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In Vivo Evaluation of *Jatropha curcas* L (Euphorbiaceae) Leaves Acute and Subacute Toxicity in Mice

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Abstract

Jatropha curcas is a medicinal plant whose dead leaves are particularly used in arterial hypertension and diabetes treatment. The purpose of this study is to conduct an acute and subacute toxicity study of *Jatropha curcas* leaves aqueous extract (JCLAE) using OECD method. For acute toxicity, dose limits of 2000 and 5000 mg/kg were used. In subacute toxicity study 4 batches were constituted including a control batch that received distilled water for 28 days and the other 3 batches, JCLAE doses of respectively 200, 400, 800 mg/kg for the same duration. The LD_{50} was determined and the hematological, biochemical and histological parameters were analyzed in mice. The LD_{50} is greater than 5000 mg/kg. HDL-C is the only biochemical parameter that has experienced significant rise variation. Hematological analysis showed a decrease in mean platelet volume and Platelets number. The histological study revealed cases of hepatic cellular apoptosis and kidneys tubular necrosis among animals treated with highest dose. JCLAE is less toxic than *Jatropha curcas* leaves aqueous extract and high-dose JCLAE also has a moderate toxic effect on thrombocyte line and a protective effect on cardiovascular system.

Keywords: *Jatropha curcas*; Cardiovascular system; Toxicity; Apoptosis.

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1. Introduction

Traditional medicine offers recipes based on medicinal plants and some of them may be toxic. *Jatropha curcas* is a medicinal plant whose leaves are used in many pathologies treatment. In Cameroon *Jatropha curcas* leaves are used to treat arthritis and abscess in stomach [1]. In Benin this leaf decoction is used to treat edema and cough [2].

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In Burkina Faso *Jatropha curcas* is used to treat several diseases such as sickle cell disease, diabetes, gout, tumors, oral candidiasis, skin diseases, malaria, intestinal parasitosis, fever cases, and liver disorders. The dead leaves are particularly used to treat arterial hypertension and diabetes [3].

Jatropha curcas is a shrub that can be reproduced by seeds and cuttings. It is believed to be native to tropical America and is often found in wetlands, in tropical countries stream banks. Despite this plant various virtues and its use in traditional medicine, very few studies were focused on its toxicity. To contribute to the plant innocuousness establishment, we have carried out this acute and subacute toxicity study of its leaves aqueous extract.

2. Materials and Method

2.1. Plant material

Jatropha curcas leaves yellowing on the plant were collected at Kombissiri, a village situated at 45 km from Ouagadougou (Burkina Faso). This identification was carried out by the Plant Biology and Physiology Laboratory of the University of Ouaga I Pr. Joseph KI-ZERBO. Those *Jatropha curcas* leaves were dried in shade for fourteen days and then ground using an electric grinder to obtain a powder which was sieved.

2.2. Leaves total aqueous extract preparation

150g vegetable powder was added and homogenized in 1500 mL of distilled water, and left to macerate with magnetic stirring for 24 h at room temperature.
Then the macerate was filtered three times on hydrophilic cotton, then lyophilized and shielded from light in non-transparent flasks. The yield was 8.2%.
Yield = (Mass of extract/mass of dry plant) × 100

2.3. Animal material

9 week-old NMRI strain mice, weighing between 25 and 35 g were used to test acute and subacute toxicity. These male and female mice were taken from UFR/SVT breeding farm at the University of Ouaga I Pr Joseph KI-ZERBO. The mice were raised in stable temperature rooms (24 ± 2 °C) where they had free access to water and granular.

2.4. Acute toxicity

An orientation study was initially carried out and consisted in dividing 6 female mice into 2 batches of 3. The first and second batches respectively received a unique dose of 2000 mg/kg and 5000 mg/kg by tube-feeding using a probe. A main study was conducted following that orientation study with a test limit dose of 5000 mg/kg in accordance with

OECD Guideline 420 [4]. These mice were fasted 4 h before extract administration and 1 h after they were observed for 2 weeks to record any behavioral changes and to count the dead to determine lethal dose 50 (LD_{50}).

