

THE IMMUNOSTIMULATORY POTENTIAL OF FERMENTED BENISEEDS (*SESAMUM INDICUM LINN*) LIQUOR ON ALBINO RATS

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ABSTRACT

The immune-stimulatory potential of natural liquid fermentation of beniseed liquor was assessed *in-vivo* using young adult albino rats. They were fed with a specific volume of the liquor at 100mg/ml for a period of three weeks after which their blood was subjected to haematological and biochemical analyses. The haematological assay for the biosafety analyses of the liquor on the albino rats showed that the liquor significantly increased the blood parameters when given a dose of 0.75ml at 100mg/ml daily. The biochemical analyses of the blood of the albino rats used showed that the liquor caused significant increase in the level of bicarbonate, urea, uric acid and calcium. The liver functioning tests for the rats showed that the liquor caused a little increase in the serum total protein and alkaline phosphatase without any significant rise in the cholesterol level. The results obtained from these *in-vivo* analyses of fermented beniseed liquor has shown that beniseeds possess excellent nutraceutical properties with good immunostimulatory potential and that it can be used as a good anti-diarrhoeagenic agent.

KEYWORDS: The immune-stimulatory potential, ferment beniseed(*Sesamum Indicumlinn*)

INTRODUCTION

Beniseeds, which serves as food in various parts of the world is known to have medicinal properties (Odugbemi, 2006). The plant belongs to the family *Pedaliaceae* and is an annual crop that grows in tropical areas (Dutta, 2004 and Dan *et al*, 2004). The seeds are tiny, flat ovals measuring about 3mm (Oshodi *et al*, 2010). The plant's roots and leaves are used for treating migraine, hypertension, ulcers, constipation, chicken pox and piles (Odugbemi, 2006). 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 'gorigou'. The fermented form of the paste has antibacterial activity from previous work. Though not documented, the Ebara 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 (Oyetayo, 2009). This research is therefore focused on the assessment of the immune-stimulatory potential of fermented beniseed liquor on albino rats.

MATERIAL 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.

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 and the filtrate divided into two. It was stored in refrigerator at 40C. It was allowed to settle for 3 hrs before using the liquor for antibacterial sensitivity testing and isolation for day 0 to day 7.

Hematological assay

The animals were sacrificed and their blood was collected by cervical collection into labeled EDTA bottles. The various analyses carried out on the blood of the animals are as follow:

i. Erythrocyte Sedimentation Rate (ESR)

A wintrobe tube was filled to the top 0 mark and one end of it blocked with plastacine. It was stand in an upright position undisturbed for 60minutes (1hr). The distance of the fall of red cells in it was read and expressed as the mm fall in an hour as the ESR

ii. Packed Cell volume (PCV)

Blood collected into anticoagulant bottle was mixed and a capillary tube was filled up to 75% (3/4) of its length and placed in the micro-haematocrit centrifuge with the sealant at the outer end and centrifuged at 12,000 rpm for 5 minutes. The result was read as a percentage of packed red cells to total volume of whole blood using a haematocrit reader.

iii Red Blood cell count (RBC)

The blood sample was diluted 1:200 and mixed properly. 0.02ml of the blood was pipetted into 4ml of diluting fluid in a bijou bottle and washed thoroughly by alternately drawing up and expelling the diluting fluid. A fine Pasteur pipette was used to fill the counting chamber and counted using a counter under ×40 objective.

iv White Blood cell count (WBC)

The blood was first diluted in ratio 1:20 and 0.05ml of the blood pipetted into 0.95ml of diluting fluid. A little portion was charged into the counting chamber and observed using $\times 10$ objective to count the white cells/cubic mm.

v Haemoglobin (Hb)

