CEDTECH International Journal of Bioscience and Biotechnology
Volume 3, Number 2, June 2022
http://www.cedtechjournals.org
ISSN: 2756-4568

TOXICITY STUDIES OF *BOSWELLIA DALZIELII* AGAINST CANDIDA AND ASPERGILLUS SPECIES IN KEBBI STATE, NIGERIA

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ABSTRACT

Plant extracts are commonly used as herbs all over the world. The practice, in most underdeveloped countries, is with little or no regulation from constituted authorities. Some of these herbs though, probably effective, may be toxic. Toxicological investigations of these herbs need to be conducted to establish their safety and dosages. In this study, the toxicological examination of a widely used plant for herbs in Sokoto State of Nigeria was conducted. The acute and sub-acute toxicity tests of the methanol leaf extracts of *Boswellia Dalziel* were performed on experimental rats. Tests were conducted to find derangement in the liver function of animals administered with crude extracts during the acute and sub-chronic toxicity studies. The study showed that the extracts did not create any adverse effects on the tested animals. Both the acute and sub-chronic tests of *Boswelliadalzielii* methanol extracts produced no harmful effects on the tested animals.

INTRODUCTION

Herbalism as complementary medicine has always been the backbone of traditional medicine to treat mild, acute and chronic diseases or even as preventive medication. Herbs have been used from time immemorial and in virtually all cultures as a source of medicine (Cragg and Newman, 2001). The World Health Organization estimated that the size of the world population that relies chiefly on traditional medicines could be as high as eighty percent (Cragg and Newman, 2001).The world body has accepted the use of herbal products with recommendations for national policies and regulatory measures to guide the administration of traditional drugs. It also promotes research and the evaluation of the safety and efficacy of herbal products (Farnsworth, 1984).Farnsworth (1984) reported that of the 119 plants derived drug listed by WHO study, 74% were discovered as a result of chemical studies to isolate the active

compounds responsible for the use of original plant in traditional medicine.

Zerabruk and Yirga (2012) have however noted that the rationale for the utilization of medicinal plants has rested largely on long-term experience with little or no scientific data on their efficacy and safety. The need to validate the folkloric usage of these herbs through scientific investigation is very important (Aisha *et al.,* 2018; Sofowora, 1993). This investigations help on the choice of whether or not a new drug should be adopted for clinical use (Anisuzzaman *et al.,* 2001). Toxicological studies may be based on the dose of the substance, the toxic properties of the substance and the duration of exposure of animal to the drug. Based on these the study could either be acute, sub-acute or chronic (Baki*et al*., 2007). The relationship between these factors is significant in the consideration of therapeutic dosage in pharmacology and herbalism (Hayes, 2001). Acute toxicity test is suitable for single exposure for a short period (UWCSDG, 1999) and is normally carried out in rodents. It is useful in determining the lethality or life-threatening toxicity of a drug (Commission Directive, 2003). Acute toxicity studies are commonly used to determine LD_{50} of drugs or chemicals (Baki*et al*., 2007). The acute study provides a guideline for selecting doses for the sub-acute and chronic low-dose study, which may be clinically more relevant (Janbaz*et al*., 2002; Hasumura *et al*., 2004).

In this study, a toxicological investigation of Boswelliadalzielii, a plant commonly used as herb in Kebbi State of Nigeria, was undertaken. The outcome may help establish the safety and probable dosages of the herb. The acute and sub-acute toxicity tests of the methanol leaf extracts of the plant were performed on experimental rats. The plant itself is mostly found in Africa with so much research on its effectiveness in curing ailments such as rheumatism, pain and inflammation (Moll *et al*., 2000; EMEA, 2004). The plant which grows to as much as 13 metres is widespread in parts of West Africa (Moll *et al.,*2000, Álvarez-Sánchez *et al.,* 2006). The plant has been shown to possess important phytochemicals and antimicrobial capabilities (Moll *et al.,* 2000; Álvarez-Sánchez *et al* 2006; EMEA, 2004; Gokbulut*et al.,* 2014; Marrugal-Lorenzo, 2019).

METHODOLOGY

Collection, processing and Extraction of Plants

Fresh leaves of *Boswelliadalzielii* were collected from Zuru area of Kebbi State of Nigeria. The leaves were taken to the Herbarium of the Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, and Sokoto for identification and authentication. The *Boswelliadalzieli* sample was given voucher number UDUH/ANS/0069. The leaves collected were air-dried pulverized into a fine powder using a pestle and mortar and stored in sealed containers. The extraction was done using the maceration method as described by Abdullahi and Lawal (2010).

