[Downloaded free from http://www.toxicologyinternational.com on Wednesday, April 27, 2016, IP: 14.139.224.243]

Original Article

www.toxicologyinternational.com

Biochemical Changes after Short-term Oral Exposure of *Jatropha curcas* Seeds in Wistar Rats

Vijeyta Awasthy, V. P. Vadlamudi, K. M. Koley, B. K. Awasthy, P. K. Singh

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Anjora, Durg - 491001, Chhattisgarh, India

ABSTRACT

Jatropha curcas (Euphorbiaceae) is a multipurpose shrub with varied medicinal uses and is of significant economic importance. In addition to being the source of biodiesel, its seeds are also considered highly nutritious and could be exploited as a rich and economical protein supplement in animal feeds. However, the inherent phytotoxins present in the seed is the hindrance. The toxicity nature of the seeds of the local variety of *J. curcas* is not known. Therefore, investigations were undertaken to evaluate the short-term oral toxicity of the seeds of locally grown *J. curcas*. Short-term toxicity was conducted in rats by daily feeding the basal diet (Group I), and the diet in which the crude protein requirement was supplemented at 25% (Group II) and 50% (Group III) levels through *Jatropha* seed powder. The adverse effects of *Jatropha* seed protein supplementation (JSPS) were evaluated by observing alterations in biochemical profiles. The biochemical profile of rats fed on diet with JSPS at both the levels revealed significant reduction in plasma glucose and total protein and increase in plasma creatinine, transaminases (Plasma glutamic pyruvic transaminase and Plasma glutamic oxaloacetic transaminase), and alkaline phosphatase.

Key words: *Jatropha* seed protein supplementation, phytotoxins, Wistar rats

DOI: 10.4103/0971-6580.72673

INTRODUCTION

Jatropha curcas (*Euphorbiaceae*), locally known as 'Ratanjyot,' is a multipurpose shrub with varied medicinal uses and is of significant economic importance. In addition to being the source of biodiesel, its seeds are also considered highly nutritious and could be exploited as a rich and economical protein supplement in animal feeds (53 – 58% crude protein [CP] in defatted meal), if the toxins are removed.[1] A recent study[2] revealed that *J. curcas* seed meal reduced of its phorbol ester level to a tolerable level of 0.09 mg/g had 68% CP, much higher than most of the oilseed meals. There are reports of existence of nontoxic varieties of the plant and variation in toxicity potential of the plant in different geographical regions.[3] The toxicity nature of the seeds of the local variety of *J. curcas* is not known. Therefore, investigations were undertaken to evaluate the short-term oral toxicity of the seeds of locally grown *J. curcas*, which is in turn characterized by changes in biochemical profiles. This toxicity study will help us to evaluate the possibility, if any, of their utility as a source of protein supplement in animal feeds.

MATERIALS AND METHODS

Plant material

The seeds of *J. curcas* were locally collected in bulk in the month of March-April from the premises of College of Veterinary Science and A.H., Anjora, Durg campus. The plant species was authenticated after botanical identification. The seeds were properly cleaned to free from any extraneous dust or other material. The cleaned seeds were shade dried and reduced to fine powder with the help of an electrical grinder. The seed powder so obtained was stored in airtight containers and used as such for preparation of the experimental diet whenever required.

Address for correspondence: Dr. Vijeyta Awasthy, Department of Pharmacology and Toxicology, Nagpur Veterinary College, Seminary Hills, Nagpur - 440006, India. E-mail: vijeytavet@gmail.com

[Downloaded free from http://www.toxicologyinternational.com on Wednesday, April 27, 2016, IP: 14.139.224.243]

Preparation of experimental animal diets

The experimental diets for feeding the animals were prepared after consulting with the Department of Animal Nutrition, College of Veterinary Science and A.H., Anjora, Durg. The control diet (standard diet) provided 22% CP containing normal feed ingredients. The modified diets were prepared by replacing the normal feed ingredients to an extent of 25% and 50% of the CP with *J. curcas* whole seed powder (CP = 18%). The composition of the experimental diets is shown in Table 1.

Short-term toxicity study

Young weaned Wistar rats (60 – 75 g) of either gender were obtained from a laboratory animal breeder. The animals were kept in the Laboratory Animal House under normal housing conditions and fed with standard feed with clean drinking water *ad libitum*. After sufficient period of acclimatization to the experimental conditions, the animals were randomly selected for the short-term toxicity testing to find out the effect of phytotoxins present in *J. curcas* seeds on various systems of the body following repeated exposure at sub-toxic doses. For this purpose, 52 weanling albino rats of either gender were taken and randomly assigned to three groups (i.e., Group I, II, and III) having 20 (10 males and10 females), 16 (8 males and 8 females), and 16 (8 males and 8 females) animals, respectively. The rats in Group I were given normal or standard diet. The Group II and III rats were fed on diet where the CP requirement was supplemented at 25% (Group II) and 50% (Group III) levels through *Jatropha* seed powder for 21 days. The adverse effects of *Jatropha* seed protein supplementation (JSPS) were evaluated by observing the alterations in biochemical profiles.

