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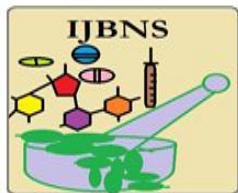
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TOXICOLOGICAL EVALUATION OF FERMENTED LIQUOR AND METHANOLIC EXTRACT OF BENISEEDS IN ALBINO RATS

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ABSTRACT:

The toxicological evaluation of fermented beniseed liquor and the methanolic extract were assessed in-vivo using young albino rats. They were fed with a dose of 0.75ml at 100mg/ml of the extract and 0.75ml at 100mg/ml of the fermented liquor respectively for three weeks (21 days) after which their vital organs were assessed histopathologically. The result of the histopathological analyses showed that the extract and fermented liquor caused no form of distortion, necrosis, lesions or haemorrhage on the heart, the cardiac muscles' striata were more strengthened by the fermented liquor when compared with the control and the one fed with the extract which showed homogenous muscle fibres and slight inflammatory cell infiltrations. The liver had its sinusoids in place and showed no form of necrosis, neither karyolysis nor any thickening nuclear structures. The kidneys had normal interstitial cells without tubular necrosis and intestine showed a well formed intestinal villi without any erosion of the cells while the spleen showed well formed dark green cells. Therefore, both the beniseed methanol extract and fermented liquor had no deleterious effect on vital organs and are fit for consumption.

KEY WORDS: Tablets, coated tablets, uncoated tablets, coating elements, various coatings

1. INTRODUCTION:

The need to take care of our vital organs such as the liver, heart, kidneys and intestine have been well emphasized by medical sciences for ages. According to [12] and Rob, [19] diseases in these organs are often terminal, leading to the eventual death of the person or animal. Beniseeds, which serves as food in various parts of the world is known to have medicinal properties [11]. According to [3] beniseed paste, when added to local kunun-zaki drink increased the protein, fat and energy content by over 20%. The plant belongs to the family *Pedaliaceae* and is an annual crop that grows in tropical areas. The seeds are tiny, flat ovals measuring about 3mm [14]. The plant's roots and leaves are used for treating migraine, hypertension, ulcers, constipation, chicken pox and piles [11]. It is presently used in large quantity for the production of margarine and cooking oils. Non-

culinary uses include its use as an ingredient in soap, cosmetics, lubricants and medicines. The simplest and commonest use of sesame seeds now is sprinkling the seeds over cakes and breads, especially in Syria and Lebanon (Encyclopedia of spices-2012). In Nigeria, the local names of the seeds are 'eluru' and 'ekuku' (Yoruba). The Ebiras call it 'gorigo'. The fermented form of the paste has antibacterial activity from previous work. Though not documented, the Ebiras people in Kogi State of Nigeria use it for the treatment of intestinal disorder, especially in children, expecting mothers and young adults. They also use it for soup after grinding it into smooth paste with a grinding stone and they equally roast/fry it as snacks. Improving intestinal health using inexpensive and effective nutraceutical agents such as beniseed is presently being explored by medical sciences [15-16]. This research is therefore

focused on the toxicological assessment of fermented beniseed liquor and its methanolic extract on the vital organs (liver, heart, kidneys, intestine and spleen) of young adults albino rats.

2. MATERIALS AND METHODS:

The beniseeds were purchased at Okene central market in Kogi State of Nigeria. The seed's taxonomic identity was confirmed at the department of Crop Science of the Federal University of Technology, Akure, Ondo State.

2.1 Fermentation of Seed

500g of the seed was soaked in 1000ml of water for 3 days and grounded into a smooth paste. It was then filtered using muslin bag. It was stored in refrigerator at 4°C after allowing it to settle for 3hrs. The liquor was allowed to undergo natural fermentation at that temperature for 3days before using it to feed the albino rats according to the method of Adebolu *et al.*, 2011.

2.2 Extraction with methanol

Ninety-eight percent methanol was used to extract the active components of beniseeds according to the method of Ogundare, 2006.

2.3 In-vivo analysis

In-vivo feeding of methanol extract and fermented beniseed liquor was done by grouping the albino rats into different groups for the doses of 0.75ml at 100mg/ml daily. They were fed for a period of 3weeks (21 days) after reconstituting with tween-twenty according to the method of Dehanuker *et al.*, (2001) for the methanol extract.

