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Evaluation of The Effect of Legend Flusher Bitters on Liver And Kidney of Wistar Rat

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ABSTRACT

Legend Flusher bitters is a poly-herbal formulation used for various ethno-medicinal purposes in Nigeria and Ghana. This study was undertaken to evaluate the toxic effect of legend flusher bitters on the liver and kidney of wistar rats through biochemical and histopathological studies. Adult wistar rats of either sexes weighing between 57g-96g were divided into three groups of five animals per group. Group1 was the control and was given standard feed and distilled water only.Group2 and Group 3 received 2.5mL/kgBw and 5.0mL/kgBw of Legend flusher bitters respectively by oral gavage. The administration lasted for 15days, after which the animals were anesthetized, bled and blood sample collected and then sacrificed. The liver and kidneys were excised, fixed in formalin, cleared and prepared for microscopic examination. The results showed elevated levels of AST, ALT, creatinine and bilirubin in rats treated with 5.0mL/kgBw of Legend flusher bitters when compared with the control; while administration of 2.5mL/kgBw of the herbal bitters (LFB) showed non-significant changes in some biochemical parameters. Histopathological studies revealed normal, preserved liver and kidney architecture in wistar rats treated with 2.5mL/kgBw of Legend flusher bitters but treatment with 5.0mL/kgBw (LFB) caused some pathologic changes in liver and kidney morphology when compared with the control. Thus, Legend flusher bitters may have adverse effects on liver and kidney when taken at high dose especially in individuals with liver and kidney diseases.

Keywords: Herbal medicine; Legend Flusher bitters; Toxicity; Biochemical assay; Histopathology

INTRODUCTION

Herbal mixtures have played a crucial role in health management in both under-developed and developed countries including Nigeria and Ghana. Herbal preparations are also used for maintaining body functions as well as treatment of varied disease conditions

Bitters are natural remedies that are commonly used in developing countries as a cure for indigestion and other stomach ailments and for treatment of various diseases (Bussmann *et al*., 2010). Bitters generally have been reported to prevent kidney and bladder infections, help to regulate **blood pressure** and dilate arteries, facilitate digestion, prevent disorder like ulcers, gastritis, insomnia, stress and depression and prevent overweight and excess body fat (Ogbonnia *et al*., 2010).

Legend Flusher Bitter (LFB) is poly-herbal formulation used for various ethno medicinal purposes in Nigeria and Ghana. This is excellent herbal formula for Treatment of chronic pile, low ejaculation, sexual weakness, stomach Trouble Indigestion. It also burns fat, boosts Man Power, lower blood pressure, diabetes, cleans Menstrual Period, Prevent kidney and Bladder infection, waist pain; free Bowels, Reduces too Much sugar in The Body.

Statement of the Problem

Herbal medicine and supplements have played a crucial role in health management in both under-developed and developed countries. The commercialization of herbal products and patronage it has attracted has tremendously increased. Yet, there is little or no scientific data to their acclaimed therapeutic potentials. The belief that herbal product are relatively safe because their ingredients are of natural plants is really misleading. Recently several studies have demonstrated adverse and toxic effects of some herbals including bitters, which have made some of them to be withdrawn from the market. Since there is paucity of scientific information on the potential toxicity profile of the Legend Flusher Bitters, toxicity data, are therefore required to predict its safety on short and long term usage

Aim and Objective of the Study

The main aim of this study is to determine the toxicity effect of legend flushers bitters on Wistar rats.

The objective of this study is to determine the effect of legend flushers bitters on Biochemical parameters and on the histology of liver and kidney of wistar rats

Materials and Equipment

- ✓ Weighing balanced,
- ✓ Standard rat feed
- ✓ Water
- ✓ Metallic Cage,
- ✓ Injecting string,
- ✓ Water container,
- ✓ Lithium Heparine bottle,
- ✓ Cotton wool
- ✓ Cannula
- ✓ Universal bottle
- ✓ Centrifuge (Multex MSE, England).

The Herbal Drug

Legend Flusher Bitters purchased at Bill Pharmacy Limited at Rumuokwuta, Port Harcourt, Rivers State.

Chemicals and Reagent

- ✓ Hematoxylin and Eosin (B.D.H Ltd England)
- ✓ Chloroform (May and Baker), formalin

Experimental Animal

Adult wistar rat of either sexes weighing 57-96g were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt. The animals were maintained in a well ventilated room and fed with standard laboratory animal feed and clean water ad libitum. The animals were housed in small groups of five per cage and were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the treatment. All procedures were done in conformity to standard of laboratory animal care; under 12/12hr light-dark cycle and controlled temperature (22 ± 3^0C) (OECD, 2008).

