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SAFETY EVALUATION OF WISTAR ALBINO RATS TREATED WITH METHANOL EXTRACTS OF JATROPHA CURCAS LEAF FOR ANTI-DIARRHOEAL ACTIVITIES

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ABSTRACT

The safety Assessment of anti-diarrhoeal activity of methanol extract of J.curcasleaf at different dose levels on castor-oil induced diarrhoea in albino rats was carried out. Thirty (30) albino rats were divided into 5 groups of 6 rats each. Three groups of the albino rats were administered with 3 different doses of the extracts and the fourth and fifth groups received diphenyloxylate (5mg/kg bdy wt.) and 0.5% v/v aqueous tween 80(1ml) orally respectively. Sixty (60)minutes after the drug treatment each animal was administered with 1ml of castor-oil orally and observed for defecation for about four hours. The animals were sacrificed and the effect of the leaf extract on biochemistry and Histopathological examination of the kidney and liver tissues were assessed using standard techniques. The results showed that, there was no significant difference($P>0.05$) in the biochemical parameters: total protein, albumin, globulin, alanine amino transferase (ALT) and Aspartate Amino transferase (AST), between the experimental animals and the control. Histopathological assessment of the liver and kidney tissues showed mild alterations of the histological features. The findings of this study showed that the leaf extract of J. curcas has no adverse effect on the experimental animals.

Keywords: Safety Assessment, Anti-diarrhoeal , Methanol Extract.

1. INTRODUCTION

The use of medicinal plants is increasing worldwide, in-view of the tremendous expansion of traditional medicine and growing interest in herbal treatments. Plants are used in medicine to maintain and augment health physically, mentally and spiritually as well as to treat specific condition and ailments (Obute, 2005).

The plant kingdom contributed immensely to human health when no concept of surgery existed (Nwala*et al.*, 2013a). There is therefore need toconserve these plants associated withindigenous knowledge for our development of good health. Synthetic drugs gained popularity against green remedies because of their fast-acting effects; however, people have begun to realize the benefits associated with natural remedies (Igoli*et al.*,2005). Chemically-prepared drugs may act quickly but they have side effects which affect our body negatively in the long run, whereas medical plants work in an integrated or probiotic approach with little or no adverse effects on the body (Babu&Madhavi, 2001). For example, a regular intake of garlic can control high cholesterol and

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high blood pressure within a moderate period of time, but taking synthetic drugs make the person's body completely dependent on that particular medicine (Babu&Madhavi, 2001).

Medicinal plants are now in a comeback phase within the last two decades, seeing people shifting their focus back to the forgotten traditional natural green remedies (Nwala, 2013). Countries which are endowed with diverse and imported medical plants can grow these traditional herbs for export to developed countries, earning valuable foreign exchange. Moreover, as people learn more about the nutritional and medicinal value of plants, they increase their consumption, resulting in improved health (Sofowora, 1993).

Thereare considerable documentations of the traditional uses of various parts of the*Jatropha* plant (Egbunefu *et al.*, 2020). Some of these uses have been verified by research. Preparations of all parts of the plants, including seeds, leaves and stem back, fresh or as a decoction are used in traditional medicine and for veterinary purposes (Egbunefu *et al.*, 2020).

Traditionally, herbs have been considered to be non-toxic and have been used for treating various problems by the general public and/or traditional medicine practitioners(herbalists) worldwide (Nwala*et al.*, 2013a; Nwala *et al.*, 2013b). Documented severe toxicityresulting from the use of herbs on the many occasions abounds, yet the potential toxicity of herbs has not been recognized by the general public or professional groups of traditional medicine practitioners (Nwala*et al.*, 2013b). The leaf of *Jatropha curcas* when boiled in water has been used by some local healers in Etche Local Government Area of Rivers State Nigeria for treatment of diarrhoea (Egbunefu*et al.*, 2020). Though little research has been done on the anti-diarrhoea activity of extract from some parts of curcas plant according to Mujundar, *et al.*(2000) and Egbunefu, *et al.*(2020), no work has been done so far on the safety implications of such treatments, even though there has not been noticeable adverse effect on the users of these plant. It is on this premise that the study is conducted or designed to investigate the histopathology of the liver and kidneys tissues of albino rats treated with the leaf extract of *J. curcas*, using some biochemical parameters, with a view to assessing the safety status of the extracts.