The blood samples were collected at different time intervals (i.e., on day 0, 7, 14, and 22) through cardiac puncture in properly heparinized vials**.** The different biochemical parameters (i.e., glucose, creatinine, total protein, GPT, GOT, and alkaline phosphatise [AP]) were estimated using blood plasma by standard procedures, using the diagnostic kits (Bayer's Diagnostics, Baroda, India) with the help of a semiautomated analyzer (RA-50 chemistry). Pretreatment values (normal/control) were determined in randomly selected five rats of control group.

Table 1: Composition of experimental diets

Ingredient	Proportion (100 g)		
	Control diet	Diet with *Jatropha* protein supplementation (%)	
		25	50
Maize	52.0	29.0	8.0
Soyabean	34.0	20.0	16.0
Rice polish	6.0	4.0	5.0
Groundnut cake	7.0	14.0	7.0
Jatropha seed	0.0	32.0	63.0
Vitamin-mineral premix	1.0	1.0	1.0
Total	100	100	100

Statistical analysis

The data are expressed as mean ± SE. The data were subjected to ANOVA to find out statistical variation between the mean values of different groups at different intervals of the observation period using the Software SPSS-10 for windows.

RESULTS AND DISCUSSION

All the rats fed with 25% JSPS (Group II) survived and were healthy till the end of the experiment. However, mortality was recorded in rats fed with 50% JSPS (Group III). Four rats of Group III died during 13th day of the trial. Furthermore, two rats of Group III succumbed to death on 16th day of treatment. Table 2 summarizes the effect of JSPS on different biochemical profiles of rats.

Effect on plasma glucose

There was no significant difference ($P \leq 0.05$) in glucose levels during pretreatment and at all the three intervals in

Table 2: Effect of feeding JSPS diet on plasma biochemical parameters (mean ± SE) of Wistar rats

Parameter	Period (days)	Groups		
		Control	25% JSPS	50% JSPS
Plasma glucose (mg/dl)	Pretreatment		56.2 ± 1.78	
	7th day	56.3 ± 1.95	55.4 ± 0.50	54.7 ± 1.11
	14th day	56.5 ± 1.98	52.9 ± 0.72	45.5 ± 1.21*
	22nd day	57.0 ± 1.67	47.4 ± 0.30*	–
Plasma protein (gm/dl)	Pretreatment		6.8 ± 0.35	
	7th day	7.3 ± 0.19	5.5 ± 0.36*	4.7 ± 0.16*
	14th day	6.7 ± 0.30	4.9 ± 0.41*	4.1 ± 0.07*
	22nd day	6.9 ± 0.36	4.1 ± 0.05*	–
Plasma creatinine (mg/dl)	Pretreatment		1.3 ± 0.24	
	7th day	1.4 ± 0.26	2.2 ± 0.17*	2.6 ± 0.28*
	14th day	1.5 ± 0.23*	3.0 ± 0.19*	3.2 ± 0.20
	22nd day	1.4 ± 0.21	3.2 ± 0.20*	–
Plasma GOT (U/l)	Pretreatment		36.8 ± 2.70	
	7th day	41.2 ± 1.39	37.4 ± 1.77	46.0 ± 0.94*
	14th day	42.6 ± 0.97	51.0 ± 0.91*	72.7 ± 2.17*
	22nd day	45.2 ± 1.59	71.6 ± 2.02*	–
Plasma GPT (U/l)	Pretreatment		35.6 ± 0.67	
	7th day	35.8 ± 0.73	38.4 ± 1.16	45.8 ± 0.96*
	14th day	40.0 ± 1.70	44.1 ± 1.95*	50.5 ± 0.64*
	22nd day	38.8 ± 0.86	50.6 ± 1.76*	–
Plasma ALP (U/l)	Pretreatment		133.0 ± 1.48	
	7th day	136.8 ± 0.73	144.6 ± 1.02*	146.6 ± 2.61*
	14th day	137.7 ± 1.43	149.2 ± 1.10*	152.0 ± 1.17*
	22nd day	141.0 ± 2.02	152.0 ± 2.08*	–

n (no. of observations) = 5; *Indicates significant ($P \leq 0.05$) difference level; SE - standard error; PGOT - plasma glutamic oxaloacetic transaminase; PGPT - plasma glutamic pyruvic transaminase; ALP - alkaline phosphatase