Experimental design

The experimental rats were divided into three groups of 5 animals per group

Group 1 received standard feed and distilled water only.

Group 2 received 2.5ml/kg body weight of Legend flusher bitters

Group 3 received 5.0ml/kg body weight of Legend flusher bitters

The administration lasted for 15days, at the end blood sample was collected by cardiac puncture into lithium heparin bottles and centrifuges at 4200 rpm for 5minutes to separate plasma for the determination of biochemical parameters. The liver and kidneys were excised and prepared for microscopic examination.

Biochemical Assay

Alanine aminotransferase and aspartate aminotrasferase activities were determined according to the method of Reitman and Frankel (1957). Total protein and albunin concentration were estimated by Biuret method and Bromocresol Green (BCG) binding method respectively. Total bilirubin (Jendrassik and Grof, 1938), concentration of Urea (Patton and Crouch, 1977) and Creatine level. (Bowers and Wong, 1980)

Histopathological Examination

The animals were dissected and the liver and kidney collected for histopathological studies as described by Krause (2001).The liver and kidney were fixed in freshly prepared 10% buffered formalin, then with increasing concentration of ethanol and cleared in xylene. After clearing, the tissues were embedded in molten paraffin wax in

the oven at 40ºC. The tissues were subjected to automated tissue processing machine, thin sections of 5-micrometres were made using microtome and later stained with hematoxylin and Eosin stain ready for microscopic examination.

RESULT

Clinical symptoms and Mortality

The result of this study revealed that there was no mortality recorded at the doses of 2.5ml/kgbw and 5.0ml/kgbw for the period of 15days treatment with Legend Flusher Bitters. Other parameters analyzed as presented below.
The results of the effect of 15days administration Legend Flusher Bitters on body weight are presented in table I.
The result showed steady increase in body weight of the animals in both treatment groups (2.5ml/kgbw and 5.0ml/kgbw) but was markedly increased in the control.

Table 1: The effect of Legend Flushers Bitters (g) on body weight of wistar rats

Groups	Initial weight (g)	Final weight (g)
Control	71	114.25
Group 1	71	88.50
Group 2	84.75	107.0

Values are mean of four replicates.

The effect of 15 days administration of Legend Flushers Bitters on the some Biochemical parameters of wistar rats

The result of the effect of 15 days administration of legend flushers on some biochemical parameters is presented in table 2 below. The result revealed minimal difference in the concentration of urea and albumin between treated groups and when compared to that of the control.

There was marked increase in creatinine concentration of group 2 rats (98.6µmol/l) as compared to the control (83 .10µmol/l) meanwhile, group 1 rats showed observable decrease (65.70µmol/l) as compared to group 2 and control

AST activity was notably higher in group 2 (125.40µ/l) compared to the control (91.94µ/l) inversely, AST activity was markedly reduced in group 1 (74.50µ/l) compared to that of the control.

ALT activity of group 2 rats showed marked increase (47.1µ/l) compared to the control (38. 80µ/l) and group 1(35.0µ/l), but there was minimal difference in ALT activity between group 1 and control. Total protein of group 1 rats showed a marked increase (70.43g/l) compared to that of control group (59.81g/l), but minimal difference was observed between group 3 and the control. As compared with the

control and group 2 treated rats, group 1 rats showed very low concentration of total bilirubin (1.5µmol/l). These results are presented in table 2.

Table 2: The effect of Legend Flushers on some Biochemical parameters

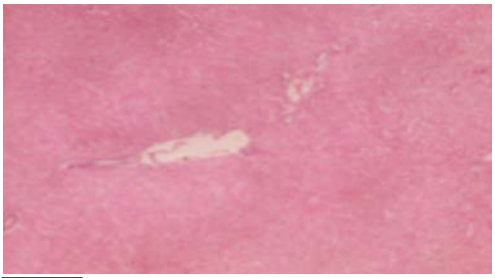
Parameters	Control	Group 1	Group 2
Urea (mmol/l)	6.27	5.90	5.47
Creatinine (µmol/l)	83.10	65.70	98.6
AST (µ/l)	91.94	74.50	125.4
ALT (µ/l)	38.80	35.00	47.1
Total protein (g/l)	59.81	70.43	60.37
Albumin (g/l)	35.96	33.97	32.78
Total bilirubin (µmol/l)	17.25	1.5	24.5

Values are mean of three replicates.

Effect of Legend Flusher Bitters on Liver and Kidney Histology

The histopathology of the liver section of the control group showed normal histology, without any pathological abnormalities. The group treated with 2.5ml/kgbw of Legend Flusher Bitters showed relatively normal hepatocytes (Plate b) which was not so different from section from the control, while treatment with 5.0ml/kgbw of Legend Flusher Bitters showed constricted sinusoidal spaces and central vein, mild portal inflammation but no evidence of necrosis (Plate c).