Using mouthpiece, sucker and a 0.02ml pipette, blood was withdrawn and expelled into 4ml Drabkin's solution in a tube. The tube was stoppered, mixed and allowed to stand for 5 minutes for full colour development. A standard blood sample of known haemoglobin concentration was prepared. Using a green (624) filter, the calorimeter was set to zero using plain Drabkin's solution as a blank. The readings of the sample and the standard were taken and the result calculated as follows: $\text{sample haemoglobin concentration} = \frac{\text{Reading of test} \times \text{standard haemoglobin concentration}}{\text{Reading of standard}}$

vi White Blood cell Differential (WBC Differential)

These are divided into granulocytes and agranulocytes. The granulocytes are further divided into three which are neutrophils, eosinophils and basophils. These were counted after staining with Giesma stain and their numbers recorded. The agranulocyte are equally further divided into two, which are lymphocytes and monocytes.

Biochemical analyses

This was done according to the method of Monica, (2004).

Liver Functioning Tests (LFT)

These were done according to the methods outlined by Baker et al., (2006).

Statistical analysis of results

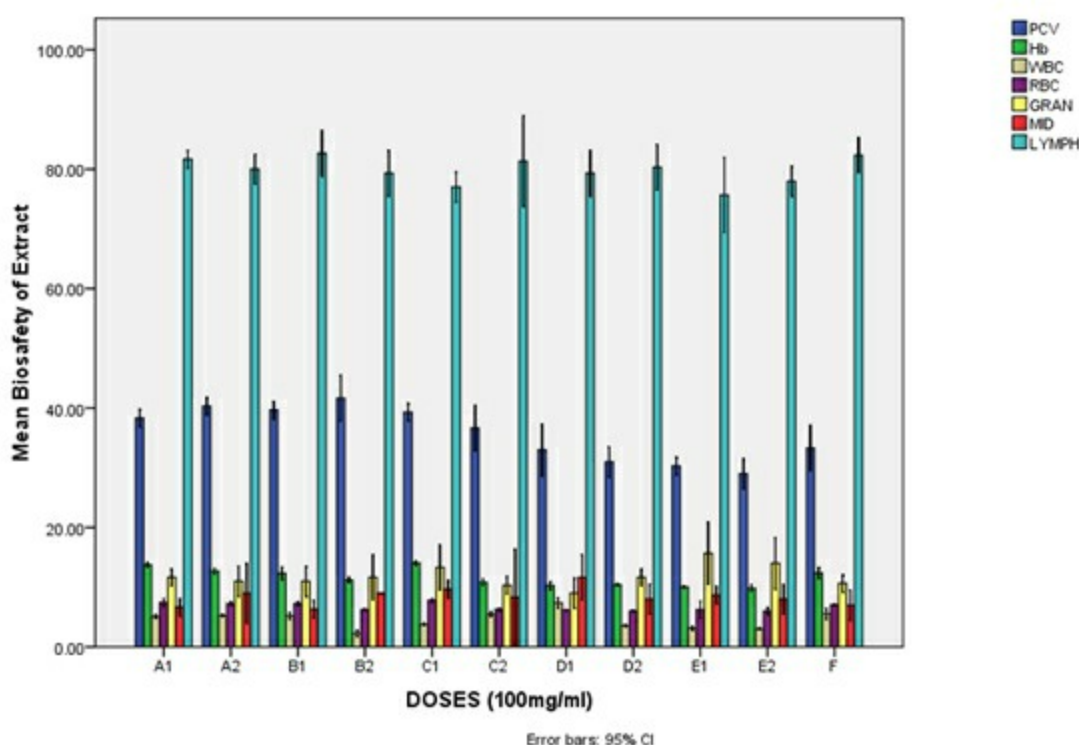
Results obtained will be subjected to descriptive one way analyses of variance, SPSS version 10 Microsoft windows 7 and Duncan multiple range test will be used as follow up test.

RESULTS

The results of the haematology showed that the fermented liquor increased the blood parameters. The group of rats fed with 0.75ml at 100mg/ml once a day had the highest PCV values as well as the highest lymphocyte value. However, the value decreased as the dose increases (but still higher than that of the control). This is shown in figure 1.

