuts were separated from seed coat.12 kg of raw areca nut was taken, to which four litre of distilled water was added (the ratio of raw areca nut and distilled water was 3:1) and mixed properly. The contents were boiled for six hours. After boiling, the contents were filtered through sieve. The Filtrate was collected and stored in refrigerator. The dry matter content of raw areca nut extract was analyzed by drying the sample to a constant weight in a forced hot air oven at 105^{o} C[1]. Repeated dose 28-day oral toxicity study of raw areca nut extract was conducted in both male and female Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals, Repeated dose 28-day oral toxicity study (OECD 407)[2]. Healthy Wistar albino male and female rats aged around 6-9 weeks weighing 120 to 150 g were acclimatized to the laboratory conditions for seven days prior to the study. Animals were divided into four groups each containing six rats of both sex separately. Satellite group of control and high dose groups were maintained for both male and female rats separately as per the study protocol. These groups were maintained for further two weeks after the 28 day period without administering the raw areca nut extract. The animals were administered with the raw areca nut extract at a different concentration, where the highest dose was chosen with the aim of inducing toxicity but not death or reverse suffering. Thereafter, a descending sequence of dose was selected with a view to demonstrate any dose related response and No Observable Adverse Effects Level (NOAEL) at the lowest dose. The animals were administered daily single oral dose and the volume of administration was maintained at 1 ml per animal through proper dilutions of raw areca nut extract for a period of 28 days.

The group details and doses administered to male and female rats

Group Male rats	Dose type	Concentration (mg/kg)		Group Female rats	Dose type	Concentration (mg/kg)
Group I	Control	Distilled water		Group I	Control	Distilled water
Group II	Low dose	100		Group II	Low dose	100
Group III	Medium dose	250		Group III	Medium dose	250
Group IV	High dose	500		Group IV	High dose	500

Serum biochemical parameters (AST, ALT, BUN and Cr) were estimated from the serum samples and haematological parameters (TEC, TLC, PCV and Hb) were estimated using blood samples collected on day 0, 7, 14, 21 and 28 during the study period by retro-orbital plexus puncture technique using microhaematocrit capillary tubes under ketamine (40 mg/kg, I.P) and xylazine (10 mg/kg, I.M) anaesthesia. The animals were weighed individually at the beginning of the study and at weekly intervals till the end of study. Feed consumption measurements were made once in a week throughout the study period. At the end of study period, the rats were weighed and sacrificed on day 29 and necropsy was conducted for any gross and histopathological changes. The liver, spleen, kidney, heart, lung, stomach, intestine and brain were separated, weighed and collected in 10% neutral buffer formalin for histopathological study. Organ to body weight

ratio i.e., organ weight/body weight was calculated and expressed in percentage[3]. The data were analyzed by using two-way ANOVA, Bonferroni post-test. Mean values and standard error of mean were calculated and all the values are expressed as Mean ±SEM.

Table 1: Effect of raw areca nut extract on aspartate aminotransferase (AST) in male and female rats in repeated dose 28-day oral toxicity

Groups	Male rats (U/L)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	54.81±0.60	55.33±0.65	55.53± 0.73	56.20± 0.49	56.83± 0.79	56.20±0.49	56.40±0.46
Group II	55.81±0.33	58.58±0.80	60.16± 1.59	59.01± 1.59	61.66±2.45*	-	-
Group III	55.85±0.51	59.46±0.86	58.80± 1.75	60.76± 1.32	62.68±0.84**	-	-
Group IV	55.98±0.46	56.76±0.84	56.46± 2.80	62.73±0.66**	62.68±1.06**	61.73±1.70*	59.73±1.72

Groups	Female rats (U/L)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	55.80±0.47	56.70±0.62	56.25±0.35	57.88±0.65	56.00±0.25	54.90±0.70	55.25±0.40
Group II	55.46±0.45	55.83±0.94	60.25±1.20	60.03±1.48	62.03±3.24*	-	-
Group III	55.21±0.45	58.93±0.64	62.11±1.50*	61.85±2.03	63.30±2.07**	-	-
Group IV	55.13±0.28	58.96±0.75	63.15±1.68**	64.33±1.44**	63.66±1.73***	60.33±1.46**	58.12±1.58

Values are Mean± SEM, * P<0.05, ** P<0.01, ***P<0.001, n=6, the values on day 35 and 42 pertain to satellite group

Table 2: Effect of raw areca nut extract on alanine aminotransferase (ALT) in male and female rats in repeated dose 28-day oral toxicity

Groups	Male rats (U/L)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	24.65±0.70	24.73±0.71	25.55±0.59	26.26±0.96	25.70±1.21	25.14±0.90	25.45±0.60
Group II	24.73±0.73	25.81±0.77	25.45±0.98	27.38±0.60	26.28±0.32	-	-
Group III	24.13±0.84	26.26±0.80	28.18±0.91	27.63±0.49	29.33±0.47*	-	-
Group IV	23.70±1.21	25.88±0.54	28.38±1.01	30.60±0.36**	32.41±1.16***	28.50±0.28**	27.82±0.90