2. MATERIALS AND METHODS

2.1 Plant Materials

The *Jatropha curcas* leaves used for the work were obtained from Igbo in Etche Local Government Area of Rivers State, Nigeria, where the plant is normally used as life tree to make fence that demarcate plots of land (Egbunefu*et al.*, 2020). The leaves were sun dried and pulverized and stored in an air-tight container for further use. About 300g of the pulverized leaf sample was extracted with methanol (Nwala*et al.*, 2013c). The extract was filtered using Whitman's No. 1 filter paper and the filtrate was concentrated to dryness in vacuo using arotary evaporator to remove the methanol.

2.2 Experimental Animals

Thirty (30) white albino rats (of mass between 150g and 200g) obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria were

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usedfor the studies. They were divided into five groups of six (6) each (i.e. n=6) and were housed individually in separate laboratory conditions for acclimatization period of seven (7) days prior to the commencement of the experiment. The animals were fasted for 18 hours prior to the experiment (Egbunefu*et al*., 2020).

2.3 Castor Oil-induced Diarrhoea

The doses of methanol extract of sample were selected on a trial basis and administered orally (100, 200 and 400mg/kg weight) by gavages to three groups of animals.The fourth group received diphenoxylate (5mg/kg body weight) orally and the fifth group received neither drug nor extract but 0.5% v/v aqueous tween 80(1ml) only and served as a control. After 60 minutes of drug treatment, each animal was administered 1ml of castor oil orally by gavage and observed for defecation up to 4 hours after castor oil administration.Characteristic diarrhoeal droppings were noted in the transparent plastic dishes placed beneath the individual perforated rat cages. The mean number of wet faeces was calculated from the diarrhoeal dropping in the transparent plastic dishes (Egbunefu *et al*., 2020).

At the end of the exercise (experiment), the wistar albino rats were sacrificed and the effects of treatment with extracts of *J.curcas*leaves on blood biochemistry and histopathological examination assessed as described by Oduola*et al*. (2007) earlier.

2.4 Determination of Serum Biochemical Parameters

Total protein was measured using biuret reagent while albumin was measured by colourimetric estimation using the stigma diagnostics albumin reagent (Sigma diagnostic, U.K), which contained bromocresol Green (BCG). Globulin was obtained from the difference between total protein and albumin. Aspartate AminoTransferase (AST), and Alanine Amino Transferase (ALT) were also measured.Aspartate Amino Transferase (AST) was determined by monitoring the concentration of Oxaloacetate Hydrazone formed with 2,4-dinitrophenyl hydrazine and ALT by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenyl hydrazine(Adedapo *et al.*, 2004).

2.5 Histopathological Evaluation

The animals (Wistar Albino Rats) used for the study were finally sacrificed and effect of the extract of *J. curcas*leaves on the organs (kidney and liver) examined as described (Nwala*et al*., 2013a; Nwala *et al*.,2013b; Arhoghro*et al*., 2009).

2.6 Statistical Analysis

The data were analyzed using tables, range, means, percentages, standarddeviation and hence standard error (S.E). Also all the data obtained were subjected to analysis of variance (ANOVA) using computer aided science planning and scheduling system (SPSS) using Ducan's multiple range test as described by Nwala (2013), at 5% level of significance.

3. RESULTS

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3.1 Biochemical Parameters

The total protein content was highest in the group treated with 400mg methanol extract (74.03 ± 0.18g/l) which was significantly ($p<0.005$) elevated, relative to the treatments with 0.5% tween 80(68.23 ± 0.34g/l), diphenoxylate (68.00 ± 0.58g/l) and 200mg methanol extract (66.95 ± 0.48g/l). The albumin content of the rats ranged from 3.47 ± 0.27g/dl (100mg methanol extract) to 4.97±0.56g/dl (0.5% tween 80). Other albumin contents were 4.00 ± 0.29g/dl (diphenoxylate), 3.60 ± 0.81g/dl (200mg methanol extract) and 4.33 ± 0.19g/dl (400mg methanol extract).