2.5. Subacute toxicity

The subacute toxicity study was conducted in accordance with the OECD protocol 407 [5,6]. Mice were divided into 4 batches of 8 animals each (4 males and 4 females). Three batches were injected daily through tube-feeding for 28 days with *Jatorpha curcas* leaves aqueous extracts, JCLAE (200, 400 and 800 mg/kg). A control batch was treated with distilled water over the same period. Each animal's weight was taken at the beginning and every week until the treatment end.

After 28 days each animal was anesthetized with ketamine plus xylazine as 2 volumes of ketamine for 1 volume of xylazine and administered at a dose of 1 ml/kg. Blood sample was taken in a tube containing an anticoagulant (K3EDTA) to determine hematological and biochemical parameters [7,8]. These haematological parameters were evaluated by a mindray BC-3000Plus brand haematology counter: White Blood Cells or Leukocytes (WBC), lymphocytes (lymph), les Granulocytes (Gran), les Monocytes (mono), Red Blood Cell (RBC), Hemoglobin (HGB), Hematocrit (Ht), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin concentration (MCHC), Platetet (PLT) and Mean platelet volume (MPV).

These biochemical parameters were determined using RMS/BCA 201 spectrophotometer: Creatinine, Alanine aminotransferase (ALAT), Aspartate aminotransferase (AST), according to kinetic method; urea, total proteins (TP), total Cholesterol (T cholest), Triglycerides (TG), High Density Lipoprotein (HDL), by colorimetric method. Low Density Lipoproteins (LDL) was determined by Friedewald formula. Glucose was measured using the Codefree Glucose Meter. The kidneys, liver, heart, lungs and spleen were removed and weighed. The kidneys and liver were fixed with 10% formalin for histopathological examinations.

2.6. Statistical analysis

The results of statistical analyzes were carried out using Graph Pad Prism 5.00 software. Values are presented as mean ± standard error on average. Significance degrees between treated and control batches were measured by one way analysis of variance (ANOVA) followed by Dunnett's *t*-test to compare mean effect among different groups to that of control groups. If $p < 0.05$, the difference is considered significant.

3. Results

3.1. Acute toxicity

Acute toxicity results did not show any sign of toxicity following administration of JCLAE by oral route dose 5000 mg/kg body weight (bw). All mice survived after 14 days of testing. JCLAE LD_{50} is therefore greater than 5000 mg/kg of body weight.

3.2. Subacute toxicity

No abnormal behavior was observed over 28 days of treatment. Their body weight did not change significantly with time (Table 1), as well as the relative weights of organs taken at the end of treatment (Table 2).

The haemogram did not show a significant change in the number of white and red blood cells. In contrast, mean platelet volume decreased significantly at 800 mg/kg BW dose (Table 3). Platelets number has decreased significantly for doses 400 mg/kg and 800 mg/kg compared with control batch. The results presented in Table 4 show that the JCAE has been without significant effect on certain plasma parameters such as: glucose, creatinine, urea, transaminases as well as total proteins, total cholesterol and LDL, triglycerides. However, HDL-C increased very significantly (Table 4).

Liver histopathological analyses show regular hepatocytes, numerous apoptotic patterns and extensive lesions of punctate lobular necrosis with vascular congestion and hemorrhagic suffusion consistent with moderate hepatotoxicity. Kidney histopathological examination shows lesions of subacute interstitial nephritis with vascular congestion, consistent with toxic etiology.

Table 1. *Jatropha curcas* leaves aqueous extract effect on mice body weight over 28 days of treatment.