Acute Toxicity Tests of the Various Plants (Extracts) on Experimental Rats

This was carried out following the procedure of Mirtese*t al.,*(2011). A total number of (40) Albino rats were used for the test. The rats were obtained from the animal house Department of Biological Science, Usman Danfidiyo University Sokoto. The rats were caged differently under (4) groups, ten in each cage. They were housed in a well-ventilated house at a temperature of 27-30°C. The rats were allowed to acclimatize for 3 days before the experiment. The experimental rats were weighed before the onset of the experiment. Doses of the extract (5ml each) were administered orally to the rats in four groups consisting of ten rats in a group. The rats in groups B, C and D were given 1000, 2000, and 3000mg/kg body weight of plant extracts respectively. The rats in the fourth group which were administered with 0.5ml of distilled water through the same route served as a positive control. The rats were weighed after hours of the administration of the drug. The rats were first observed, 4 hours after administration of the extract. The observation was then carried out every 8 hours for the next 7 days. The toxic symptoms observed were calmness, change in eye colour, convulsion, stretching of furs, mouth or nose and then mortality of the rats (OECD 2001).

Sub-acute Toxicity Study

A total of 40 Albino rats were divided into 4 groups comprising 10 rats in each group. Rats in groups B, C, and D were administered orally with 5ml of graded doses of plant extracts (1000, 2000 and 3000 mg\kg body weight per day) once daily for 28 days respectively. The control group (group A) received 0.5ml of distilled water under similar experimental conditions and through the same route. Subsequently, all the animals from each group were killed by decapitation and blood samples were

carefully taken at the slaughter point for biochemical evaluation of the liver function.

Biochemical Evaluation (Liver Function Tests)

The tests conducted to investigate derangement in the liver function of animals administered with crude extracts during the acute and sub-chronic toxicity studies are; Serum bilirubin, Serum enzymes, Alkaline phosphatase, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Serum total protein. Serum total bilirubin was estimated by a modification of the Jendrassik and Grof method (Cheesbrough, 1991). Serum total protein and albumin were determined using the biuret method and Bromocresol green (BCG) Bind respectively. Serum globulin level and albumin/globulin (A/G) ratio were estimated from the value obtained for total protein and albumin (Cheesbrough, 1991). Serum AST and ALT activities were determined using the Reitman-Frankel method (Saidu, 2005). Alkaline phosphatase activity in the serum was estimated following the procedure of Saidu, (2005) by the Nitrophenyl phosphate method.

Measurement of Serum Total and Direct Bilirubin

Total Bilirubin

Two sets of test tubes, labeled sample blank and sample were set up from each of the samples. To the sample test tube, 200µl of reagent 1(29mmol/l sulphanilic acid containing 0.17M HCL), 50 µl of reagent 2 (25mmol/l sodium nitrite) , 1ml of reagent 3(0.26mol/l caffeine) and 200ul of the sample was added. The sample blank was prepared likewise except that the reagent 2 was not added. All the tubes were mixed and allowed to stand at 25°c for 10 minutes. One milliliter of 0.93mol/l of tartrate containing 1.9M NaOH was added to all the test tubes, mixed, and allowed to stand at 25°c for about 10minutes and the absorbance of the samples (A_{TB}) read against their respective sample blanks at 580nm.

The concentration of total bilirubin was calculated as:

total bilirubin (umol/l) = $185XA_{TB}$.

Where185 is the conversion factor.

Direct bilirubin:

Two sets of test tubes, labeled sample blank and sample were setup for each of the samples. To the sample, test tube was added 200ul of 29mmol/l of 29mmol/l of sulphanilic acid containing 0.17M HCL (reagent 1), 50ul of 25mmol/l sodium nitrite (reagent 2), 2ml of 9g/l

NaCL and 200ul of the sample. The sample blank was prepared likewise except that reagent 2 was not added. All the tubes were, mixed, allowed to stand at 25°c for exactly 5 minutes and the absorbance of the 'samples' (A_{DB}) read against their respective sample blanks at 580nm.
The concentration of direct bilirubin was calculated as:
Direct bilirubin (umol/l) = $246XA_{DB.}$
Where 246 is the conversion factor

Determination of Serum total Protein (Biuret Method)
A total protein kit was used for the analysis. 0.05cm each of serum, standard protein (bovine serum albumin) and water was added into three labeled test tubes containing 2.5ml Biuret reagent each. The tubes were incubated at 37°c for 10min and the absorbance of the standard and test was read against the blank at 540nm.
Serum total protein was calculated as:

$$\text{Total protein (g/l)} = \frac{\text{Abs. of test x conc. of STD (g/l)}}{\text{Abs. of STD}}$$

Determination of serum albumin (Bromocresol Green Method)
Into a labelled test tubes containing 4ml BCG reagent, 0.02ml of serum was added, standard protein (bovine serum albumin) and water for the test, standard and blank respectively. The tubes were mixed properly while avoiding frothing and the absorbance of the standard and test were read against the blank at 632nm. Serum albumin was calculated as below:

$$\text{Serum albumin (g/l)} = \frac{\text{Abs. of test x conc. of STD (g/l)}}{\text{Abs. of STD}}$$

Estimation of serum Globulins
Serum globulin concentration was estimated as the difference between serum total protein and serum albumin concentrations.
Albumin Globulin ratio
A: Gratio was calculated by dividing serum albumin by serum globulin concentrations.