Histopathology of the rats' kidney section from group 2, treated with 5.0ml/kgbw of Legend Flusher Bitters showed evidence of mild glomerular damage, tubular and interstitial alterations (Plate f), but section from the control showed normal kidney histology. The histopathological sections are shown below:



a

Plate (a) Photomicrograph of rat liver in control group showing normal histology, portal tract and distinct central vein (H&E x400)

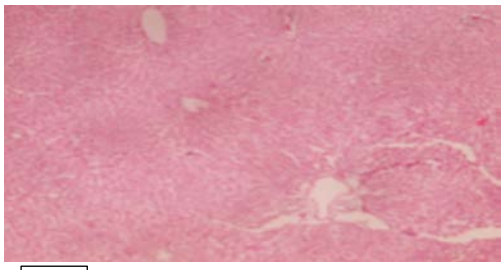


Plate (b) Photomicrograph of rat liver in group administered 2.5ml/kgbw of LFB showing hepathocytes which are distinct and relatively normal, sinusoids, portal tract and radiating hepatic plates (H&E x400)

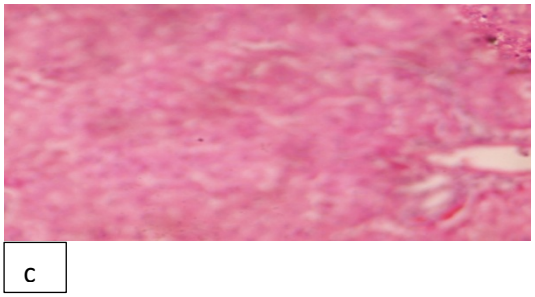


Plate (c): Photomicrograph of rat liver in group administered 5.0ml/kgbw of LFB showing constricted sinusoidal spaces and central vein, but no evidence of necrosis (H&E x400)

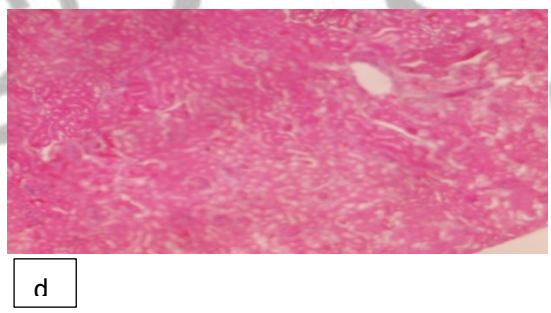


Plate (d) Photomicrograph of rat kidney in control group showing normal histology, glomeruli, tubules, interstitial blood vessels (H&E x400)

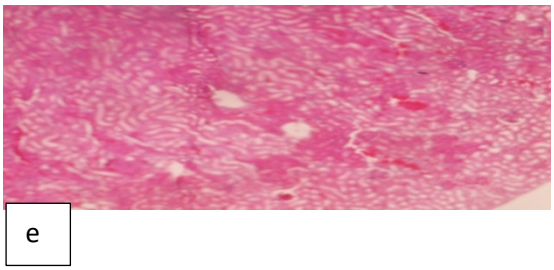
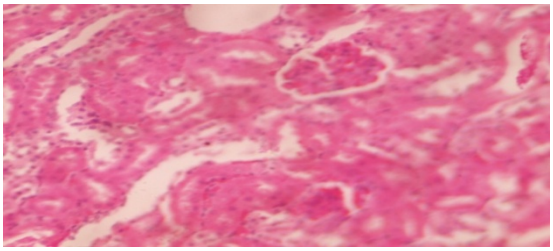


Plate (e) Photomicrograph of rat kidney in group administered 2.5ml/kgbw of LFB showing relatively normal glomerular, interstitium, tubules and blood vessels. (H&E x400)



f

Plate (f) Photomicrograph of rat kidney in group administered.5.0ml/kgbw of LFB showed mild glomerular, interstitial and tubular alterations (H&E 400)

Discussion

Legend flusher bitters is a poly-herbal mixture of various plant roots, bark, seeds etc. extracted in alcohol and sold as 'cure all' patent medicine. The patronage that this herbal bitters has attracted calls for this investigation into its toxicity profile.

Body weight measurement is important in monitoring the health status of an individual or animal. Loss of body weight is often the first indicator of the onset of adverse effect of a substance or drug (Tanuja, *et al.,* 2016). The weight gain observed in this study coupled with no sign of toxicity and any mortality within the 15 days treatment suggests that the herbal product was well tolerated and non-toxic. This weight gain can be said to be a healthy weight gain which is expected of animals that are fed ad libitum (Anionye, *et al.,* 2015).