Doses (mg/kg de PC)	0	7	14	21	28
0	33,5 ± 1,66	33,62 ± 1,64	33,87 ± 1,90	34,25 ± 1,82	33,6 ± 1,82
200	32 ± 1,53	32,37 ± 1,75	33,37 ± 1,88	33 ± 1,93	32,82 ± 1,98
400	31 ± 1,26	30,87 ± 1,56	31,62 ± 1,63	31,75 ± 1,52	30,81 ± 1,41
800	31,25 ± 1,55	29,12 ± 2,13	30,12 ± 1,85	30,37 ± 1,63	30,3 ± 1,65

Table 2. *Jatropha curcas* aqueous extract effects on relative weight of organs taken from mice after 28 days of treatment.

Doses (JCLAE)	0 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Liver	3,79 ± 0,06	3,85 ± 0,15	3,84 ± 0,09	4,31 ± 0,44
Heart	0,45 ± 0,03	0,38 ± 0,02	0,44 ± 0,02	0,45 ± 0,01
Lungs	0,54 ± 0,02	0,55 ± 0,03	0,66 ± 0,04	0,79 ± 0,08
Spleen	0,48 ± 0,08	0,45 ± 0,06	0,43 ± 0,04	0,46 ± 0,06
Kidneys	1,15 ± 0,06	1,08 ± 0,09	1,15 ± 0,05	1,10 ± 0,03

Table 3. *Jatropha curcas* aqueous extract effects on blood figurative elements taken from mice after 28 days of treatment.

Doses (JCLAE)	0 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
WBC (10ˆ 9/L)	4,4 ± 0,28	4,74 ± 1,14	3,84 ± 0,41	3,46 ± 0,56
Lymph (%)	71,4 ± 1,89	71,2 ± 2,42	70,2 ± 1,56	67,4 ± 2,69
Gran (%)	25,4 ± 1.80	25,6 ± 2,38	26,8 ± 1,46	30 ± 2,34
Mono (%)	3,2 ± 0,58	3,2± 0,37	3 ± 0,32	2,8 ± 0,37
RBC (10ˆ12/L)	7,09 ± 0,32	7,48 ± 0,31	7,67 ± 0,22	7,24 ± 0,12
HGB (g/dl)	11,32 ± 0,53	11,64 ± 0,59	12,5 ± 0,32	11,4 ± 0,32
HCT (%)	34,22 ± 1,17	35,18 ± 1,19	36,08 ± 1,00	33,76 ± 0,78
MCV (Fl)	48,4 ± 0,65	47,14 ± 0,56	47,26 ± 0,31	46,6 ± 0,39
MCH (pg)	15,9 ± 0,17	16,1 ± 0,69	16,26 ± 0,07	15,66 ± 0,19
MCHC (g/Dl)	33 ± 0,60	32,96 ± 0,70	34,48 ± 0,24	33,72 ± 0,36
PLT (10ˆ9/L)	770 ± 74,99	671,4 ± 72,37	527 ± 76,17 *	532,2 ± 31,18 *
MPV (fL)	7,04 ± 0,22	6,06 ± 0,32	6,52 ± 0,42	5,74 ± 0,17*

White Blood Cells or Leukocytes(WBC), lymphocytes (lymph), les Granulocytes (Gran), les Monocytes (mono), Red Blood Cell (RBC), Hemoglobin (HGB), Hematocrit (Ht), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin concentration (MCHC), Platetet (PLT) and Mean platelet (MPV).

Table 4. *Jatropha curcas* aqueous extract effects on mice biochemical parameters after 28 days of treatment.

Doses (EAFJC)	0 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Glucosa (mmol/L)	13,12 ± 1,33	13,63 ± 1,53	14,88 ± 0,95	15,56 ±0,92
Creatinine (µmol/L)	43,1 ± 9,44	35,66 ± 4,43	42,44 ± 5,17	33,3 ± 3,71
Urea (mmol/L)	8,56 ± 0,97	7,98 ± 0,53	7,77 ± 0,55	8,56 ± 1,01
ALAT (UI/L)	147,54 ± 21,21	122 ± 26,26	148,52 ± 27,17	160,16 ± 29,50
ASAT (UI/L)	233,26 ± 22,29	182,22 ± 25,60	233,6 ± 11,27	189,04 ± 26,60
P T (mg/dL)	5,25 ± 0,21	5,08 ± 0,07	5,42 ± 0,28	5,28 ± 0,17
Cholest T (mmol/L)	1,71 ± 0,18	1,65 ± 0,25	1,87 ± 0,24	2,45 ± 0,39
T G (mmol/L)	0,72 ± 0,10	0,75 ± 0,16	0,93 ± 0,15	0,51 ± 0,08
HDL (mmol/L)	0,19 ± 0,02	0,17 ± 0,07	0,27 ± 0,05	0,49 ± 0,05**
LDL (mmol/L)	1,19 ± 0,18	1,13 ± 0,16	1,17 ± 0,22	1,73 ± 0,33