The globulin content was highest in the group treated with 100mg methanol extract (32.50 ± 0.29g/dl), which was significantly ($p<0.05$) different from the groups treated with 0.5% tween 80 (25.77 ± 0.44g/dl), diphenoxylate (20.87 ± 0.50g/dl), 200mg (20.13 ± 0.08g/dl) and 400mg (31.17 ± 0.10g/dl) methanol extracts. The group treated with 0.5% tween 80 had the highest level of ALT (19.50 ± 0.29µ/L) which was significantly ($P<0.05$) different from those treated with diphenoxylate (6.33 ± 0.17 µ /L), 100mg (11.00 ± 0.58 µ /L), 200mg (9.50 ± 0.29 µ/L) and 400mg (7.50 ± 0.46 µ /L) methanol extracts. The AST levels of the groups were 35.00 ± 0.40 µ /L for 0.5% tween 80, 20.32 ± 0.24µ/L for diphenoxylate, 25.32 ± 0.30 µ /L for 100mg extract, 23.00 ± 0.59µ/L for 200mg extractand 20.33 ± 0.19µ/L for 400mg extract. The group treated with 0.5%tween 80 (35.00 ± 0.40µ/L) was significantly ($P<0.05$) elevated, relative to the other group. The above result is presented in Table 1 below.

Table1:Results of Biochemical Parameter used to evaluate the effect of treatment with extracts of *Jatropha curcas*leaf (At different levels) on castor-oil induced diarrhoea in Albino Rats.

Groups	Total Protein g/L	Albumin g/dL	Globulin g/dL	ALT m/L	AST m/L
0.5% tween 80	68.23± 0.34^{abd}	4.79± 0.56^{a}	25.77±1 0.44^{a}	19.50± 0.29^{a}	35.00± 0.40^{a}
Diphenoxylate	68.00± 0.58^{abd}	4.00± 0.29^{a}	20.87± 0.50^{bd}	6.33± 0.19^{be}	20.32± 0.24^{be}
Methanol Extract 100mg	72.17± 0.10^{ce}	3.47± 0.29^{a}	32.50± 0.29^{c}	11.00± 0.58^{cd}	25.32± 0.30^{cd}
Methanol Extract 200mg	66.95± 0.48^{abd}	3.60± 0.81^{a}	20.13± 0.08^{bd}	9.50± 0.29^{cde}	23.00± 0.59^{cd}
Methanol Extract 400mg	74.03± 0.18^{ce}	4.33± 0.19^{a}	31.17± 0.10^{c}	7.50± 0.46^{e}	20.33± 0.19^{be}

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Values are means ± standard deviation of triplicate determinations means in the same column with different superscript letters were significantly different at the 0.05 level.

3.2 Histological Parameters of Albino Rats Treated with Extract of *Jatropha curcas* Leaf at Different Dose Levels) on Castor-oil Induced Diarrhoea

The kidney of the group treated with 100mg leaf extract showed glomerular inflammation, fibrin material within the Bowman's capsule, interstitial inflammation and normal vessels. The liver showed mild portal tract inflammation.

The group treated with 200mg leaf extract had kidney tissues that showed glomerularinflammation with Bowman's capsule containing fibrinous materials. There werealso tubular damage and interstitial inflammation. The liver showed mild portal tract inflammation, the cytoplasm of the hepatocyte contains eosinophilic amorphous material.The 400mg leaf extract treated group had kidney tissues that showed glomerular inflammation, tubular damage and interstitial inflammation. The liver showed mild portal inflammation. There were infiltrations of the parenchyma by inflammatory cells.

The group treated with diphenoxylate (standard drug) showed mild glomerular inflammation, interstitial inflammation and normal vessels. The liver showed mild portal and lobular inflammations. The kidney of the group treated with 0.5% tween 80 solutions (control) showed severe glomerular damage with deposition of fibrin within the Bowman's capsule. There were also tubular damage and interstitial inflammations.The liver showed lobular and portal inflammations. The result is presented in the Table 2.

Table 2: Results of Histological Examinations of Rats treated using Extracts of *Jatropha curcas* leaf at different Doses on Castor-oil Induced Diarrhoea

TISSUE SLIDE	TREATMENT GROUPS	DESCRIPTION OF HISTOLOGICAL EXAMINATION
	100mg leaf extract	
Kidney	→	Showed glomerular & interstitial Inflammation with normal vessels.
Liver	→	Showed mild portal tract inflammation.
	200mg leaf extract	
Kidney	→	Showed glomerular inflammation, tubular damage with interstitial inflammation.