The Urea and Creatinine assays are considered as good prognostic indicator of renal dysfunctions, but creatinine is a more useful biomaker of renal function. This study revealed marked increase in creatinine level of rats treated with 5.0ml/kgbw of Legend flusher bitters as compared to the control rat, whereas, the administration of 2.5ml/kgbw of the herbal bitters caused significant reduction in creatinine level when compared to the control. On the other hand, the level of urea in both treatment groups was slightly lower than that of control rats. Renal dysfunction is usually evident when there is rise in serum and urea creatinine level. As the kidneys become impaired, creatinine level in the blood increases due to damages to the functional nephrons and consequently poor clearance by the kidney (Aliyu, *et al.,* 2006).

It is also reported that creatinine level that is within normal reference range does not equate to normal functioning renal system (Burtis *et al.,* 2008). It is pertinent to note that the concentration of urea is dependent on protein intake, the body's capacity to catabolize protein and adequate excretion of urea by renal system.

Serum bilirubin concentration is sometimes, considered as the real liver function biomarker, which measures the ability of the liver to clear bilirubin from the blood as it circulate through the liver. Findings from this study indicated that rats fed with 2.5ml/kgbw of Legend flusher bitters showed very low concentration of bilirubin as compared to the control, but administration of 5.0ml/kgbw of the herbal bitters caused a spike in the level of bilirubin when compared to the control. Lower than normal bilirubin levels are usually not a concern, but elevated levels of bilirubin may indicate lyses of red blood cell since it is made from hemoglobin (Kpomah *et al.,*

2012). Liver function test gives information about the state of the liver, its functionality and cellular integrity.

Liver cell damage is characterized by a rise in some plasma enzymes (AST and ALT). The result of this study revealed that AST and ALT activities were considerably higher in group treated with 5.0ml/kgbw of legend flusher herbal bitters when compared to the control, this observable rise or changes in AST and ALT activity may be due to hepatocellular damage. AST is more intracellular than ALT and is found in liver cells, brain, kidney, pancreas, cardiac and skeletal muscle (Kasper *et al.,* 2005), on the other hand, ALT is present in high concentration in the liver (Emeka and Obidia, 2009). Clinically, circulatory shock, trauma, hemolytic disease, myocardial infarction are associated with elevated ALT (Allston, 1993).

Almost all blood proteins are synthesized in the liver. Studies have pointed out that increased in total protein and albumin levels indicate liver dysfunction. (Tanuja *et al.*, 2016).Thus, increase in total protein and minimal changes in albumin concentrations as observed in this study in the two treatment groups could suggest non-toxic effect of herbal bitters on the liver. Albumin plays a major role in maintaining plasma osmotic pressure as well as transportation of liquid and hormone (Saidu, *et al.,* 2000). Protein intake is directly linked to the levels of total protein and albumin. Increase in total protein could also come from protein rich feed.

Histopathological examination is a standard for evaluating treatment induced pathological changes. It is also done to correlate result obtained from serum biochemical and enzymes assays. The histopathology of the liver section of the control group showed normal histology, without any pathological abnormalities. The group treated with 2.5ml/kgbw of Legend Flusher Bitters showed relatively normal and preserved cytoarchitecture with hepatocytes radiating from central vein.(Plate b), while treatment with 5.0ml x/kgbw of Legend Flusher Bitters showed constricted sinusoidal spaces and central vein, mild portal inflammation but no evidence of necrosis.

Histopathology of the rats kidney sections showed evidence of mild glomerular damage, tubular and interstitial alterations and presence of eosinophilic substances in the tubules in rats treated with 5.0ml/kgbw of Legend Flusher Bitters (Plate f), but the control rats showed normal histology. These histopathological findings support the altered plasma level of creatinine observed in group 2. Creatinine has been found to be a fairly reliable indicator of kidney function as it is less influenced by other factors such as diet and hydration. (www.medicinenet.com/creatinine)

Conclusion

It can be said that low dose (2.5ml/kgbw) of the Legend flusher bitters caused no adverse effects on the liver and kidney histology of the treated albino rats but administration of Legend Flusher Bitters at high dose (5.0ml/kgbw) as used in this study may produce undesirable outcomes such as alteration in biochemical parameters with degenerative changes in liver and kidney, in spite of its beneficial therapeutic potency. Thus, caution should be applied when used for health treatment at high dose especially in individuals with underlying health issues.

Recommendations

Based on findings in this study, it is recommended that sub-chronic and chronic toxicity studied be carried out to clearly define some observed alteration in biochemical parameters and some histological changes observed in liver and kidney sections.

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