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EVALUATION OF BENISEED EXTRACT AND FERMENTED LIQUOR IN TREATMENT OF DIARRHOEA IN ALBINO RATS INFECTED WITH *SALMONELLA TYPHI*.

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Abstract: *The efficacy of fermented beniseed liquor and the methanol extract in treating diarrhoea caused by Salmonella typhi in albino rats orogastrically infected with the bacteria was assessed. At the end of the experiment, haematological, biochemical and liver functioning tests were performed on the blood from the animals. The results obtained from this analysis showed that the selected organism have high infectivity dose, caused a significant reducing effect on the weight of the albino rats and negative effect on the hematological and biochemical parameters assessed. Treatment of infected animals with the fermented liquor and methanol extract of beniseeds showed that both caused a significantly quick recovery of the infected animals from diarrhoea within five days of treatment. However, the rate of recovery was faster with the group of infected rats treated with the fermented beniseed liquor than the extract. Also, treatment with methanol extract of beniseeds also caused a significant increase in the cholesterol level of the blood from the animals. The results obtained from these analyses showed that beniseed have therapeutic properties and that the fermented form is more effective and can be used to treat diarrhoea caused by the selected bacteria used in this study in albino rats.*

Keywords: Therapeutic; beniseeds; infection; diarrhoea; albino-rats.

INTRODUCTION

Diarrhoea, a condition that is characterized by frequent passage of watery stool that may in some cases contains mucus and blood is on the increase and has become worrisome (Brook *et al*, 2010). Acute diarrhoea is the most frequent health problem of children especially after weaning. It is also a problem of travelers to developing countries and HIV-infected persons. Diarrhoea is difficult to treat because most of the antibiotics normally used may induce diarrhoea (Prescott *et al*., 2008). Also, most of the causative organisms have become resistant to available antibiotics (Pool, 2002). According to European Food Safety Authority (EFSA) in 2006, Salmonellosis remained the second most frequent diarrhoea and is most frequent with people who consume foods from infected fresh poultry, water, fruits and vegetables. *S. typhi* is the most frequently isolated Salmonella serovar from diarrhoea patients in Europe and America, especially from patients who were infected after consumption of bovine meat, raw milk, cheese and dairy products, other or mixed meat (The EFSA Journal, 2007). Beniseeds serves as food in various parts of the world and is an oil seed of worldwide significance. It is known to have medicinal properties. For example, the plant roots and leaves are used in treating migraine, hypertension, ulcers, constipation, chicken pox and piles (Odugbemi, 2006). According to Ayo et al., (2010),

beniseed paste, when added to local kunun-zaki drink increased the protein, fat and energy content by over 20%. The seeds are tiny, flat ovals measuring about 3mm and are contained in the pods (Oshodi *et al*.,2010). 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 Beniseeds are 'eluru' and 'ekuku' (Yoruba). The Ebiras call it 'gorigo'. In French it is called 'sesame', 'tahini' in Arabic while the Japanese call it 'goma'. (http: // plants. Jstor. Org, 2010). The plant belongs to the family Pedaliaceae and is mucilaginous in nature. Previous work on beniseeds showed that the fermented liquor and methanol extract of the seeds have antibacterial and immunostimulatory potentials. This research is therefore focused on the use of the fermented liquor and methanol extract of beniseeds to treat albino rats infected with *Salmonella typhi* (a diarrhoeagenic bacterium).

MATERIALS AND METHODS

Collection of Beniseeds

Beniseeds was purchased at Okene central market in Kogi State, Nigeria. Its identity was confirmed in the Department of Crop Science of the Federal University of Technology, Akure, Ondo State, Nigeria.

Collection of test organism

The test organism (*Salmonella typhi*) was collected from the Microbiology Department, University College Hospital, Ibadan, Oyo State, Nigeria. The identity of the organism was confirmed using biochemical and morphological characteristics before storing on agar slant and kept in the refrigerator at 4^0C.

