WWJMRD 2019; 5(3): 11-14
www.wwjmrd.com
International Journal
Peer Reviewed Journal
Refereed Journal
Indexed Journal
Impact Factor MJIF: 4.25
E-ISSN: 2454-6615

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The Evaluation of Combined Crude Root Extract of *Sphenocentrum jollyanum* and *Baphia nitida* on Some Liver Enzymes in Male Wistar Albino Rats.

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Abstract
The recent study investigated the effect of combined crude root extract of *Sphenocentrum jollyanum* and *Baphia nitida* on some liver enzymes in 15 male Wistar rats (body weight 150-200g). The rats were assigned into three groups. First and second groups were fed with 200mg/kg and 400mg/kg of crude extract of *Sphenocentrum jollyanum* and *Baphia nitida* respectively, via orogastric feeding for 60 days while the third group serves as control. Result was analyzed using One way ANOVA, followed by post hoc multiple comparisons and level of significance set at $p<0.05$. Liver enzymes tests were performed in the animals before and after the experiment using Randox diagnostic reagent kits. Results showed that mean serum ALT levels of rats fed with 200mg/kg were significantly ($P<0.05$) elevated compared 400mg/kg fed group and control. Mean serum AST of 200mg/kg fed rats had significant ($P<0.05$) reduction compared to 400mg/kg fed rats and control. Mean serum ALP levels of extract fed rats were elevated compared to control. However, mean ALP levels of rats fed with 200mg/kg had significant ($P<0.05$) increase than group fed with 400mg/kg extract. Furthermore, levels of the parameters before and after extract administrations show a significant decrease in ALP activity in the groups after administration compared to its initial levels. However, ALT and AST showed significant increased levels in the groups after administration compared to before administration. The changes in the liver enzyme levels may suggest possible effect of *Sphenocentrum jollyanum* and *Baphia nitida* and its ingestion in excess may likely lead to impaired liver function.

Keywords: *Sphenocentrum jollyanum, Baphia nitida,* liver enzymes, Orogastric feeding and Randox diagnostic reagent kits

1. Introduction

The *Sphenocentrum jollyanum* is a deciduous shrub that belongs to the family of menispermacea. It is about 1.5m tall with gray bark and its leaves are spirally arranged with smooth appearance on both sides [10, 9]. The stem is thinly short hairy when young, later glabrous, [22]. It is grown naturally along the west coast sub region of cote d ivories with expense from Cameroon across Nigeria to Sierra Leone [12]. It has shown to posses' antihypertensive, antioxidant, antinociceptive, antiviral and antiangiogenic effects in animals [13]. It has been documented to have emetic and purgative effects while the sap is believe to relive stomach ache and constipation and to boost appetite and sexual drive [8]. In Nigeria a decoction of the root is applied to treat topical ulcer and the edible fruit is taken against fatigue [3]. It has been reported that the ethanol root extract of *Sphenocentrum jollyanum* increased the testosterone levels in a dose-dependent manner and also reduces the count, motility and viability of spermatozoa in albino rat [20].

Baphia nitida (camwood or barwood) and also known as African sandalwood is a shrubby, leguminous, hard wooded tree from central Africa the wood of a very fine colour and is used in woodturning for making knife handles [2]. *Baphia nitida* is a wide spread forest plant which is commonly distributed globally especially within the coastal region of West Africa [16]. It is about 9m (30ft) and produce small fragrant pea flowers, white with yellow centers from February to May it also has pointed pods 7cm (3inch) long which ripen in October and

split open to release one or two dark brown shiny seeds [17]. It is applied against ringworm, stiff joints, sprains and rheumatic pains and used in the treatment of constipation, [15]. The dye of camwood possesses some level of antimicrobial activity and can be used as a remedy for pathogenic infections [16]. The leaves of *Baphia nitida* have been reported to be good inhibitors for mild steel corrosion in acid medium [14]. It has been reported to be beneficial against atherosclerosis, osteoporosis, diabetes mellitus and breast cancer [25]. Its flavonoids constituent has been suggested to have antibacterial, antioxidant and anti–inflammatory activities [26].