[Downloaded free from http://www.toxicologyinternational.com on Wednesday, April 27, 2016, IP: 14.139.224.243]

a control group. Following protein supplementation at 25% level in the feed with *Jatropha* seed protein, the 22nd day glucose level was significantly lower than that of the pretreatment level. Following protein supplementation at 50% level in the feed with *Jatropha* seed protein, the 14th day glucose level was significantly lower than the pretreatment level. The glucose levels in the three groups at pretreatment and on 7th day were statistically similar. The glucose level on 22nd day in Group II was significantly lower than the control Group (I). Similarly on the 14th day, glucose level in Group III was also significantly lower than the 14th day glucose level in control group and Group II. The reduced blood sugar levels of rats following JSPS might be due to impairment of carbohydrate digestion because of the presence of antinutrient amylase inhibitor in the seeds and absorption of glucose. The amylase inhibitors (tannins/polyphenols) in legume feeds were also reported to hinder carbohydrate (starch) digestion and glucose metabolism in animals.[4,5] Decrease in hepatic glycogen (liver biopsy) and reduced blood sugar levels were also observed in Nubian goats after feeding with *J. curcas* seeds @ 0.25 to 10 g/kg/day.[6]

Effect on plasma creatinine

In control group, the creatinine levels before and during the 7th to 22nd day plasma were identical. The rats in Group II (25% JSPS) during different treatment intervals showed creatinine levels in the range of 2.2 ± 0.17 to 3.6 ± 0.20 mg/dl which were significantly higher than the pretreatment level, and their 14th or 22nd day creatinine levels were also higher than that of the 7th day treatment level. In Group III (50% JSPS), the creatinine levels at both the treatment intervals were also significantly higher than pretreatment level. The 14th and 22nd day treatment levels in Group II and 7th and 14th day treatment levels in Group III were significantly higher than control group (I). The blood creatinine or urea nitrogen levels are indicative of renal function.[7,8] The elevated plasma creatinine levels are indicative of renal impairment following JSPS.

Effect on plasma protein

The protein levels during pretreatment (6.8 ± 0.35 g/dl) and at the three treatment intervals were statistically nonsignificant (6.7 ± 0.30 to 7.3 ± 0.19 g/dl) in a control group. Following 25% JSPS (Group II), the differences between pretreatment and different intervals were significant, where the levels on day 7th and 14th were significantly lower than at pretreatment, and that on the 22nd day was also lower than that of pretreatment and 7th day. The protein levels of Groups II and III were lower during different treatment intervals as compared with respective levels of control group (I). Furthermore, on 7th and 14th days, protein levels of Group III were also significantly lower than the respective levels of Group II. The hypoproteinemic effect of JSPS may also correlate to the presence of antinutrients such as trypsin inhibitor and tannins in the *Jatropha* seeds. The trypsin inhibitor interferes with digestion of dietary protein,[9,10] and the tannins complex with it and inactivates proteins in general, including the digestive enzymes that help in protein digestion and utilization.[11,12]

Effect on transaminases

In control group (I), the GOT enzyme activities at different intervals (36.8 ± 2.70 to 45.2 ± 1.59 U/l) were statistically similar. In Group II (25% JSPS), the GOT level at 14th day was significantly higher than pretreatment and on 7th day, and the GOT activity on 22nd day was significantly higher than pretreatment and on 7th and 14th day. In Group III (50% JSPS), the GOT levels on 7th day were significantly higher than that at pretreatment, and on 14th day activity was also significantly higher than at pretreatment and 7th day. The enzyme activity in Group II on 14th and 22nd day was significantly higher than that of control group. Similarly, the 7th and 14th day plasma glutamic oxaloacetic transaminase activities of Group III were also significantly higher than the activities in Group II and control group (I).

In control group (I), the GPT enzyme activity at different intervals (35.8 ± 0.73 to 40.0 ± 1.70 U/l) were statistically similar to the pretreatment activity (35.6 ± 0.67 U/l). In Group II (25% JSPS), the GPT levels on 14th and 22nd day were significantly higher than at pretreatment or on 7th day. In Group III (50% JSPS), the GPT levels on 7th and 14th day of treatment were significantly higher than at pretreatment, and the 14th day activity was also higher than that on 7th day. The enzyme activity on 22nd day in Group II was significantly higher than the respective level of control group. Furthermore, the 7th and 14th day enzyme activity of Group III was also significantly higher than the respective values of control group and Group II.

Alkaline phosphatase

The plasma alkaline phosphatase (PAP) activity in control group before treatment and at different treatment intervals did not vary significantly (133.0 ± 1.48 to 141. 0 ± 2.02 U/l). The enzyme activity in Group II (25% JSPS) and the AP at the three treatment intervals were significantly higher than that of pretreatment. Furthermore, the AP levels on 14th and 22nd day in Group II were also significantly higher than the PAP level on 7th day. The enzyme activity in both the JSPS groups at different intervals was significantly higher than the respective AP activities of control group.