Fermentation of Beniseeds.

Five hundred grammes of the seeds was soaked in 1000ml of water for 3 days and grounded into a smooth paste using thoroughly washed electrical grinding machine according to the method of Adebolu (2007). It was then filtered using muslin bag. The filterate was allowed to undergo fermentation for 3days in a refrigerator. The fermented liquor was poured in air-tight container and stored in the refrigerator at 4^0C to maintain its potency through the period of the treatment.

Preparation of Beniseeds extract

Ninety-eight percent methanol was used to extract the active components of beniseeds according to the method of Ogundare (2006) after pounding the dry form of beniseeds.

Determination of Infectivity dose (ID) of the test organism.

This was done using standard method described by Adebolu *et al.* (2011) for the test organism.

Infection of rats with test organism

The infection of the animals was done using the infectivity dose of the organisms by orogastrically dosing them according to the method of Adebolu *et al*. (2011).

Treatment of infected rats.

A specific volume (0.75ml) of the extract and liquor was administered to the animals for 7days after infection has set in according to Oladunmoye (2007) and Erah *et al.* (1996). The following tests were carried out on the blood sample of the animals as:

a. Biochemical tests such as determination of bicarbonate, creatinine, calcium, uric acid and urea level were done according to Baker *et al.*, (2006).
b. Liver functioning test (LFT) such as the total bilirubin, serum total protein, serum albumin, serum globulin and alkaline phosphate were done to ascertain the liver functioning extent according to Zotta *et al*., (2008).
c. Haematological tests such as PCV, HB, RBC, ESR and WBC differential count were done according to Cheesbrough (2006).
d.

The effect of the infection on the weight of the animals during the experiment was monitored using KERRO LAB DIGITAL SCALE (KERRO BLG 2000 Electronic scale series). Model BLC-20001.

Statistical analysis of results

Results obtained will be subjected to descriptive one way analyses of variance, SPSS version 14 Microsoft windows 7 and Duncan multiple range tests will be used as follow up test.

RESULTS

Table 1: Results of infectivity dose of test organisms on albino rats

Microorganisms	**Infectivity dose (cfu/mL)**	**Duration for infection to set in**	**Animal weight (g)**
S.typhi	2.7×10^3	24-48 hours	68-70

Effects of treatment on health and weight of infected rats

Signs and symptoms of infection on animals infected with the bacteria include watery or unformed stool, weakness characterized by slow movement, lack of appetite and falling of fur. There was also loss of weight in the animals within 24-36hrs after infection. The animals were able to recover from the infection within five days after treatment began with both the extract and liquor respectively.

Table 2: Effect of infection and treatment with fermented beniseed liquor on the weight of albino rats infected *S. typhi*.

Day	A	B	C	D
0	70.33±0.58^{ef}	70.00±0.00^{c}	70.00±1.00^{a}	70.67 ±1.15^{a}
2	71.07±0.67^{g}	68.67±0.58^{b}	72.67±0.58^{b}	71. 33±1.15^{ab}
4	70.60±0.05^{f}	67.33±1.15^{a}	74.33±0.58^{c}	72. 67±0.58^{b}
6	70.00±1.00^{e}	67.00±0.00^{a}	76.33±0.58^{d}	74.00 ±0.00^{c}
8	68.33±0.58^{d}	68.67±1.52^{b}	78.00±1.00^{e}	74.00±0.00^{c}
10	67.00±0.58^{c}	69.33±2.08^{bc}	81.67±0.58^{f}	75.33±0.58^{d}
12	65.07±1.15^{b}	71.00±2.00^{d}	82.33±0.58^{fg}	76.00±0.00^{e}
14	64.00±2.00^{a}	73.00±2.00^{e}	83.33±0.58^{g}	76.33±0.58^{e}

Values followed by the same letter in a column are not significantly different at P≤0.05.
Key: A=Infected and not Treated, B=Infected and Treated, C=Not infected but Treated, D=Not infected and not Treated.