2.4 Histopathological Analysis

Histopathologic tests were carried out on the organs of the laboratory animals as follows: the organs of the animals were collected and fixed in 10% formalin to prevent decay. They were dehydrated in

different percentage (50%, 70%, 80% and 100%) of alcohol 1 ½ hours each. After dehydration they were cleared with 100% xylene and left for 2hours to remove any remnant alcohol and impregnated in liquid wax for 2 hours for embedding. The embedded organs were sectioned using microtome and were stained with haematoxylin-eosin (Silva *et al.*, 1999). Excess stain was removed with tap water. After clearing in xylene, Canada balsam was added and cover slips placed on the slides. The preparations were left in the oven at 40°C and then placed under the microscope with a digital camera connected to a computer system to be examined by an expert and take the photographs according to Dehanuker *et al.*, 2001. Interpretation of results were done according to the methods.^{[4][9]}

2.5 Statistical analysis of results

Results obtained will be subjected to descriptive one way analyses of variance, SPSS version 10 Microsoft windows 7 and Duncan multiple range test will be used as follow up test.

3. RESULTS AND DISCUSSION:

The Histopathological examination of organs of the rats used for these biosafety analyses of both the extract and the fermented liquor of beniseeds showed no form of distortion of any of the vital organ analyzed for. There was no form of encephalopathy, necrosis, haemorrhage, oedema, infiltration of cells, and neither dilation of liver sinusoid nor infarction of cardiac muscles. The heart of the control showed little form of haemorrhage at the apex/distal end, little wider tubular holes of the heart and a more spacious cellulotic space in the spleen. These histopathology results are shown in Fig:3.1-Fig:3.5 (plates 1-5)

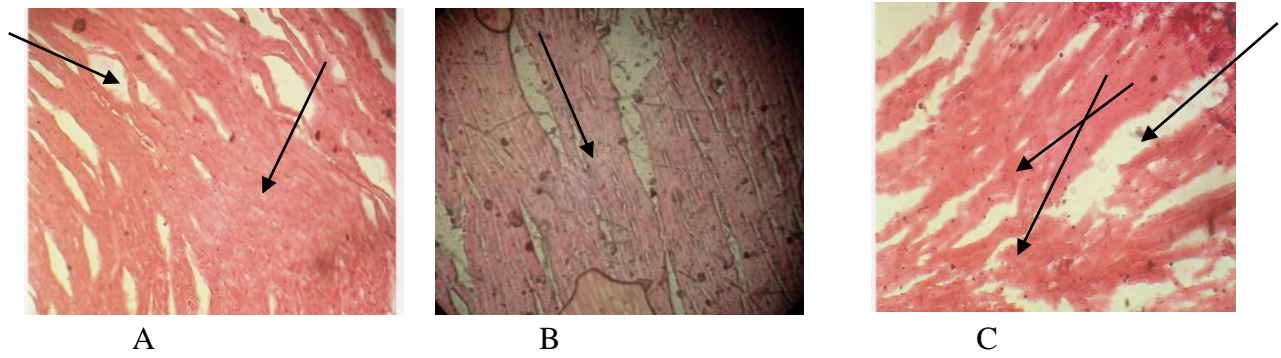


Fig: 3.1- Plate 1: Histopathology of the liver of albino rats fed with methanol extract and fermented liquor of beniseeds and their control.