Measurement of Serum Aspartate Aminotransferase Activity
Glutamic oxaloacetic acid transaminase (GOT) reagent kits were used, and to test tube labeled reagent blank and sample blank were added with 0.1ml of distilled water and serum respectively. 0.5ml of solution 1 containing (100mmol/l phosphate buffer, pH 7.4, 100mmol/l L-aspartate and 2mmol/l α-ketoglutarate) was added to each of the tubes, mixed and

incubated for 30 minutes at 37oc in a water bath. 0.5ml of solution 2 (2mmol/l 2,4-dinitrophenylhydrazine) was added to each of the tubes, mixed, allowed to stand for 5minutes and the absorbance of the sample (A_{SAMPLE}) was read against the reagent blank at 540nm.
The enzyme activity, which was expressed in U/I was obtained from a calibration curve using the values supplied along with the kits.

Measurement of serum alanine aminotransferase (ALT) activity
Glutamic-pyurvic transaminase kit (GPT) was used for the assay activity of ALT. into test tubes labeled 'reagent blank' and 'sample blank' were added 0.1ml of distilled water and serum respectively. 0.5ml of solution 1 containing (100 mmol/l phosphate buffer, Ph 7.4, 200mmol/l L-alanine and 2mmol/l α-ketoglutarate was added to each of the tubes, mixed and incubated for 30minutes at 37oc in a water bath. 0.5ml of solution 2(2mmol/l 2,4-dinitrophenyldrazine) was added to each of the tubes, mixed and allowed to stand at room temperature for 20 minutes. 5ml of 0.4mol/l NaOH was added to each of the tubes, mixed allowed to stand for 5minutes and the absorbance of the samples (A_{sample}) was read against the reagent blank

Measurement of serum Alkaline phosphatases (ALP) activity
ALP reagent kit was used for the assay activity of ALP. To labeled tube containing 20µl of the serum sample, 1cm³ of alkaline phosphatase working reagent was added. The content was mixed and initial absorbance was read against air at 405nm. This was taken as 0min and the timer started immediately and the absorbance would be read again at 1, 2 and 3 mins. The alkaline phosphate activity was calculated as:

ALP= 2760 X Absorbance of the sample

RESULTS DISCUSSION
RESULTS
Acute Toxicity Results
The results presented in Table 1to Table 4 are that of acute toxicity test on the rats. The signs of toxicity observed are: calmness, change in eye colour, convulsion, stretching of furs, mouth or nose and then the mortality rate of the rats.

Table 1: The result of the calmness of the methanolic leaves extract of *Boswelliadalzielii*on Albino rats after acute toxicity test

Group	No. of animals used	Dose (mg/kg)	Sign of calmness
A (control)	10	-	Calm
B	10	540	Calm
C	10	1080	Calm
D	10	2160	Calm

Table 2:the result of the change in eye colour of the methanolic leaves extract of Boswelliadalzielii on Albino rats after acute toxicity test.

Group	No. of animals used	Dose (mg/kg)	Change in eye colour
A (control)	10	-	None
B	10	540	None
C	10	1080	None
D	10	2160	None

Table 3:the result of the change in the convulsion of the methanolic leaves extract of Boswelliadalzieliion Albino rats after acute toxicity test.

Group	No. of animals used	Dose (mg/kg)	Sign of convulsion
A (control)	10	-	None
B	10	540	None
C	10	1080	None
D	10	2160	None

Table 4:the result of the change in Unusual stretching of furs, nose or mouth of the methanolic leaves extract of *Boswelliadalzielii*on Albino rats after acute toxicity test.

Group	No. of animals used	Dose (mg/kg)	Un usual stretching of furs, nose or mouth
A (control)	10	-	None
B	10	540	None
C	10	1080	None
D	10	2160	None

Table 5: Acute toxicity on mortality of Albino rats due to the administration of methanolic leaves extract

Group	No. of animals used	Dose (mg/kg)	Mortality
1 (control)	10	-	0/10
2	10	540	0/10
3	10	1080	0/10
4	10	2160	0/10
5	10	3240	0/10

Biochemical Test Result

The results of the acute and sub-acute biochemical studies of the animals are presented in Tables 6 and Tables 7

TABLE 6: Effect of leave of *Boswelliadalzielii* methanol extract on liver function parameter of rats treated with acute oral doses.