Table 3: Effect of infection and treatment with methanol extract of beniseed on the weight of albino rats infected *S. typhi*.

Day	A	B	C	D
0	72.67±0.58^{h}	71.00±1.00^{ef}	75.00±1.00^{a}	75. 00±0.00^{a}
2	70.67±1.15^{g}	70.67±1.52^{e}	77.33±0.58^{b}	76. 33±0.58^{b}
4	70.33±0.58^{f}	68.00±1.00^{b}	78.67±1.15^{c}	77. 00±1.00^{c}
6	68.00±0.00^{e}	67. 00±1.00^{a}	79.00±1.00^{d}	77. 33±1.00^{c}
8	65.33±0.58^{d}	68.67±0.58^{bc}	80.67±0.58^{e}	79.67±1.15^{d}
10	64.00±1.00^{c}	69.33±0.58^{c}	81.67±1.15^{f}	80.00±1.00^{d}
12	63.33±1.53^{b}	70.33±0.58^{d}	82.33±0.58^{g}	81.33±0.58^{e}
14	61.67±2.08^{a}	72.00±0.00^{f}	84. 67±0.58^{h}	81.00±1.00^{e}

Values followed by the same letter in a column are not significantly different at P≤0.05.
Key: A=Infected and not Treated, B=Infected and Treated, C=Not infected but Treated, D=Not infected and not Treated.

Table 4: Results of biochemical analyses of infected albino rats with *S. typhi* and treated with fermented liquor of beniseeds.

Parameters	A	B	C	D
Bc	23.22±0.0^{a}	26.00±0.00^{c}	27.00±0.11^{d}	24.00±0.02^{b}
Cr	0.05±0.00^{a}	0.06±0.00^{a}	0.10±0.02^{b}	0.06±0.08^{a}
Ua	2.00±0.40^{c}	0.35±0.00^{b}	0.30±0.00^{a}	0.25±0.06^{a}
U	5.20±0.50^{c}	3.70±0.30^{b}	3.60±0.00^{a}	3.50±0.05^{a}
Ca	3.25±0.50^{c}	2.40±0.00^{b}	2.30±0.05^{a}	2.40±0.02^{b}

Values followed by the same letter in a row are not significantly different at P≤0.05.
Key: A=Infected and not Treated, B=Infected and Treated, C=Not infected but Treated, D=Not infected and not Treated. Bc=Bicaronate, Cr=Creatinine, Ua=Uric acid, U=Urea,Ca=Calcium.

Table 5: Results of biochemical analyses of infected albino rats with *S.typhi* and treated with methanol extract of beniseeds.

Parameters	A	B	C	D
Bc	22.43±0.90[a]	24.90±1.00[c]	26.40±0.01[c]	24.00±0.02[b]
Cr	0.05±0.00[a]	0.08±0.00[a]	0.08±0.01[a]	0.06±0.08[a]
Ua	2.00±0.00[c]	0.30±0.10[b]	0.29±0.06[b]	0.25±0.06[a]
U	7.50±0.09[d]	4.00±0.00[c]	4.23±1.20[b]	3.50±0.05[a]
Ca	4.90±0.50[a]	2.90±0.67[c]	2.60±0.00[b]	2.41±0.02[a]

Values followed by the same letter in a row are not significantly different at P≤0.05.
Key: A=Infected and not Treated, B=Infected and Treated, C=Not infected but Treated, D=Not infected and not Treated. Bc=Bicaronate, Cr=Creatinine, Ua=Uric acid, U=Urea,Ca=Calcium.

Table 6: Liver functioning results of infected albino rats with *S. typhi* and treated with fermented liquor of beniseeds.