The elevated serum or plasma transaminases (GPT and GOT) and AP activities are suggestive of hepatic impairment,[13,14] as these enzymes are organ specific in rats. Marked rise in serum arginase and GOT was also reported in Nubian goats fed with *J. curcas* seeds @ 0.25 to 10 g/kg/day up to 21 days,[6] in calves orally administered with water

[Downloaded free from http://www.toxicologyinternational.com on Wednesday, April 27, 2016, IP: 14.139.224.243]

in which *J. curcas* seeds were suspended @ 0.25, 1 or 2.5 g/kg within 10 or 14 days,[15] or in desert sheep fed with the seeds @ 0.05, 0.5, and 1 g/kg/day.[16] While investigating the short-term oral toxicity (14 days) of crude extract of some plants of *Euphorbia*, Adedapo *et al*.[17] also reported significant elevation in serum transaminase activities (GOT and GPT).

CONCLUSION

From the present investigation it could be concluded that the seeds of local variety of *J. curcas* at 50% protein supplement level cause biochemical alteration and mortality in rats during feeding trial. Hence, feeding of seed powder at 25% protein supplement level could be advocated with caution. Removal of antinutritic factors or detoxification of the seeds should be attempted before conducting further studies.

ACKNOWLEDGEMENT

The authors thank the Dean, College of Veterinary Sciences, Anjora for providing necessary facilities for this study.

REFERENCES

1. Becker K, Makkar HP. Effects of phorbol esters in carp (Cyprinus carpio L.). Vet Hum Toxicol 1998;40:82-6.
2. Aregheore EM, Becker K, Makkar HP. Detoxication of a toxic variety of Jatropha curcas using heat and chemical treatments, and preliminary nutritional evaluation with rats. South Pac J Nat Sci 2003;21:50-6.
3. Makkar HP, Aderibigbe AO, Becker K. Comparative evaluation of non-toxic and toxic varieties of Jatropha curcas for chemical composition, digestibility. Protein degradability and toxic factors. Food Chem 1998;62:207-15.
4. Glik Z, Joslyn MA. Food intake, growth depression and metabolic effects of tannic acid in the rat. J Nutr 1970;100:509-11.
5. El-Sayed MM, Ramadan ME, Ali HM, El-Morsi EA. Biochemical studies on alpha-amylase inhibitors in some legume seeds. Ann Agric Sci 1997;35:357-74.
6. Adam SE, Magzoub M. Toxicity of Jatropha curcas (Euphorbiaceae) for goats. Toxicology 1975;4:347-54.
7. Owen LN. Identifying and treating cancer in geriatric dogs. Vet Med 1991;86:55-66.
8. Finco, DR. Kidney function. In Clinical Biochemistry of Domestic Animals. In: Kaneko JJ, Harvey JW, Bruss ML, Editors. 5th ed. Singapore: Harcourt Brace and Company Asia PTE. Limited; 1997. p. 441-84,
9. Liner IE. Toxic factors in edible legumes and their elimination. Am J Clin Nutr 1982;11:281-4.
10. Salunkhe DK, Jadhav SJ, Kadam SS, Chavan JK. Chemical, biochemical and biological significance of polyphenols in cereals and legumes. Crit Rev Food Sci Nutr 1982;17:277-83.
11. Arora SK, Luthra YP. The in vitro digestibility of promising Indian varieties of soybean and its relation with tannin content. Indian J Nutr Diet 1974;11:233-4.
12. Satwadhar PN, Kadam SS, Salunkhe DK. Effect of germination and cooking on polyphenols and in vitro protein digestibility of horse gram and moth beans. Plant Foods Hum Nutr 1981;31:71-4.
13. Denman B, Hamdi E, Fox F, Frajola W. Carbon tetrachloride toxicity - Hepatostructural and enzymatic change. Arch Environ Health 1983;7:630-46.
14. Tennant BC. Hepatic function. In Clinical Biochemistry of Domestic Animals, In: Kaneko JJ, Harvey JW, Bruss ML, Editors. 5th ed. Singapore: Harcourt Brace and Company Asia PTE. Limited; 1997. p. 327-52.
15. Ahmed OM, Adam SE. Effects of Jatropha curcas on calves. Vet Pathol 1979;16:476-82.
16. Ahmed OM, Adam SE. Toxicity of Jatropha curcas in sheep and goats. Res Vet Sci 1979;27:89-96.
17. Adedapo AA, Abatan MO, Olorunsogo OO. Toxic effects of some plants in the genus Euphorbia on haematological and biochemical parameters of rats. Vet Arhiv 2004;74:53-64.

Source of Support: Nil, **Conflict of Interest:** None declared.