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Research Article

Acute and Chronic Toxicity Studies of *Tuba-tuba Jatropha curcas L.* (1753) Leaf Extract on Albino Rats (*Rattus norvegicus)*

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ABSTRACT

Herbal treatments are the most popular form of traditional medicine and it is very rampant in the Philippines since most of the Filipinos prefer to be treated the old natural way. *Jatropha curcas* is a plant that produces high oil content seeds used as biodiesel which is not edible because it contains toxic Phorbol ester. The leaves contain apigenin, vitexin, isovitexin which along with other factors enable them to be used against malaria, rheumatic and muscular pains. When drunk as a tea it helps with the reduction of fevers and also helps jaundice and gonorrhea. A lot of concrete evidences support the therapeutic activity of the plant. However, only little information can be provided regarding the possible toxicity of the plant. So the researchers conducted acute and chronic toxicity testing of *Jatropha curcas* decoction and ethanol leaf extract using rats as the test animals, grouped and administered with 200mg/kg, 500mg/kg and 1000mg/kg dose assessed thru organ weighing and behavioral changes. Results showed that extracts were not acutely toxic with 0% mortality but lethal to the rats when taken for longer period of time marked by behavioral changes that signifies chronic toxicity such as weight loss, anorexia, reluctance to move and restlessness, there was a change in organ weight in an average of more than 10% for all the organs weighed when compared to the organs of the untreated animals and mortality. *Jatropha curcas* is relatively safe when administered orally for acute use but not safe when used chronically.

Keywords: *Jatropha curcas* leaf extract; acute toxicity; chronic toxicity.

INTRODUCTION

Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. The use of traditional herbal medicine in the Philippines is very rampant, since most of the Filipinos prefer to be treated the old natural way. The use of herbal medicines has its advantages and disadvantages. An advantage of using these herbs is due to their availability, they are easily cultivated, cheap and effective. The downside of using natural medicinal herbs is their capacity to cause toxicity, the potency is not determined and the some of the constituents present in it may cause potential damage to human health[1]. The use of herbs has long been the center of interest by traditional practitioners for treating various body conditions. A lot of people from different countries opt for herbs as medicines because aside from being inexpensive; they have been believed to be safe. However, latest reports indicate that some of the plants used traditionally as medicines showed adverse effects. Thus, it should be clearly emphasized that traditional use of any plant as medicines should guarantee safety[2]. *Jatropha curcas* has been touted as a wonder plant. It is a plant that produces seeds with high oil content that can be used as biodiesel. The seeds are not edible and contain phorbol ester that is toxic[3,4]. The uses of various parts of Jatropha plant are well known from ages. The oil from seeds is used in relieving rashes and parasitic skin diseases. Sap form the bark is used to dress wounds and ulcers and can also be used to stop bleeding. Jatropha is most commonly used to purge the stomach, causing vomiting and diarrhea. When drunk as a tea, it can help with the reduction of fevers and will also help with jaundice and gonorrhea. Many people chew on the seeds to aid in constipation[5]. The leaves contain apigenin, vitexin, isovitexin which along with other factors enable them to be used against malaria, rheumatic and muscular pains. Many of these traditional medicinal properties of *Jatropha curcas* need to be investigated in depth for the marketable therapeutic products vis-à-vis the toxicological effects thereof[6]. A lot of concrete evidences support the therapeutic activity of the plant. However, only little information can be provided regarding the possible toxicity that the plant may cause to the consumers. Thus, acute and chronic toxicity studies on medicinal plants should be done in order to increase the confidence in their safety to humans, particularly for use in the development of pharmaceuticals. Sufficient data should support that toxicity of the plant does not outweigh its therapeutic use. This study was conducted since there was no available data regarding toxicity of its leaf extract. This study evaluated the acute and chronic toxicity of *Jatropha curcas* leaf extracts.

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Table 1: No. of Deaths during Chronic Toxicity Testing.

Test. Test Group (Water-Free Extract)	Total Population	No. of Dead Test Animals	No. of Survival Days
200mg dose	5	2	21
500mg dose	5	1	6
		1	23
		2	35
1000mg dose	5	4	6
Test Group Ethanol-Free Extract)	Total Population	No. of Dead Test Animals	No. of Survival Days
200mg dose	5	2	34
500mg dose	5	4	6
1000mg dose	5	4	4
		1	12

Table 2a: Vital Organ Weights on Different Test Groups with water-free extract.