Parameters	**A**	**B**	**C**	**D**
BT	9.00±0.00[a]	10.70±1.00[c]	13.50±0.21[d]	10.50±0.20[b]
STP	70.25±0.50[c]	61.85±1.15[a]	67.10±0.51[b]	62.00±0.15[a]
AST	27.00±0.00[d]	23.10±0.30[a]	22.54±0.15[b]	24.08±0.68[c]
ALT	36.45±0.43[c]	29.00±0.00[b]	26.00±0.60[a]	28.70±0.12[b]
Alk.Phos	31.00±0.00[c]	27.50±0.05[b]	23.95±0.32[a]	26.45±1.22[b]
Cholesterol	2.25+0.50[d]	1.60±0.44[c]	1.30±0.00[a]	1.50±0.02[a]

Values followed by the same letter in a row are not significantly different at P≤0.05.
Key: A=Infected and not Treated, B=Infected and Treated, C=Not infected but Treated, D=Not infected and not Treated. BT=Bilirubin total, STP=Serum total protein, AST=Asparate transferase test, ALT=Antilymphocyte transferase test, ALK=Alkaline phosphatase.

Table 7: Liver functioning results of infected albino rats with *S. typhi* and treated with methanol extract of beniseeds.

Parameters	**A**	**B**	**C**	**D**
BT	9.00±0.00[a]	11.00±0.00[c]	13.50±0.12[d]	10.50±0.20[b]
STP	70.25±0.50[d]	64.00±0.00[b]	69.23±0.11[c]	62.00±0.15[a]
AST	27.00±0.00[d]	25.00±0.00[c]	20.50±0.45[a]	24.08±0.68[b]
ALT	36.45±0.43[d]	26.00±0.00[a]	29.80±0.00[c]	28.70±0.12[b]
Alk.Phos	31.00±0.00[d]	27.75±0.25[c]	25.90±0.67[a]	26.45±1.22[b]
Cholesterol	2.25±0.50[b]	2.50±0.57[c]	3.28±0.65[d]	1.50±0.02[a]

Values followed by the same letter in a row are not significantly different at P≤0.05.
Key: A=Infected and not Treated, B=Infected and Treated, C=Not infected but Treated, D=Not infected and not Treated. BT=Bilirubin total, STP=Serum total protein, AST=Asparate transferase test, ALT=Antilymphocyte transferase test, ALK=Alkaline phosphatase.

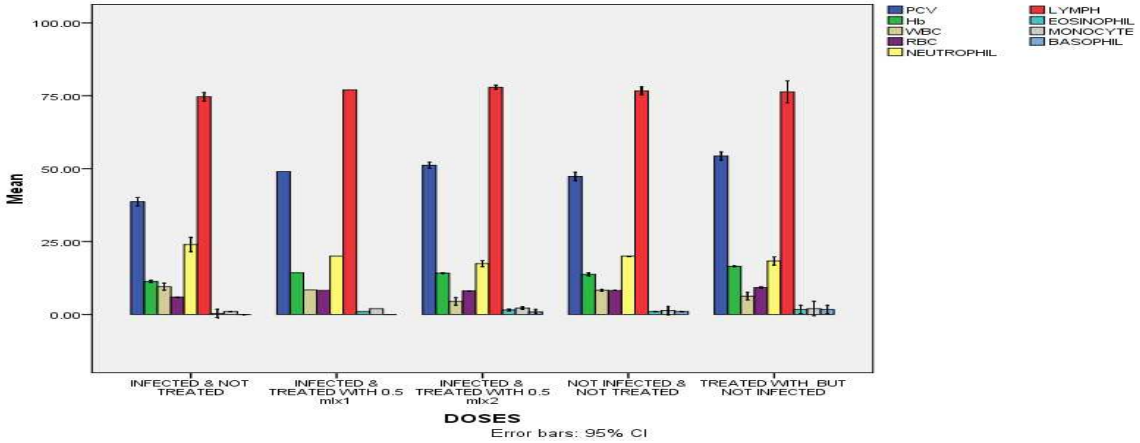

Fig 1: Haematology result of rats infected with *S.typhi* and treated with fermented beniseed liquor.