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Liver	→	Mild portal tract inflammation, the hepatocyte contain eosinophilic material
	400 mg leaf extract	
Kidney	→	Showed glomerular & interstitial inflammations with tubular damage.
Liver	→	Showed mild portal inflammation. There were infiltrations of the parenchyma by inflammatory cells.
	Diphenoxylate	
Kidney	→	Showed mild glomerular & interstitial inflammations with normal vessels.
Liver	→	Mild portal & lobular inflammations.
	0.5% tween 80	
Kidney	→	There were tubular damage and interstitial inflammation, sever glomerular damage.
Liver	→	Showed lobular & portal inflammations.

The photomicrographs shown in Plates 1-10 represents the kidney and liver tissues respectively of the five (5) different treatment groups for the leaf extract of *Jatropha curcas*.

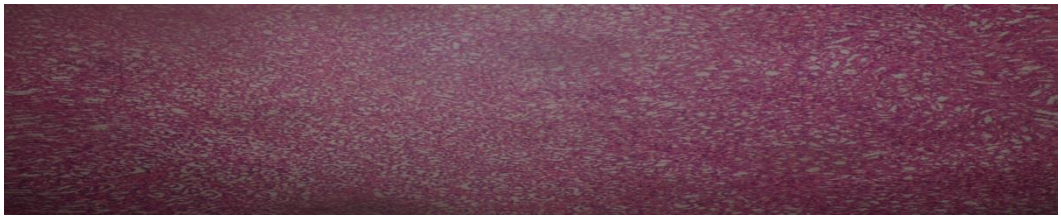

Plate 1: Kidney treated using 100mg of Leaf extract (glomerula and interstitial inflammations with normal vessels)

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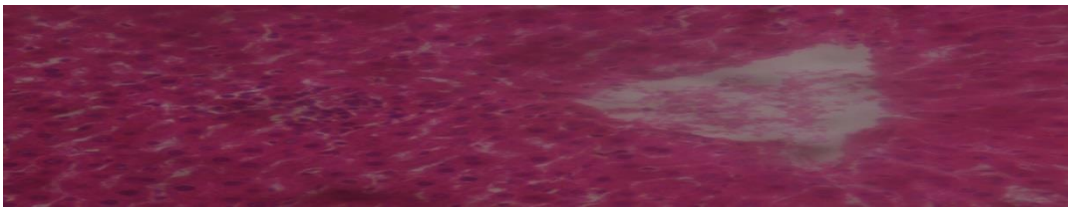


Plate 2: Liver treated using 100mg of Leaf Extract (mild portal inflammation)

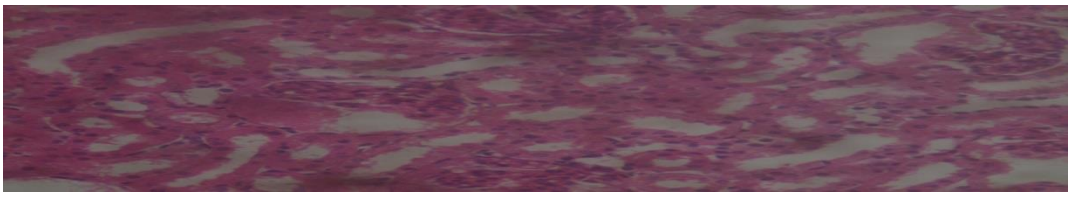


Plate 3: Kidney treated using 200mg of leaf Extract (glomerular inflammation, tubular damage with interstitial inflammation)

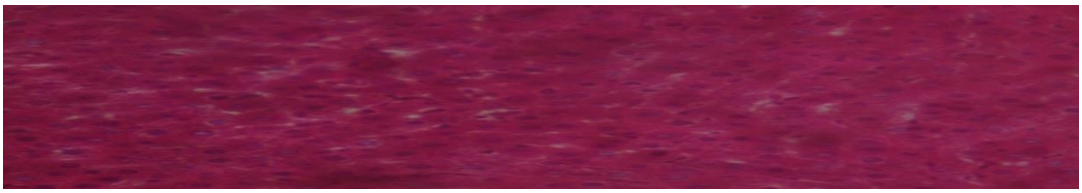


Plate 4: Liver treated using 200mg of leaf Extract (mild portal tract inflammation as hepatocyte contains eosinophilic material)