Water-Free Extract 200mg/kg	Actual Weight	Ideal Weight	Weight Difference	% Difference	Weight Difference Remarks	Possible Correlation
A (155.3g)						
heart	0.81 g	0.94 g	0.13 g	13.83%	decreased	anorexia
kidneys	0.92 g	0.60 g	0.55 g	53.33%	increased	renal toxicity
liver	6.3 g	4.75 g	1.55 g	32.63%	increased	enzyme induction
lungs	1.4 g	1.29 g	0.11 g	8.53%	increased	enzyme induction
spleen	1.0 g	0.52 g	0.48 g	92.31%	increased	immune toxicity
B (126.1g)						
heart	0.5 g	0.77 g	0.27 g	35.06%	decreased	anorexia
kidneys	0.78 g	0.49 g	0.52 g	59.18%	increased	renal toxicity
liver	5.3 g	3.86 g	1.44 g	37.31%	increased	enzyme induction
lungs	1.3 g	1.05 g	0.25 g	23.81%	increased	enzyme induction
spleen	0.8 g	0.42 g	0.38 g	90.47%	increased	immune toxicity
C (133.1g)						
heart	0.48 g	0.81 g	0.33 g	40.74%	decreased	anorexia
kidneys	0.63 g	0.51 g	0.12 g	23.53%	increased	renal toxicity
liver	6.63 g	4.07 g	2.56 g	62.29%	increased	enzyme induction
lungs	1.21 g	1.10 g	0.11 g	10%	increased	enzyme induction
spleen	0.74 g	0.45 g	0.29 g	64.44%	increased	immune toxicity
500mg/kg (138.4g)						
heart	0.76g	0.85g	0.09	10.59%	decreased	anorexia
kidneys	0.70g	0.53g	0.17g	32.07%	Increased	Renal toxicity
liver	5.74g	4.24g	1.50g	35.38%	Increased	Enzyme induction
lungs	1.01g	1.15g	0.14g	12.17%	Decreased	Weight loss
spleen	0.57g	0.45g	0.12g	26.67%	Increased	Immune toxicity
1000mg/kg (169g)						
heart	0.62g	1.03g	0.41g	39.81%	Decreased	Anorexia
kidneys	0.66g	0.65g	0.01g	1.54%	Increased	Renal toxicity
liver	5.84g	5.17g	0.67g	12.57%	Increased	Enzyme induction
lungs	1.83g	1.40g	0.43g	30.71%	Increased	Enzyme induction

MATERIALS AND METHODS

Collection and Preparation of Sample

Fresh mature leaves of the plant, *Jatropha curcas Linn.* was collected from Toledo, South of Cebu City. They were cleaned with running water to remove dirt and foreign particles present. The leaves collected were dried using a clean cloth and cut into small pieces using a pair of scissors. The plant sample has been authenticated at the University of San Carlos, Department of Biology, Cebu City.

Extraction of the Plant Sample

Decoction

About 250 grams of the leaves were weighed and boiled with 800ml distilled water for 15 minutes, cooled and strained; the leaves were pressed to collect all the possible liquids. The collected extractives were evaporated to dryness using water bath. The resulting extract was labelled as water – free extract.

Maceration

About 250 g of the leaves were weighed and placed in an Erlenmeyer flask, and ethanol was added enough to cover the leaves. The flask was covered and allowed to stand at room temperature for a period of at least three days with frequent agitation to ensure complete extraction. The mixtures were strained and the extractive was concentrated

Table 2b: Vital Organ Weights on Different Test Groups with ethanol-free extract.