Glucosa, Creatinine, urea, Alanine aminotransferase (ALAT), Aspartate aminotransferase (AST), total proteins (TP), total Cholesterol (T cholest), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoproteins (LDL)

4. Discussion

Oral acute toxicity study of JCLAE showed a lethal dose 50 (LD_{50}) greater than 5000 mg/kg body weight. Dabiré *et al.* [9]; Nga *et al.* [10] and Ping *et al.* [11] used the same method and showed that LD_{50} of *Excoecaria grahamii (Euphorbiaceae)* and *Alchornea cordifolia* (Euphorbiaceae) leaves aqueous extract; and *Euphorbia hirta L (Euphorbiaceae)* methanolic extract are greater than 5000 mg/kg body weight.

Studies conducted by Dangambo *et al.* [12] on *Jatropha curcas* leaves aqueous extract acute toxicity showed an LD_{50} of 2792.85 mg/kg body weight. This result shows that dead leaves aqueous extract would be less toxic than *Jatropha curcas* leaves aqueous extract.

Hematological analyses showed that total *Jatropha curcas* leaves aqueous extract did not cause significant changes in the number of erythrocytes and leucocytes. On the other hand, platelets number and mean platelet volume decreased significantly between batch I, batch III and IV respectively, and between batch I and batch IV. The decrease in MPV and PLT indicates that JCLAE has a toxic effect on platelet line. *Mukinda* and *Eagles* [13] reported that *Polygala fruticosa* extract causes a decline in platelet production.

Biochemical analyses showed a very significant increase in HDL-C of treated batch (800 mg/kg) compared with the control ones.

Angiotensin converting enzyme (ACE) inhibitors are known to increase in HDL-C [14]. Segura-Campos *et al.* [15] have shown that a peptide isolated from *Jatropha curcas* has an angiotensin converting enzyme inhibitory activity. HDL-C increase in our study could be related to this molecule presence in JCLAE.

HDL-C, also known as "good cholesterol", is a High Density Lipoprotein (HDL) that transports cholesterol from the tissues to the liver for elimination, therefore preventing accumulation of cholesterol on artery walls, thus decreasing atherosclerosis risk [16]. JCLAE may contain protective substances for cardiovascular system.

Apoptosis or programmed cell death is an important process in regulating cellular homeostasis and may limit tumor growth [17]. *Jatropha curcas* leaves anticancer properties have been demonstrated by *Balaji et al.* [18]. Molecules with anticancer properties were isolated from *Jatropha curcas* leaves [19,20]. These molecules anticancerous activity could justify the high amount of apoptotic features in the liver histopathological analysis. *Jatropha curcas* leaves still retain their anticancerous properties. Subacute interstitial nephritis lesions with vascular congestion observed in the kidney histopathological analysis show that JCLAE at 800 mg/kg has a toxic effect on kidneys.

5. Conclusion

JCLAE acute and subacute toxicity study by oral route showed that this extract is less toxic than green leaves aqueous extract and this justifies its use in traditional medicine. In addition, this extract may have a protective effect on cardiovascular system and retain its anticancerous properties. However, at high dose JCLAE has a toxic effect on thrombocyte number, liver and kidneys. Further work is needed to confirm its angiotensin converting enzyme inhibitory activity.