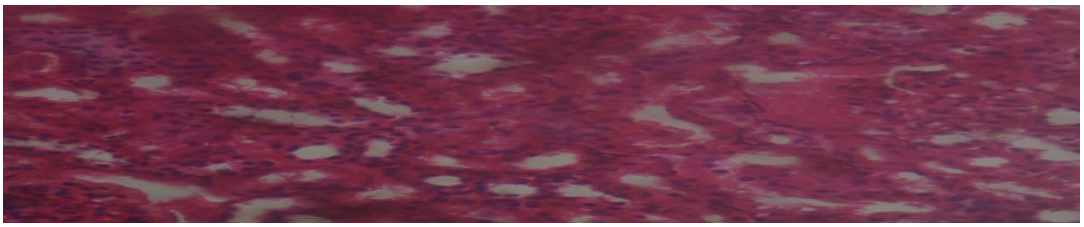


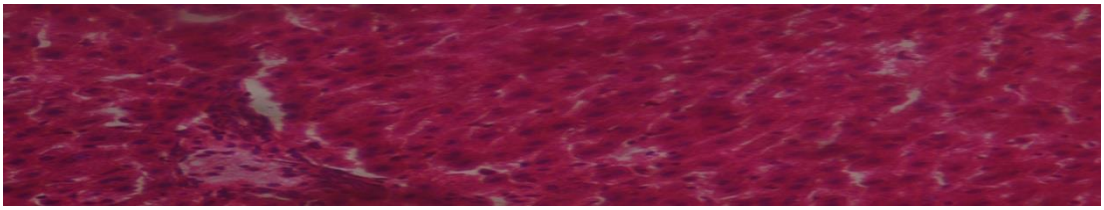
Plate 5: Kidney treated using 400mg of leaf Extract (glomerular, interstitial inflammations with tub
ular

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Plate 6: Liver treated using 400mg of leaf Extract (mild portal inflammation and infiltration of the parenchyma by inflammatory cells

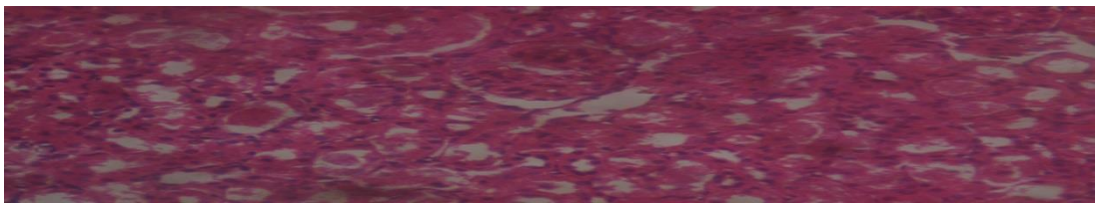

Plate 7: Kidney treated using diphenoxylate (mild glomerular and interstitial inflammations with normal vessels)

Plate 8: Liver treated using diphenoxylate (mild portal and tubular inflammations)

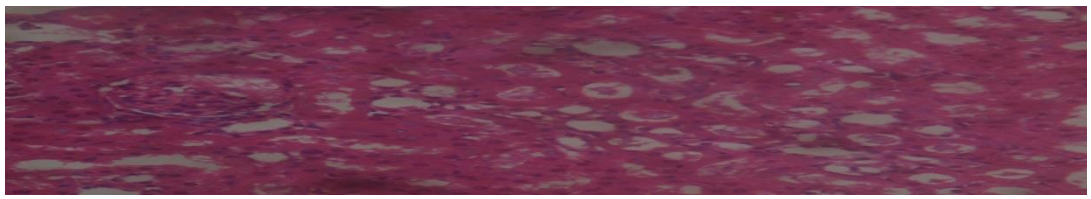

Plate 9: Kidney treated using 0.5% tween 80 (tubular damage, interstitial inflammation and severe glomerular damage)