The liver is a vital inner organ that performs numerous functions.[7] They include secretion of bile, excretory function, synthetic function, metabolic function, hemopoietic function, hemolitic function, heat production, defense and detoxification function[7], clotting factors and albumin synthesis,[19]. The human liver consists of numerous enzymes that aids necessitate chemical processes in the body, [18, 19]. The most commonly used indicators of liver functions are the Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) [5] and alkaline phosphatase concentration. Variations in the concentrations of these enzymes have many implications. Increased levels of ALT and AST are indications of hepatocellular disease, active cirrhosis, metastatic liver tumor, toxic hepatitis, severe, pancreatitis, myocardial infarction (heart attack), trauma, severe burns, acute hemolytic anemia, crushing injuries and shock. [18] Alkaline phosphatase is commonly used to access obstruction in the biliary system. Its increase is majorly detected in biliary tumor, gallstone disease, alcohol abuse and drug-induce hepatitis. [24].

Materials and Methods

Materials: Syringes, Filter paper, Electronic weighing balance (Arman, England), orogastric cannula, plain and EDTA sample bottles, normal saline, chloroform anesthesia, water bath (Kutterman, U.K), bucket centrifuge machine (Labman, U.K), Spectrophotometer (Jenway, England), Dissecting kits (Haweley, England) and rat specific kits for liver enzymes.

Ethanoic Preparation of *Sphenocentrum jollyanum* and *Baphia nitida* root

The fresh root of *Sphenocentrum jollyanum* and *Baphia nitida were* collected and sent to a Botanist in Pharmacology Department Madonna University Nigeria for identification. They were chopped into pieces and air dried (36-39^{o}c). The dried root was ground to a coarse powder with grinder and stored in a closed earthenware pot which was left open when placed on a fire without adding water. The 1000g and 500g of the powered *Sphenocentrum jollyanum* and *Baphia nitida* respectively were extracted with 100% ethanol within 72h. The ethanol filtrate was concentrated to obtain a crude extract and evaporated to dryness in water bath at 60^{0}C for 36hours. The brownish residue were weighed and kept in an air tight bottle in the refrigerator until use. This method was recently used by (Mbaka, *et al*, 2011), [10].

Laboratory animals

Fifteen (15) male albino Wistar rats aged 8 weeks and weighing 150–200g were used for this study. The animals were housed in the Department of Biochemistry Animal house, Madonna University, Elele, Rivers State, Nigeria. Standard animal cages with wood dust as bedding were used in keeping the animals. They were allowed *ad libitum* access to mice specific feed and clean water, and exposed to 12/12-hr light/dark cycle. The animals were acclimatized for 7 days. The animals were kept in line with laid down principles for animal care as prescribed in Helsinki's 1964 declaration. The animal ethics committee of Madonna University approved our study protocol graciously.

Experimental design and *Spbenocentrum jollyanum* and *Baphia nitida* Extract Administration

The animals were randomly assigned into three (3) groups of five animals each. First and second groups serve as low dose and high dose and were fed with combined crude extract of *Spbenocentrum jollyanum* and *Baphia nitida* at the doses of 200mg/kg and 400mg/kg body weight respectively. The third group serves as control and receives only feed and water. Administration of *Spbenocentrum jollyanum* and *Baphia nitida* crude extract was done via orogastric feeding once daily for 60 days after which the animals were sacrificed under chloroform anaesthesia and blood sample was collected for liver enzyme assay. Method of administration recently used by, (Mobisson, *et al.*, 2018), [11].

Determination of liver enzymes

ALP was measured according to standard procedure.[1] P-nitrophenyl phosphate was hydrolysed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01ml in a test tube was mixed with reagent (0.5ml) containing the substrate p nitrophenyl phosphate and kept at room temperature. The solution was mixed, initial absorbance read after 1 minute. The reaction was allowed to stand for 3 minutes and the absorbance read again at 405nm. Alkaline phosphate activity was calculated from.

UL = 2760 x ΔA nm/minute micro

Where UL = Unit of alkaline phosphatase affinity

ΔA = Change in absorbance

Serum AST and ALT levels were determined using endpoint colorimetric diagnostic kit (Randox Labouratories, UK) based on Reitman and Frankel's method.[21] The pyruvate produced by transamination reaction between L-alanine and ketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a coloured hydrazone, and was used to measure alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine was used to measure aspartate aminotransfarase (AST). Both ALT and AST were read at 540nm wavelength. [1]

Statistical Analysis

All results are presented as mean ±standard error of mean (SEM). One way analysis of variance (ANOVA) was utilized in comparing the difference within groups, followed by post hoc multiple comparisons. The level of significance was placed at $p<0.05$.