References

1. J. M. Watt and M. G. Breyer-Brandwijk, 2nd Edition (S. Livingstone, Ltd., Edindurgh, 1962) pp. 420-422.
2. H. D. Neuwinger (Chapman and Hall, New York, 1996) pp. 500–509.
3. O. G. Nacoulma / Ouedraogo, PhD thesis, Univ. Ouagadougou, France (1996).
4. Organization of Economic Co-Operation and Development (OECD), OECD Guideline for Testing of Chemicals: 420-Acute Oral Toxicity, OECD, Paris (1001) pp. 1–14.

5. OCDE. Étude de toxicité orale à dose répétée pendant 28 jours sur les rongeurs. In Lignes directrice de l'OCDE pour les essais de produits chimiques. OCDE, (2008) pp 1-14.
6. B. Gandhare, S. Kavimani, and B. Rajkapoor. J. Sci. Res. **5**, 315 (2013) http://dx.doi.org/10.3329/jsr.v5i2.11800
7. E. Sacchetto , D. Ali, E. Dumontet, D. Le carrer, J. –L. Orsonneau, O. Delaroche, and E. Bigot-Corbel, Ann. Biol. Clin. **72**, 337 (2014).
8. H. Kamali, M. Mohri, Revue Méd. Vét. **166**, 275 (2015).
9. A. P. Dabiré, Y. Ouedraogo, R. G. Belemtougri, S. Sawadogo, and M. Tiendrebeogo, Int. J. Pharm. Sci. Res. **2**, 12 (2017).
10. E. N. Nga, J. Yinyang, E. Baran, G. Etame-Loe, and S. D. Dibong, J. Appl. Biosci. **109**, 10649 (2017). http://dx.doi.org/10.4314/jab.v109i1.9
11. K. Y. Ping, I. Darah, Y. Y. Chen, S. Sreeramanan, and S. Sasidharan, BioMed. Res. Int. Article ID 18206 (2013) https://doi.org/10.3390/molecules17077782
12. M. A. Dangambo, H. T. Kabara, A. J. Alhassan, M. S. Sule, M. K. Atiku, A. M. Wudil, L. A. Mashi, Brit. J. Pharm. Res. **5**, 52 (2015). https://doi.org/10.9734/BJPR/2015/13105
13. J. T. Mukinda and P. F. Eagles, J. Ethnopharmacol. **128**, 236 (2010). https://doi.org/10.1016/j.jep.2010.01.022
14. D. Gude, J. Sci. Soc. **41**, 59 (2014).
15. M. R. Segura-Campos, F. Peralta-Gonzalez, A. Castellanos-Ruelas, L. A. Chel-Guerrero, and D. A. Betancur-Ancona, BioMed. Res. Int. Article ID 541947 (2013). http://dx.doi.org/10.1155/2013/541947
16. M. Arock, K. Chevet, R. Couderc, A. Del Corso, V. Ducros, R. Garnotel, I. Gastin, P. Gillery, N. Kapel, L. Kramer, A. Legrand, G. Le moël, C. Morin, C. Queyrel, J. C. Renversez, N. Schneider, P. Therond, H. Tronel, I. Villena, and J. P. Zarski, Le guide des examens biologiques, Le Quotidien du Pharmacien (2008).
17. C. M. Payne, H. Bernstein, C. Bernstein, and H. Grewal, Ultrastruct. Pathol. **19**, 221 (1995). https://doi.org/10.3109/01913129509064227
18. R. Balaji, N. Rekha, M. Deecaraman, and L. Manikandan, Afric. J. Pharmacy Pharmacol. **3**, 547 (2009).
19. R. Staubmann, M. Schubert-Zsilavecz, A. Hiermann, and T. Kartnig, Phytochem. **50**, 337 (1999). https://doi.org/10.1016/S0031-9422(98)00531-7
20. S. A. Al-ssa, Saudi Pharm. J. **21**, 305 (2013). https://doi.org/10.1016/j.jsps.2012.09.002