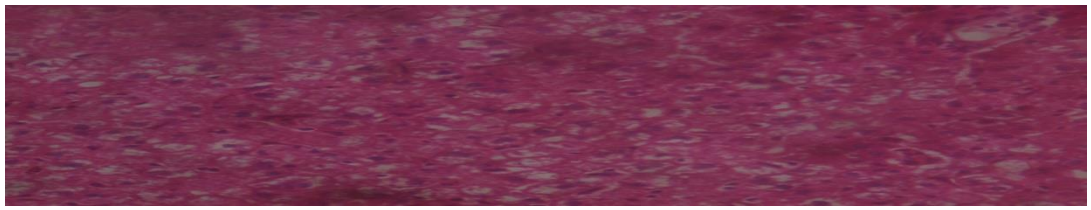

Plate 10: Liver treated using 0.5% tween 80 (lobular and portal inflammations)

4. DISCUSSION

The traditional practitioners of Igbo in Etche Local Government Area of Rivers State, Nigeria, use the water extract of leaf of *J. curcas* obtained in boiled water for the treatment of diarrhoea without recourse to safety considerations.The use of the leaf extract of this plant has been reported by Egbunefu*et al.*(2020), Srinivas, *et al*. (2008) and Maiti,*et al*. (2007) as an anti-

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diarrhoea agent, but not much has been done on the safety aspect. But from the present study, the total protein of rats treated with methanol extracts of *J. curcas* leaf sample on castor oil induced diarrhoea was highest in the group treated with 400mg/kg methanol extract (74.03 ± 0.18g/L) and this valueis not significantly different ($p<0.05$) from that of the group treated with 100mg/kg methanol extract (72.17±0.10g/l).The disparity between the total proteins contents of the control group treated with the standard drug, diphenoxylate, from the other treatment groups was not very much pronounced, hence an indication of the fact that no serious change occurred in these experimental animals. The observed concentrations here were higher than the values reported by Adeoyeand Oyedapo(2004) and Adedapo*et al*. (2004) on similar studies.The albumin levels of all the treatment groups were non-significantly ($p>0.05$) different from each other, though the group treated with 0.5% tween 80 has the highest level or concentration of albumin. The albumin contents reported here were lower than the ones reported by Adedapo*et al.*, (2004) in similar research study. The globulin level of the groups treated with 100 and 400g/kg methanol extracts were elevated more than the other treatment groups. According to Nwala, *et al.* (2013c), this may mean arise in the immune competence of the animals concerned. For the rest groups, their immune system may be said to be normal since their globulin concentrations were comparable to that of the control group. The Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) of the rats were highest in the group treated with 0.5% tween 80 (control), and lowest in the group treated with diphenoxylate (standard drug). The concentration of these enzymes were significantly ($p<0:05$) different from the other treatment groups for each parameter (ALT and AST). The levels were lower than that reported by Nwala*et al.* (2013a) and Arhoghro*et al*. (2009) in other studies. The level of these enzymes were decreased in comparison with the control (0.5% tween80), which may be an indication that no severe damage was done to the organs (liver & kidney), where this enzymes have been released from the damaged tissue (Nwala*et al*., 2013c). The photomicrographs of the rats treated with the different doses of the extract (100mg/kg, 200mg/kg and 400 mg/kg) showed kidney tissues with glomerular and interstitial inflammations and normal vessels, while the liver tissue showed wild portal tract inflammations. The effect of the treatment with these extract were worse in the kidney tissues than as was seen in the liver tissues. The group treated with the standard drug (diphenoxylate) had mild alterations for both kidney and liver tissues. For the group treated with 0.5% tween 80, the effect of the treatment on the tissues of these organs (kidney & liver) was also mild alterations. These results obtained indicates that the use of extracts of the *J.curcas* leaf as an anti-diarrhoeal agent in the castor oil induced diarrhoea in albino rats has no pronounced safety implications on these rats as earlier corroborated (Nwala*et al.*, 2013a; Nwala *et al.*, 2013b; Nwala *et al*., 2013c; Ojiako&Nwanjo, 2006).

5. CONCLUSION

The use of the methanol extract of *J.carcus* leaf in anti-diarrhoeal activity in castor-oil induced diarrhoeal have no pronounced effect on the experimental animals and this is also in agreement with the believe of both the herbal practitioners and those that use the plant; that the plant has no known safety implications.

Conflict of Interest

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There was no conflict of interest

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