Groups	Female rats (U/L)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	23.83±0.97	25.41±0.87	26.78±0.48	25.50±0.81	25.56±0.87	24.50±0.80	24.80±0.58
Group II	23.43±0.96	26.45±0.83	25.83±0.33	27.85±0.60	28.58±0.62*	-	-
Group III	22.18±1.04	24.63±0.92	27.76±0.44	27.58±0.98	30.35±0.74***	-	-
Group IV	23.56±0.87	25.25±0.82	25.78±0.59	29.08±0.82*	30.95±1.02***	28.08±0.72**	26.78±0.55

Values are Mean± SEM, * P<0.05, ** P<0.01, ***P<0.001, n=6, the values on day 35 and 42 pertain to satellite group

Table 3: Effect of raw areca nut extract on creatinine (Cr) in male and female rats in repeated dose 28-day oral toxicity

Groups	Male rats (mg/dl)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	0.76± 0.05	0.76± 0.04	0.80± 0.05	0.78± 0.05	0.78± 0.04	0.85± 0.04	0.79± 0.05
Group II	0.76± 0.04	0.76± 0.05	0.84± 0.04	0.84± 0.02	0.80±0.04	-	-
Group III	0.77±0.04	0.84±0.04	0.83±0.04	0.77±0.03	0.84±0.05	-	-
Group IV	0.77±0.03	0.84±0.04	0.84±0.04	0.98±0.03*	0.98±0.03*	0.79±0.03	0.75±0.03

Groups	Female rats (mg/dl)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	0.77±0.04	0.77±0.04	0.79±0.05	0.79±0.05	0.81±0.03	0.79± 0.05	0.80± 0.04
Group II	0.77±0.04	0.77±0.04	0.82±0.4	0.85±0.02	0.81±0.04	-	-
Group III	0.78±0.04	0.86±0.03	0.83±0.04	0.77±0.03	0.98±0.04*	-	-
Group IV	0.76±0.03	0.85±0.04	0.85±0.04	0.98±0.03*	1.03±0.04**	0.88±0.06	0.78±0.05

Values are Mean± SEM, * P<0.05, ** P<0.01, ***P<0.001, n=6, the values on day 35 and 42 pertain to satellite group

Table 4: Effect of raw areca nut extract on body weight in male and female rats in repeated dose 28-day oral toxicity

Groups	Male rats (g)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	125.33±4.21	140.00±4.05	154.50±4.12	172.16±3.91	188.83±3.46	192.40±5.02	198.60±4.40
Group II	126.16±4.11	148.16±9.48	154.83±9.44	161.50±9.52	167.83±9.69	-	-
Group III	126.83±3.85	140.33±3.88	147.16±4.54	153.66±4.41	159.16±4.52**	-	-
Group IV	126.33±4.35	138.66±4.37	147.00±4.41	152.33±4.49	157.00±4.62***	175.20±4.80*	190.80±3.35

Groups	Female rats (g)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	126.16±4.19	140.16±4.19	154.66±4.44	172.66±3.96	187.50±3.94	196.66±3.85	204.33±3.62
Group II	126.50±4.09	147.83±9.57	153.83±9.77	161.50±9.52	167.33±9.82	-	-
Group III	127.33±3.84	141.00±3.95	146.50±4.62	150.16±4.54*	154.16±4.50***	-	-
Group IV	127.00±4.25	138.66±4.37	144.16±3.87	147.50±4.17*	151.66±3.94***	175.49±4.01**	193.22±3.67

Values are Mean± SEM, * P<0.05, ** P<0.01, ***P<0.001, n=6, the values on day 35 and 42 pertain to satellite group

Table 5: Effect of raw areca nut extract on feed consumption in male and female rats in repeated dose 28-day oral toxicity

Groups	Male rats (g/rat/day)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	10.49±0.09	14.25±0.08	16.52±0.06	18.30±0.05	18.40±0.05	20.30±0.07	18.95±0.10
Group II	10.50±0.11	14.37±0.05	16.39±0.14	18.10±0.07	18.63±0.12	-	-
Group III	11.00±0.15	14.56±0.07	16.90±0.19	17.88±0.15	17.95±0.19	-	-
Group IV	10.39±0.12	13.90±0.12	16.84±0.09	17.58±0.19**	17.79±0.18*	20.18±0.09	19.00±0.11