Keys: A=Fermented liquor, B=Methanol extract, C=Control

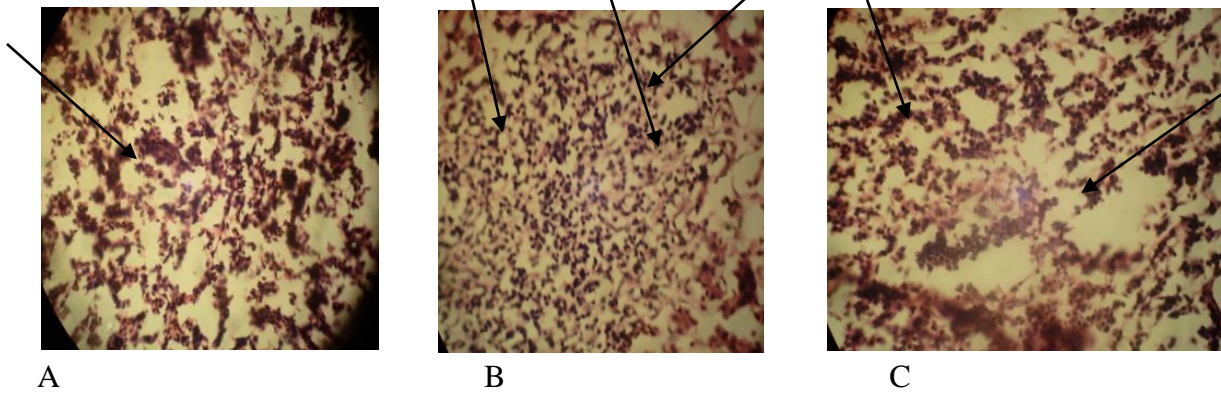


Fig: 3.2 Plate 2: Histopathology of the kidneys of albino rats fed with methanol extract and fermented liquor of beniseeds and their control.

Keys: A=Methanol extract, B=Fermented liquor, C=Control.

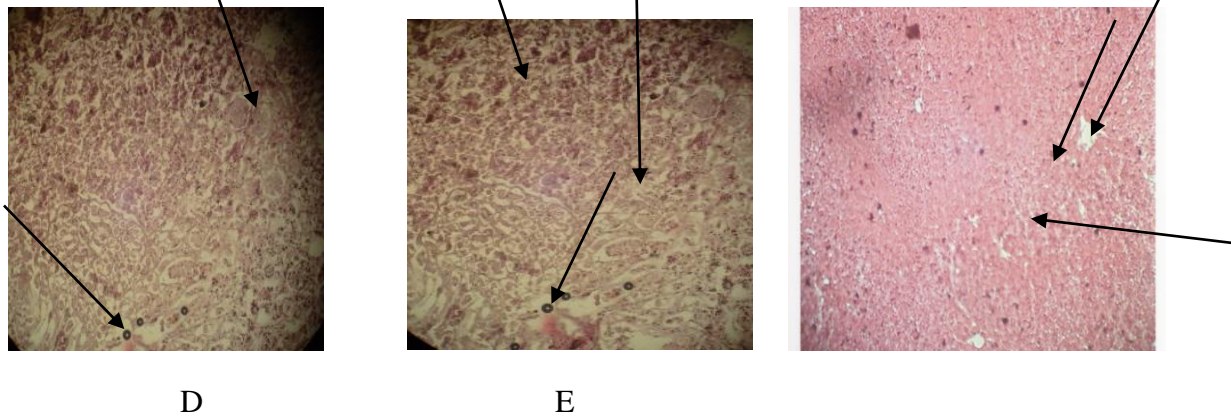


Fig: 3.3 Plate 3: Histopathology of the liver of albino rats fed with methanol extract and fermented liquor of beniseeds and their control.

Keys: A=Methanol extract, B=Fermented liquor, C=Control.