Ethanol-Free Extract 200mg/kg	Actual Weight (Treated group)	Ideal Weight (Untreated group)	Weight Difference	% Difference	Weight Difference Remarks	Possible Correlation
A (147.2g)						
Heart	0.63 g	0.77 g	0.14 g	18.18%	decreased	anorexia
Kidneys	0.75 g	0.60 g	0.15 g	25%	increased	renal toxicity
Liver	5.16 g	5.81 g	0.65 g	11.18%	decreased	malnutrition
Lungs	1.60 g	0.97 g	0.63 g	64.95%	increased	enzyme induction
Spleen	0.37 g	0.58 g	0.21 g	36.21%	decreased	cell depletion
B (150.7g)						
Heart	0.786 g	0.785 g	0.001 g	0.13%	increased	hypertrophy
Kidneys	0.76 g	0.61 g	0.15 g	24.59%	increased	renal toxicity
Liver	4.58 g	5.95 g	1.37 g	23.03%	decreased	malnutrition
Lungs	1.11 g	0.99 g	0.12 g	12.12%	increased	enzyme induction
Spleen	0.42 g	0.59 g	0.17 g	28.81%	decreased	cell depletion
C (93.2g)						
Heart	0.38 g	0.49 g	0.11 g	22.45%	decreased	anorexia
Kidneys	0.52 g	0.38 g	0.14 g	36.84%	increased	renal toxicity
Liver	4.45 g	3.69 g	0.76 g	20.60%	increased	enzyme induction
Lungs	0.75 g	0.61 g	0.14 g	22.95%	increased	enzyme induction
Spleen	0.31 g	0.38 g	0.07 g	18.42%	decreased	cell depletion
500mg/kg						
A (147.4g)						
Heart	0.61 g	0.77 g	0.16 g	20.78%	decreased	anorexia
Kidneys	0.82 g	0.60 g	0.22 g	36.67%	increased	renal toxicity
Liver	8.02 g	5.82 g	2.20 g	37.80%	increased	enzyme induction
Lungs	1.34 g	1.0 g	0.34 g	34%	increased	
Spleen	0.40 g	0.58 g	0.18 g	31.03%	decreased	cell depletion

using a rotary evaporator and evaporated to dryness using vacufuge. The resulting extract was labelled as ethanol – free extract.

Preparation of Test Solution

Solubility determination was first conducted prior to administration; one milligram of the sample per one milliliter of distilled water as the initial step while the solvent was increased by two milliliter for the succeeding steps. The same step was done to ethanol – free extract in which Tween 80 was used to dissolve the extract before adding sufficient amount of distilled water to make it into a solution. The maximum solubility of ethanol-free extract is 50mg/1.3ml Tween 80

Phytochemical Screening

One milliliter of the water-free and ethanol-free extract solution were tested for the presence of deoxysugars, tannins, alkaloids, sterols, flavonoids, unsaturated lactones, terpenes, reducing sugars and saponins

Test Animals

All test animals were housed in the University of San Carlos animal house and the research protocol was conducted under the supervision of a certified and trained animal technician ensuring proper animal handling was observed throughout the duration of the study. Thirty Albino rats of either sex weighing between 150-260 g were obtained and acclimatized for one week. The rats were housed in plastic transparent cages at room temperature and were exposed to equal light and dark cycles throughout the study, and fed on standard rodents feed. Water was given to the rats throughout the period of the study.

Determination of Acute Toxicity Activity

The rats were fasted for 12 hours and were grouped into four with five members for the treated group and one member for the untreated group. The test animals were individually weighed prior to the administration of test solutions. The three groups of rats were administered with *J. curcas* 200mg/kg ¸ 500mg/kg and 1000mg/kg orally. One untreated group was given with distilled water and the other untreated group was given with distilled water with Tween 80 for ethanol extract. After thirty minutes¸ the rats were fed with rodent pellets to ensure that the rats would not die because of starvation. For the first two hours of administration, the rats were closely observed for toxic symptoms and behavioral changes like anorexia (no fecal pellets) ¸ decrease in appetite (few fecal pellets) ¸ biting or shaking body part¸ restlessness¸ porphyrin discharge and increased respiration (red-brown pigments around eyes and nostrils). A video recorder was used to facilitate complete observation of the test animals' behavior to acquire accurate results. Mortality was recorded within 24 hours[7].

Determination of Chronic Toxicity Activity

The same test animals were used from the acute toxicity testing since none of the test animals died; the test animals had undergone a wash out period for one week to eliminate the administered test solutions from the previous test. A total of thirty-two albino rats were randomly allotted to four groups, consisting of the untreated and three extract-treated groups, 200 mg/kg, 500 mg/kg and 1000mg/kg the rats were weighed again for the preparation of the test

Table 3: Statistical Analysis Results on the organ weights ANOVA.

Source of Variation	d.f.	SS	MS	F	p-level
Between Groups	2	0.039913596	0.019956798	0.004355462	0.995656
Within Groups	12	54.98418986	4.582015822		<0.05
Total 14				55.02410346	

Table 4: Tukey HSD Test for Differences Between Means.