Biochemistry results

Fig1: Haematology result of biosafety of fermented beniseed liquor.



Keys: A1=0.5ml once daily, A2=0.5ml twice daily, B1=0.75ml once daily, B2=0.75ml twice daily, C1=1.0ml once daily, C2= 1.0ml twice daily, D1=1.25ml once daily, D2=1.25ml twice daily, E1=1.5ml once daily, E2=1.5ml twice daily and F= control.

The results of the biochemistry analysis showed that as there is increase in the dose of beniseed liquor and extract administered to the rats, there was increase in the level of

the biochemical parameters tested for. However, no record was above the normal level/range as shown in tables 1

Table 1: Results of biochemical analyses of biosafety of fermented beniseed liquor.

Parameters	A	B	C	D	E	F
Bc (mMol/L)	25.05± 0.09 ^b	26.00± 0.01 ^b	25.06±0.10 ^b	28.35±0.90 ^d	28.62±0.05 ^c	24.00±0.02 ^a
Cr (mMol/L)	0.09 ± 0.01 ^c	0.10±0.00 ^d	0.08±0.03 ^b	0.09±0.02 ^c	0.08±0.01 ^b	0.06±0.08 ^a
Ua (mMol/L)	0.20±0.0 ^a	0.40±0.02 ^d	0.30±0.00 ^c	0.40±0.05 ^d	0.30±0.00 ^c	0.25±0.06 ^b
U (mMol/L)	2.80±0.04 ^a	4.60±0.09 ^d	4.90±0.05 ^e	4.00±0.01 ^c	5.00±0.01 ^f	3.50±0.05 ^b
Ca (mMol/L)	2.30±0.00 ^a	2.90±0.10 ^d	2.60±0.05 ^c	2.50±0.03 ^c	2.60±0.00 ^c	2.40.±0.02 ^b

Values followed by the same letter in a row is not significantly different at P=0.05.

Key: A=0.50ml, B=0.75ml, C=1.00ml, D=1.25ml, E=1.50ml, F=Control.

Bc=Bicarbonate, Cr=Creatinine, Ua=Uric acid, U=Urea, Ca=Calcium.

Liver functioning test results

The results of the LFTs were all within the normal range. However, the group of rats used as the control had higher

cholesterol values than that of the group fed with the liquor. This is shown in table 2

Table 2: Liver functioning test of albino rats fed with fermented beniseeds liquor.

Parameter	A	B	C	D	E	F
BT (uMol/L)	11.6±0.03 ^d	13.5±0.21 ^e	8.0±0.09 ^a	9.5±0.35 ^b	15.7±0.11 ^f	10.5±0.38 ^c
STP (g/L)	67.5±0.72 ^c	67.1±0.51 ^c	63.9±0.39 ^a	73.4±0.25 ^d	65.7±0.60 ^b	63.6±0.31 ^a
AST(GOT) (iu/L)	17.95±0.29 ^a	22.55±0.75 ^b	24.99±0.60 ^c	29.38±0.37 ^e	25.87±0.41 ^c	25.38±0.74 ^d
ALT(GPT) (iu/L)	39.20±0.33 ^e	26.00±0.60 ^a	27.55±0.71 ^a	36.92±0.38 ^d	30.75±0.15 ^c	28.70±0.11 ^b
ALK.PHOS (iu/L)	30.90±0.20 ^c	23.95±0.32 ^a	40.50±0.65 ^f	35.88±0.25 ^d	39.00±0.00 ^e	26.45±0.38 ^a
Cholesterol (mMol/L)	1.27±0.30 ^a	1.30±0.00 ^a	1.45±0.40 ^b	1.71±0.51 ^c	2.8±0.27 ^d	1.30±0.20 ^a

Values followed by the same letter in a row is not significantly different at P=0.05.