Table 8: Haematological results of rats infected with *S. typhi* and treated with methanol extract of beniseeds

TRT	ESR	PCV	HB	WBC	RBC	LYMP	NEUT	MONO	EOSIN	BAS
A	1.00±0.00^{a}	42.33±0.58^{b}	14.13±0.12^{b}	9.00±0.44^{a}	8.43±0.06^{d}	72.67±0.58^{b}	22.33±0.58^{b}	4.00±1.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}
B	2.67±0.58^{b}	31.00±1.00^{a}	10.16±0.15^{a}	14.97±0.68^{c}	6.20±0.00^{a}	67.67±0.58^{a}	25.00±0.00^{c}	5.00±0.00^{a}	1.67±0.58^{b}	0.67±0.58^{a}
C	1.00±0.00^{a}	45.67±0.58^{c}	15.13±0.12^{c}	10.10±0.17^{b}	8.13±0.06^{c}	73.33±0.58^{b}	21.33±0.58^{a}	4.00±0.00^{a}	1.00±0.00^{a}	0.33±0.58^{a}
D	1.33±0.58^{a}	43.00±1.00^{b}	13.90±0.36^{b}	10.83±0.76^{b}	7.73±0.21^{b}	72.67±0.58^{b}	21.33±0.58^{a}	4.33±0.58^{a}	2.00±0.00^{b}	0.00±0.00^{a}

Values followed by the same letter in a column are not significantly different at P≤0.05.
Key: A=Infected and treated, B=Infected not treated, C=Treated not infected, D=Not infected not treated.

DISCUSSION

The results obtained from infection of the rats showed that the organisms caused diarrhoea in the animals within a period of 48hrs. The signs symptoms observed after infection set in were in accordance with those described by Adebolu (2007), Baker (2006) and Baker *et al*,(2007). *B. cereus* infection set in first (within 24hrs) of infection among the selected organisms that were used to infect the rats. This probably was due to the virulence of the organism in causing diarrhoea according to Oladunmoye (2007). According to Laurence *et al*, (2002), the time interval between infection and overt signs and symptoms is a function of how virulent the organism is. Also, their spore forming nature may aid their survival, reproduction and defence against the host immune system.

During infection, the group of rats fed with the extract and fermented liquor but was not infected constantly gained weight. However, weight gain was significantly more on those fed with the fermented beniseed liquor than those fed with methanol extract which is also an indication that the fermented beniseed liquor was more effective and had more positive effect on the rats than the methanol extract of beniseeds. Also, the weight of rats infected with *S. typhi* dropped significantly. This also indicates the severity of diarrhoea caused by this organism when compared with other bacteria as seen in this work. According to Hsieh *et al*, (2001), plant extract or combination of plant extracts that will have therapeutic effect on man or animals must cause a significant increase in the weight of experimental animals after administering such extract for a period of at least one week (7 days). Shittu *et al*, (2007) believes that all Sesame species have therapeutic potentials and should be harnessed for future use. According to EFSA journal (2007), the search for cheap and effective therapy for Salmonellosis is not for man alone but also for farm animals. Therefore, beniseeds in fermented form or extract can be effectively used to feed farm animals both for preventive and therapeutic purpose.

Comparatively, the therapeutic effect of the fermented beniseed liquor was more than that of the methanol extract because the rats infected and treated with liquor all recovered within a period of 5days while those treated with extract recovered after 5days. The reason for this may be the fact that the fermented beniseed liquor may be absorbed faster by the stomach walls of the intestine of the rats. According to Cutting, (2011), fermented foods, especially in liquid form are absorbed faster than the unfermented ones by the walls of the small intestine and that they get to the small intestine faster because no digestive enzyme act on them. Therefore, fermented beniseed liquor and methanol extract of it can be effectively used by man and also to feed farm animals both for preventive and therapeutic purpose for diarrhoea caused by S. typhi.

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