GROUP	DOSE (Mg/kg)	PARAMETERS								
		ALT (g/dl)	AST (g/dl)	ALP (g/dl)	TBL (µ/mol)	DBL (µ/mol)	TP (g/dl)	ALB (g/dl)	GLO (g/dl)	A:G (g/dl)
A	0	7.69	17.85	82.95	9.0	6.13	5.85	3.28	2.58	2.27
B	1000	7.62	17.84	81.16	8.27	6.36	5.95	3.15	2.44	1.29
C	2000	7.65	17.98	80.86	8.59	6.38	5.95	3.98	1.14	3.49
D	3000	7.66	17.98	81.33	8.51	6.70	5.84	3.22	2.27	1.41

Key

ALT= Alanine aminotransferase, AST= Aspertate amino transferase, T.P=Total protein, ALB=Albumin, TBL= Total bilirubin, and DBL=Direct bilirubin, GLO= Globulin, A:G = Albumin Globulin ratio

Group A=Control group

Group B= Given 1000mg/body weight

Group C= Given 2000mg/ body weight

Group D= Given 3000mg/body weight

TABLE 7: Effect of leaves of *Boswelliadalzielii* methanol extract on liver function parameter of rats treated with sub-acute oral doses.

GROUP	DOSE (Mg/kg)	PARAMETERS								
		ALT (g/dl)	AST (g/dl)	ALP (g/dl)	TBL µ/mol)	DBL (µ/mol)	TP (g/dl)	ALB (g/dl)	GLO (g/dl)	A:G (g/dl)
A	0	7.70	16.50	65.87	7.59	6.35	5.79	3.79	2.00	1.89
B	1000	7.71	16.05	66.00	7.10	6.71	5.98	3.79	2.19	1.73
C	2000	7.73	16.25	66.10	7.51	6.69	5.98	3.96	2.02	0.21
D	3000	7.73	16.20	66.05	7.10	6.82	6.00	3.98	2.02	1.97

Key
ALT= Alanine aminotransferase, AST= Aspertate amino transferase, T.P=Total protein, ALB=Albumin, TBL= Total bilirubin, and DBL=Direct billirubin, GLO =Globulin, A:G =Albumin Globulin ratio.
Group A = Control group
Group B= Given 1000mg/body weight
Group C= Given 2000mg/ body weight
Group D= Given 3000mg/body weight

DISCUSSION

The results of the observations of the tested animals after administration of the methanolic extract of *Boswelliadalzielii* are presented in Tables 1 to Table 5. As could be seen there is no adverse effect on the conditions of the animals after the administration. This shows that the lethal dose of the extracts is higher than 3000 mg/Kg body weight. The acute effects of the methanolic extract of *Boswelliadalzielii* on liver function parameters of animals treated with acute doses of the extract are presented in Table 6. Based on the parameters tested there are not many differences between the control group and the treated groups. For ALT the result indicated that the animal in group B has a little decrease in serum ALT compared to the control group (Group A, 7.69 and Group B, 7.62) followed by group C with 7.65, then group D with 7.66. In AST there is little increase in groups C and D with 17.98, compared with the control group(Group A with 17.85). Also in ALP, TBL, DBL, and ALB there is less

increase and decrease in the group compared to the respected control group.

The effect of sub-acute administration of methanolic leaves extract of *Boswelliadalzielii* on the liver function parameters of animals is presented in Table 7. For ALT the result indicated that the animal in group B has little increase in serum ALT compared to the control group (Group A, 7.70 and Group B, 7.71) followed by group C and D with 7.73 respectively. In AST there is little decrease in groups B, C and D with 16.05, 16.25, and 16.20, compared with the control group (Group A 16.50). Also in ALP, TBL, DBL, and ALB there is less increase and decrease in the group compared to the respected control group. Based on the parameters tested there are not many differences between the control group and the groups treated with the extract.

Both the acute and sub-chronic tests of *Boswelliadalzielii*methanol extract produced no harmful effects on the test animal, such as death, change in body weight, change in food and water consumption, and changes in liver function parameters(Mirte*set.al.,* 2011).

CONCLUSION

The study has been able to investigate the safety of *Boswelliadalzielii* as herbs for medication and as a vegetable for consumption. The outcome of the study indicates that the plant has no immediate or long-term adverse effect on human health.

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