Key: A=0.50ml, B=0.75ml, C=1.00ml, D=1.25ml, E=1.50ml, F=Control.

BT=Bilirubin total, STP=Serum total protein, AST=Asparate transferase test,

ALT=Antilymphocyte transferase test, ALK=Alkaline phosphatase.

DISCUSSION

Though, haemoglobin (HB) level may vary with age, gender and the altitude at which an animal may be found, the variation is negligible. According to Samson, (2002), low level of haemoglobin is an indication of anaemia and since this wasn't detected in all the animals treated with both the extract and fermented liquor of beniseeds means that beniseeds can improve the iron content (haem) of the red blood cells. The packed cell volume (PCV), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) of all the rats used for biosafety test for the extract and fermented liquor showed that both increased these parameters. Stott and Lewis, (2005) found that these parameters help to check for anaemia, dengue haemorrhagic fever and all forms of polycythaemia and that all foods that can cause increase (but not beyond their maximum values) can both cure and prevent anaemia. Therefore, fermented

beniseed liquor and its extract can cure and prevent anaemia.

According to Lewis, (2008) leukocytosis (raised WBC count) can be found in acute infections, inflammation and tissue necrosis, metabolic disorders, poisoning, acute haemorrhage, leukemia and stress conditions, while leucopenia (reduced WBC count) is associated with viral, bacterial and parasitic infections. Since beniseed liquor and extract was able to raise the WBC level in the rats that were infected and treated with it as well as maintain the level in the biosafety analysis it is a good nutraceutical food. The increase in the number of monocyte in the group of rats infected with *B.cereus* without treatment shows that beniseed liquor and extract are highly effective *in-vivo* in the treatment of infection caused by the organism. Topley, (2008) showed that increase in monocyte is an indication of chronic bacterial infections such as typhoid, food poisoning and bacterial endocarditis. The group treated

with 0.5ml and 0.75ml at 90mg/ml 100mg/ml of the extract respectively of the had the highest increase of blood parameters. Fleming et al, (2010) suggested that the dose at which an extract, preparations and byproducts of organisms yield the best result of blood parameters should be taken as the safe dose for such and that such products have positive immunostimulatory effect on animals. Therefore, beniseeds have positive immunostimulatory potential and a good nutraceutic food for animals. The bicarbonate results showed the acid-base balance in the blood gas of the rats and according to Rob (2006), an alteration or increase from the normal level of bicarbonate, urea, uric acid and creatinine is a pointer to the malfunctioning state of either the heart, liver or kidneys. Since both the extract and the fermented liquor of beniseeds did not cause any significant increase of these biochemical parameters, they have medicinal value.

The results of the liver functioning tests (LFT) showed that aspartate transferase enzyme was present in the liver, heart muscles and kidneys at a very low level. Nancy, (2008) stated that an increase in the level of this enzyme is the commonest indication of liver disease. Therefore, both the extract and fermented liquor of beniseeds are good for the liver, heart and kidney cells. Serum glutamic-oxidase transaminase (SGOT) is an enzyme excreted by damaged heart muscles and there is raised level of it in chronic myocardial infarction (Peter, 2008). This enzyme was greatly lowered by fermented beniseed liquor, an indication that fermented beniseed liquor can be used in treatment of myocardial infarction of the heart. Also, according to Robinson et al., (2006), serum glutamic-pyruvic transaminase is an enzyme excreted by the parenchymal cells of the liver and a raised level of it in the blood indicates infectious hepatitis. The administration of beniseed extract and fermented liquor showed no significant increase in the level of the enzyme in the blood, beniseeds may possess the property that can be harnessed for the treatment of infectious hepatitis. From the results obtained from this in-vivo analyses of fermented beniseed liquor, beniseeds possess an excellent nutraceutic properties with good immunostimulatory potential and is a good anti-diarrhoeagenic agent.

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