Groups	Difference	Test Statistics	p-level	Interpretation
1000mg vs 200 mg	0.11274	0.117770018	0.99626877	Insignificant
1000mg vs 500 mg	0.10578	0.11049949	0.996714364	Insignificant
200 mg vs 500 mg	-0.00696	0.007270528	0.999987099	Insignificant

solutions to be administered[8]. The doses were administered daily for 45-days through a gastric gavage using a tuberculin syringe. The rats in the different groups were observed closely for any behavioral changes like the reluctance to move¸ poor grooming, feeding and drinking habits and body weight changes with the use of a video cam to provide better results. They were later sacrificed for internal macroscopic examination to determine the increment and decrement of the vital organs; it would serve as a toxicity indicator due to possible toxin accumulation and the results were compared to that of the negative control group.

Effect on Vital Organs

At the end of the study, qualitative data on the weights of vital organs (heart, lungs, liver, kidneys, and spleen) were assessed by carefully dissecting each organ from the sacrificed animals into 10 % Normal Saline Solution contained in a Petri dish. Isolated organs were dried with a blotting paper and weighed on a sensitive balance[7]. The collected results were basically compared to the untreated group which will serve as a reference in the increment and decrement of organ weights[9,10].

Test for the Presence of Terpenes in the Blood Samples of Albino Rats (Rattus Norvegicus)

Blood samples were collected from the surviving test animals and from the untreated group thru cardiac puncture using a tuberculin injection. Test tube was in an inclined position and the blood was slowly made to slide down on the sides of the test tube. At least one-half milliliter of blood sample was added to milliliters of chloroform. With the test tube in an inclined position, concentrated sulfuric acid was made to slowly slide down the sides of the test tube and into the solution. Positive result was reddish brown interface.

RESULTS

Acute Toxicity

For the first two hours of administration, no toxic symptoms like anorexia, decrease in appetite, biting or shaking of body parts and restlessness were observed. A video recorder was used to monitor the behavior of the test animals for about ten to twenty minutes. No mortality was recorded for the whole 24 hour period after the administration of test solutions.

Chronic Toxicity

After three days of continuous administration, four rats died for 1000mg and 500mg/dose ethanol free extract groups. After six days of continuous administration, one rat died for 500mg and other four rats died from 1000mg dose of water – free extract group. After 15 days of continuous administration, one rat died for 1000mg dose ethanol - free extract group. After 21 days of continuous administration, two rats died for 200mg dose water - free extract group. After 23 days of continuous administration, one rat died for 500mg dose water free-extract group. After 35 days of continuous administration, two rats died from 500mg dose water-free extract group and another two rats died from 200mg dose ethanol - free extract group. The control for both the water - free and ethanol - free extract did not die nor showed symptoms of toxicity. A total of twenty-one rats died during the course of the 45 days period of administration (Table 1). The parameters noted were the test subject's reluctance to move, hair loss, diarrhea (wet feces) poor grooming, shaking of body part, weight loss and death. Two rats, one for ethanol free-extract 500mg dose and another rat for water-free extract 1000mg dose showed shaking of body part and after a few seconds, these rats died (these rats died on different days). The remaining rats (surviving rats) were weighed and the results were significant. All of the remaining rats had a drastic decrease or change in body weight and showed positive results for the presence of terpenes when their blood was tested, the toxicant found in *J. curcas* that is abundantly present in plant's seeds. The significant change in weight indicates chronic pain in rats.

Results on Vital Organs

There was an increase generally in the test animals' liver and kidney both in 200mg/kg, 500mg/kg and 1000mg/kg decoction extract and 200mg/kg and 500mg/kg ethanol-free extract, spleen weight also increased in 200mg/kg and 500mg/kg water-free extract test animal groups however there was a decrease in the test animals' spleen weight for the test animal given with 1000mg/kg water-free, 200mg/kg and 500mg/kg ethanol-free extract. Lungs weight increased for all of the sacrificed test animals except for the group given with 500mg/kg water free extract (Table 2).

Statistical Analysis

The One-way Analysis of Variance was used to calculate the p-level which determines the significance of the analysis. The level of confidence was set at 95%, having an allowable error of 0.05. If the calculated p-level is greater than 0.05, there is no significant difference between the given variables. If the p-level is less than 0.05, there is a significant difference (Table 3).

Tukey HSD Test for Differences between Means

A post-hoc test was used after the completion of the ANOVA. The post-hoc analysis shows that all the variables have insignificant difference; which means that the different doses administered (200mg, 500mg, and 1000mg) pose the same effects or threat to the test animals. There is no difference in the effects of the doses if administered with a 200mg dose compared to a 500mg dose or a 500mg dose from a 1000mg dose and vice versa (Table 4).