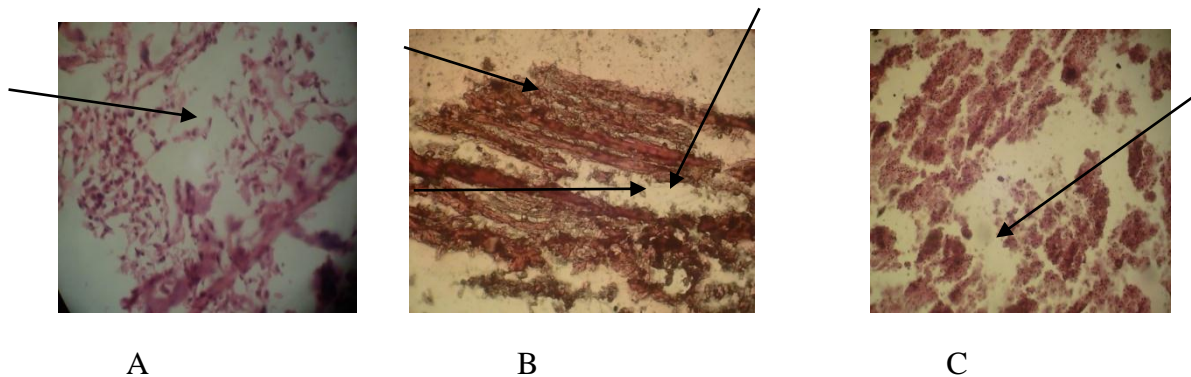
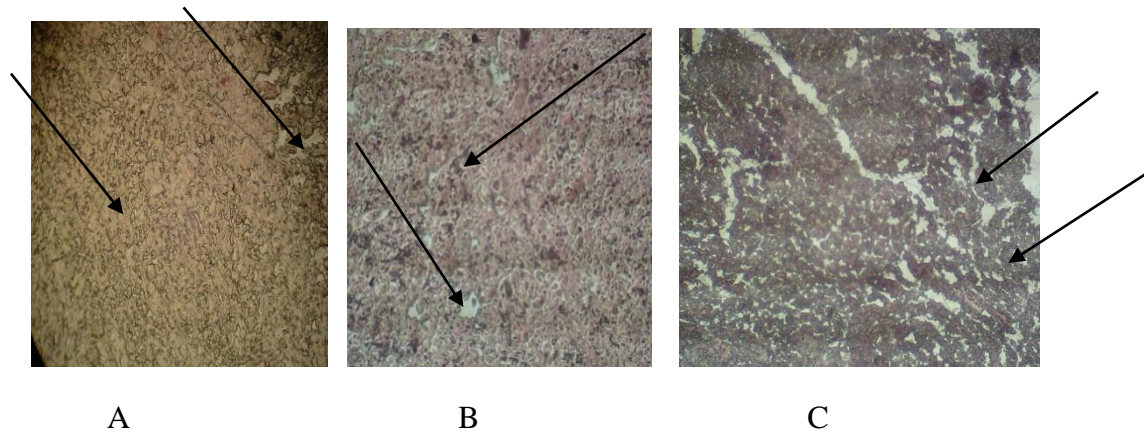


Fig: 3.4 Plate 4: Histopathology of the intestine of rats fed with the fermented liquor and methanol extract of beniseeds and their control.

Keys: A=Fermented liquor, B=Methanol extract, C=Control.



A=Liver, B=Heart, C=Intestine, D=Spleen, E=Kidney

Fig: 3.5 Plate 5: Histopathology of organs of albino rats fed with fermented beniseed liquor.

4. CONCLUSION:

The Histopathological examination of organs of rats used for this biosafety experiment of both the extract and the fermented liquor of beniseeds showed no form of encephalopathy, necrosis, haemorrhage, oedema, infiltration of cells, dilation of liver sinusoid nor infarction of cardiac muscles. The heart of the control showed little form of haemorrhage at the apex/distal end, little wider tubular holes of the heart and a more spacious cellulotic space in the spleen.

This result coincided with the work of ^[5] who observed no form of distortion in organs of rats treated with garlic extract. Also, rats infected and treated with the extract and liquor showed normal liver and heart structures as well as normal kidney nephrones. However, those infected and were not treated showed dilated liver sinusoid, infarcted myocardial heart muscles and infiltration of kidney nephrones. The group of animals infected with

S.typhi and *S.dysenteriae* had complete destruction of their villi with their lumen having infiltration of inflammatory cells and fused epithelial cells lining their villi. Since these Histopathological distortions were absent in the rats infected these organisms and treated with the extract and fermented liquor of beniseeds, then beniseeds have nutraceutic properties that can prevent and cure intestinal disorders. According to [20] [22] seeds, extracts and preparations capable of exerting these effects on vital organs have high nutraceutic properties. The spleen of the rats used for biosafety analysis of the extract and fermented liquor of beniseeds were normal without any distortions, however, the cells of the pancreas of the rats fed with the fermented liquor had dark green colour, which is an indication that the liquor is a rich blood supplement [13]. The group of rats infected and wasn't treated showed severe depletion of lymphocyte of white bulb with accumulation of hemosidrin pigments. These observations corresponded with the ones observed by method [8]. Therefore, since both the extract and liquor had no deleterious effect on these organs, they are good nutraceutic food supplements for recuperation of any of these vital organs.

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