Groups	Female rats (g/rat/day)						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I	19.00±0.09	14.55±0.08	15.90±0.07	17.53±0.06	18.00±0.07	19.53±0.05	18.84±0.07
Group II	10.70±0.12	14.20±0.05	16.11±0.15	17.69±0.07	18.13±0.02	-	-
Group III	10.53±0.13	14.11±0.08	15.98±0.17	17.55±0.15	17.38±0.19**	-	-
Group IV	10.37±0.16	14.90±0.10	16.14±0.08	17.02±0.06*	17.37±0.06**	19.60±0.08	18.90±0.07

Values are Mean± SEM, * P<0.05, ** P<0.01, ***P<0.001, n=6, the values on day 35 and 42 pertain to satellite group

RESULT AND DISCUSSION

The dry matter content of raw areca nut extract was found to be 125 mg/ml. The rats administered with raw areca nut extract showed depression, weakness, profuse salivation, diarrhoea, decreased feed intake and decreased body weight gain. The clinical signs of toxicity are possibly indicative of cholinergic stimulation by the arecoline in raw areca nut extract. The clinical signs of the toxicity in the present study were supported by similar findings reported that feeding of albino rats with a diet containing 60–100% areca nut leading to salivation, diarrhoea[4]. In the present study, there was significant increase (P<0.05) in AST, ALT and Cr values. The increase in the concentrations of serum ALT and AST can be specifically attributed to liver damage caused by the raw areca nut extract which could have caused altered permeability and leakage of the enzymes. Other possible causes are increased enzyme synthesis or decreased catabolism, which result in the release of intracellular enzymes into the blood stream[5]. There was a significant (P<0.05) difference between the high dose satellite group and control group AST and ALT values at day 35, but no significant difference at day 42 in both male and female rats, this indicative of animals under the recovery phase and thus the toxicity induced by the raw areca nut extract is reversible. The increase in creatinine concentration in these treated groups indicated possible role of toxins in causing kidney damage. There was no significant difference between the high dose satellite group and control group creatinine values at day 35 and 42 in both male and female rats, this indicative of animals under the recovery phase. There was no change in the BUN, TEC, TLC, PCV and Hb concentration of all the treated groups compared to control group. This implied absence of toxic effects on haemopoietic system in the present study. Significant decrease (P<0.05) in body weight gain and feed consumption of both male and female rats was noticed. The decrease in body weight in the present study might be attributed to the decrease in the feed consumption which might be attributed to the gastro-intestinal disturbances or due to the organ toxicity and this toxic effect might be attributed to the disturbance in digestion, diarrhoea, unthriftiness etc noticed in cholinergic stimulation. There was no change in the organ to body weight ratio of all the treated groups compared to control group. At autopsy none of the treated and control rats showed any gross pathological lesions. The histopathology of liver revealed; granular appearance of hepatocytes, congestion of vessels, decreased sinusoidal spaces, swollen hepatocytes, vacuolated cytoplasm. Kidney revealed haemorrhages and distension of tubular and glomerular epithelium, desquamation of tubular epithelium, intertubular and glomerular hemorrhagic areas, vacuolations of the glomerular epithelium. Intestine showed increased goblet cell activity. Satellite high dose group showed normal architecture of the organs in histopathological study. This indicates the possible recovery of the animals after treatment has been stopped.

Summary

There was significant increase (P<0.05) in AST, ALT and Cr of both male and female rats during the study period indicating hepatotoxicity and nephrotoxicity. Significant decrease (P<0.05) in feed consumption and body weight gain was noticed in both male and female rats during the study period. The histopathological changes of liver, kidney and intestines of both male and female rats; which are indicating mild degree of hepatotoxicity, nephrotoxicity and intestinal damage. The present study revealed the toxic nature of the areca nut extract in rats which can be attributed to its various phytochemical constituents mainly arecoline alkaloid.

REFERENCES

1. Patricia Cunniff. Official methods of analysis, In: Association of official analytical chemists. 16th ed. Washington DC, Arlington: AOAC International; 1995.
2. Draft updated test guideline (OECD 407), Adopted by the council. Organization for Economic Co-operation and Development (OECD) guideline for the testing of chemicals, Repeated dose 28-day oral toxicity study in rodents. [Internet] Available from: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg407.pdf; 1995.
3. Luna LG. Manual of histopathological staining methods of the Armed Forces Institute of Pathology.3rd ed. McGrow Hill Book Co., New York; 1968.p. 1-46
4. Saikia M and Vaidehi MP. Studies on the pathological effects of feeding betel-nut meal in albino rats. Br. J. Exp. Med.1983; 64(5): 515-517.
5. Rouiller C. The liver morphology, Biochemistry and pathology. New York, Academic Press; 1964. p. 335-476

Cite this article as:

Lohith T.S, Shridhar N.B, Dilip S.M, Pattar Jayashree and Suhasini K. Repeated dose 28-day oral toxicity study of raw areca nut extract in rats. Int. Res. J. Pharm. 2013; 4(5):238-240

Source of support: Nil, Conflict of interest: None Declared