DISCUSSION

Jatropha curcas or commonly known as tuba-tuba plant is in widespread use as a fence because no animals would eat it and strangely its leaves are used to treat various ailments in such that it used internally to relieve fever, cough, dysentery and colic, and as a purgative. Today, this plant has been the subject of many researches including its potential use against drug resistant HIV by inhibiting the entry of HIV-1 cell entry and as an anticancer agent because it was known to have anti-tumor activity with low cytotoxicity[11]. Phytochemical test using the leaf extract was conducted and detects the presence of deoxysugars, tannins, sterols, and flavonoids, unsaturated lactones, reducing sugars, saponins and terpenes for the decoction. In ethanol-free extract solution deoxysugars, flavonoids, unsaturated lactones, reducing sugars, saponins and terpenes were also detected. The presence of these constituents will support its use in folkloric medicines and as well as its pharmacologic effects such as anti-inflammatory, analgesic, antibacterial antiviral and antioxidant. One of the constituents present is terpene which is known to have a toxic derivative phorbol ester that is present in high concentrations in the seeds. However the presence of these constituents may vary from place to another depending on what constituents it needs to have more for it to survive in such situation[12]. Two studies were conducted, acute and chronic toxicity tests. None of the test animals died in acute toxicity test, so chronic toxicity testing was carried out in which animals were sacrificed after 45 days for macroscopic analysis of organs. Organ weight is one of the most sensitive drug toxicity indicators as significant differences in organ weight between treated and control animals may occur in the absence of morphological changes, and its changes often precede morphological changes[13]. Based on the results, the toxicant present in the plant greatly affects the heart, liver, kidney and spleen which was presented with an absolute difference of more than 10% in the organ weights for all of the test animals both given with water-free and ethanol-free extract with different doses. Although results showed difference in lung weight from treated and the non-treated group, these changes may not directly pinpoint organ damage, it may be because of indistinct demarcations for trimming the airways. Alterations in liver weight may suggest treatment-related changes including hepatocellular hypertrophy (enzyme induction or peroxisome proliferation or lipidosis) however elevation of liver weight signifies enzyme-induction which is one marked activity of a terpene. Decrease in liver's weight suggests animal malnutrition; maybe due to less food consumption. Changes in kidney weight may reflect renal toxicity. Evaluation of liver and kidneys are very important because of their greater sensitivity to predict toxicity, frequent target organs of toxicity and there is little inter animal variability, and is often reflective of physiologic perturbations. Additionally liver is the primary detoxification and metabolism organ. Another marked result was the decrease in heart and spleen weight for the groups received 1000mg/kg water-free extract and 200mg/kg and 500mg/kg ethanol-free extract. Heart weight and body weight decrease roughly in parallel, the latter was clearly observed after the surviving test animals were weighed before being sacrificed, under some circumstances heart weight decreases due to moderate food consumption which was shown as anorexia or loss of appetite by the test animals during the course of study. Spleen is one of the histopathological indicators for possible immune toxicity; depressed weight of spleen suggests cell depletion and is viewed as potentially immunotoxic effect but still requires more definitive testing[14]. The researchers has also have observed heavy parasitic infestation on rats' hair which accounts weakened or compromised immune system, another correlation to decreased spleen weight. A massive hair loss was also observed which may be due to a diet high in fats, a property of terpene[15]. The above discussions are the possible causes or reasons behind the changes in weight for different organs all from the test animals sacrificed. The proper interpretation and evaluation of absolute organ weights should include macroscopic and microscopic correlation through gross pathology, clinical pathology and histopathology to be done by a study pathologist. Detectable weight changes of the weight may not necessarily be treatment related or an adverse effect[16]. It is important to note that inconsistency in the change of organ weights and having survival test animals that received similar dose can be due to interspecies variations. Not all members of the population respond to the same dose identically. Some will be more sensitive to the chemical and elicit response at lower doses termed as susceptible as the more resistant members which require larger doses for the same response. These interspecies effects variations can be due to the difference in age and maturity of the test animals used. Gender difference is also accountable; some chemicals may be more toxic to one gender than the other. Certain chemicals target certain reproductive organ of either male or female. Additionally, female have larger percentage of body fat than male, they may accumulate more fat soluble chemicals. Lastly, genetic makeup influences individual responses to toxic substances. If the necessary physiologic process are diminished or defective the natural body defense are defective. The ability of an individual to metabolize substances may be different to another[17,18]. The researchers conclude that *Jatropha curcas* decoction and ethanol leaf extract are not toxic when taken for a short period of time (24 hour period). There was neither mortality nor any marked behavioral changes observed during the course of acute toxicity testing generally for the latter observation. However the risk for toxicity arises when it is being consumed frequently for

longer period of time. Statistical results regarding each doses administered whether water-free or ethanol-free extract suggest no difference in effect on the organs, which means they pose the same amount of threat to test animals. Noticeable organ weight changes, poor grooming, decreased in body weight, restlessness, reluctance to move before death and rough hair coat and hair loss were also observed aside from mortality that are other parameters to assess chronic toxicity.