Results

Table 1: Serum Liver Enzyme Levels of Rats before *Spbenocentrum jollyanum* and *Baphia nitida* crude extract administration.

Parameters Group3(control)	Group1	Group2
ALP(U/L) 99.08±15.81[ab]	103.00±0.98[a]	116.03±0.80[b]
ALT(U/L) 19.29±4.80[a]	24.09±0.04[a]	24.31±0.00[b]
AST(U/L) 17.66±1.60[c]	14.48±0.13[a]	14.48±0.13[b]

Values are expressed in mean ± SEM, n = 5.

Mean with the same superscript ([a], [b] and [c]) are significantly different. While mean with different superscript are not significantly different.

Table 2: Serum Liver Enzyme Levels of Rats fed with *Spbenocentrum jollyanum* and *Baphia nitida* combined crude extract and Control, after 60 days.

Parameters Group3(control)	Group1(200mg/kg)	Group2(400mg/kg)
ALP(U/L) 68.07±6.19[c]	74.75±0.32[a]	71.03±0.70[b]
ALT(U/L) 27.30±4.59[a]	32.86±2.07[a]	29.11±5.24[b]
AST(U/L) 42.11±1.61[c]	41.20±0.14[a]U	41.57±0.17[b]

Values are expressed in mean ± SEM, n = 5.

Mean with the same superscript ([a], [b] and [c]) are significantly different. While mean with different superscript are not significantly different.

Discussion

The roots of *Sphenocentrum jollyanum and Baphia nitida has* been in traditional medicine practice for years, [6]. The medicinal practices by some local herbal practitioners that involve the use of these plant extract for treatment of some ailments without scientific proof of effectiveness and side effects is quit alarming. Liver is a primary site of detoxification as well as the major site of intense metabolism; it is therefore prone to various disorders as a consequence of exposure to the toxins of various forms [4].

The present study investigated the effect of *Spbenocentrum jollyanum* and *Baphia nitida* combined crude extract on some liver enzyme markers. Liver enzyme markers assessed in this study include the plasma levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP). The aforementioned parameters were assessed in both the control (group3) and *Spbenocentrum jollyanum* and *Baphia nitida* combined crude extract fed groups.

The results from this study shows that serum ALP activity increases significantly ($P<0.05$) in group 1 (74.75±0.32u/l) and group2 (71.03±0.70u/l) fed with 200mg/kg and 400mg/kg combined extract of *Sphenocentum jollyanum* and *Baphia nitida* compared to group 3 which is the control (68.07±6.19u/l). Similarly ALT activity increases significantly in group 1 (32.86±2.07u/l) and group 2 (29.11±5.24u/l) compared to group 3 (27.30±4.59u/l). Furthermore AST activity decreases significantly in group 1 (41.20±0.14u/l) and group 2 (41.57±0.17u/l) compared to group 3 (42.11±1.61u/l). Then comparing the parameters before and after administrations, there was a significant decrease in the ALP activity level of the groups after the administration and there was a significant increase in the ALT and AST activity level of the groups after the administration. Increase in ALT and AST levels may likely be due to Liver damage, Osteoporosis, Biliary obstruction, Hepatitis, Diabetes, Acute hepatocellular injury and cirrhosis [27]. Furthermore decrease in ALP may likely be due to Leukemia, Hypothyrodism, Sever anemia and Magnesium deficiency, [23].

The effects produced by combined ethanoic extract of *Sphenocentrum jollyanum* and *Baphia nitida* could be attributed to the variable composition of phytochemicals which may likely produce organ damage, particularly the liver when consumed in excessive amount, [10].

Conclusion

This study shows that the consumption of these extract *Sphenocentrum jollyanum* and *Baphia nitida* often and at long period of time without safe dosage has adverse effect on the liver; therefore this extract has the potential of causing liver injury to the individual.

Recommendation

Both *Sphenocentrum jollyanum* and *Baphia nitida* and are not advised to be used in excess and without safe dosage because it might cause liver injury.Therefore, I advise that people should desist from the habit of using both the *Sphenocentrum jollyanum* and *Baphia nitida* root often and in excess for the purpose of enhancing sexual performance. Nonetheless, sufferers of liver disease may consult the physicians for proper guidance.

Acknowledgement

The authors of this study appreciate the effort of the laboratory technician, Biochemistry Department, Madonna University and the Head of department of Biochemistry. Also our profound gratitude goes to the ethical committee for authorizing us to carry out the study.

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