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REFERENCES

1. Firenzuoli F, Gori L, Lombardo G. The Medicinal Mushroom *Agaricus blazei* Murrill: Review of Literature and Pharmaco-Toxicological Problems. Evid Based Complement Alternat Med. 2008; 5(1): 3–15.
2. Pendota SC, Yakubu MT, Grierson DS, Afolayan AJ. Effect of Administration of Aqueous Extract of *Hippobromus Pauciflorus* Leaves in Male Wistar Rats. Afr J Tradit Complement Altern Med. 2010; 7(1): 40–46.
3. Griner EM, Kazanietz MG. Protein kinase C and Other Diacylglycerol Effectors in Cancer. Nat Rev Cancer 2007; 7(4):281-94.
4. Gudeta TB. Chemical Composition, Bio – Diesel Potential and Uses of *Jatropha Curcas* L. (Euphorbiaceae). American Journal of Agriculture and Forestry 2016; 4(2):35 – 48.
5. Nayak U, Javadekar BB, Bhatt SM, Patra P, Jaiswal JJ, Kulkarni A, Jetani A. Jatropha curcus Poisoning in Pediatric Patients. Gujarat Medical Journal 2009; 64(2):59 – 62.
6. Omoregie EH, Folashade KO. Broad Spectrum Antimicrobial Activity of Extracts of *Jatropha curcas*. Journal of Applied Pharmaceutical Science. J App Pharm Sci 2013; 3 (04): 83-87.
7. Joshua ZP, Timothy AG. Toxicity Studies of the Aqueous Root Extract of *Lecaniodiscus cupanioides* on Albino Rats. Science World Journal 2011; 6(4):27 – 29.
8. Yemitan OK, Ajibade AM, Adeyemi O O. Anticonvulsant activity of *Dalbergia saxatilis*. Nigerian Journal of Neuroscience 2001; 4:33-40.
9. Ajibade TO, Olayemi FO, Arowolo ROA. The haematological and biochemical effects of methanol extract of the seeds of Moringa oleifera in rats. Journal of Medicinal Plants Research 2012; 6(4): 615-621.
10. Dosseh K, Agbonon A, Gbeassor M. Antioxidant and toxicological studies of ethanolic root extract of Byrsocarpus coccineus Journal of Medicinal Plants Research 2015; 9(36):940-949.
11. Prasad DMR, Izam A, Khan MMR. *Jatropha curcas*: Plant of medical benefits. Journal of Medicinal Plants Research 2012; 6(14):2691-2699.
12. Tomar NS, Sharma M, and Agarwal RM. Phytochemical analysis of *Jatropha curcas* L. during different seasons and developmental stages and seedling growth of wheat (*Triticum aestivum* L) as affected by extracts/leachates of *Jatropha curcas* L. Physiol Mol Biol Plants. 2015; 21(1): 83–92.
13. Piao Y, Liu Y, Xie X. Change Trends of Organ Weight Background Data in Sprague Dawley Rats at Different Ages. J Toxicol Pathol. 2013; 26(1): 29–34.
14. Immunotoxicity studies. <http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM078748.pdf> accessed 24 November 2015.
15. Hairloss in Rats. <http://www.netvet.co.uk/rats/hair-loss.htm> accessed 18 September 2015.
16. Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, Perry R, Schafer K. Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. Toxicol Pathol. 2007;35(5):751-755.
17. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Julie K, Johson JK, Schafer K. Evaluation of Organ Weights for Rodent and Non-Rodent Toxicity Studies: A Review of Regulatory Guidelines and a Survey of Current Practices. Toxicologic Pathology 2007; 35:742–750.
18. Hayes AW, Kruger CL. Hayes Principles and Methods of Toxicology. Ed 6, CRC Press Taylor & Francis Group, Florida, USA, 1236 – 1239.