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Some Effects of A Diet Containing Leaves of *Anchomanes difformis* on the Liver of Adult Wistar Rats

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ABSTRACT

Crude extract of the leaves of *Anchomanes difformis* was investigated via oral route on the liver of twenty-four adult wistar rats of both sexes, weighing 200g-250g. They were randomly categorized into four experimental groups A, B, C and D; of six rats per group (n=6). Group A rats received 100% feed mash and served as the control group, while treatment groups B, C and D received mixed diet of feed mash and the crude extract of *Anchomanes difformis* at 75%, 50% and 25% w/w respectively. All groups received water ad libitum. Treatment lasted for twenty one days duration during which the weights of the animals were noted before and after the experiment. The animals were sacrificed after the duration of treatment, the liver excised, and fixed in 10% buffered formal saline and thereafter processed for routine Haematoxylin and Eosin staining. Results revealed significant difference ($P<0.5$) in the weights of the animals in group B and C compared with their initial weights. Also dose dependent histopathologic lesions such as portal congestion, chronic inflammation, periportal fibrosis and bile duct degeneration were noticed in the treatments; with group B recording the greatest severity. This suggests need for cautious use of the plant especially by practitioners of herbal remedy.

KEYWORDS: Crude Extract, Leaves, *Anchomanes Difformis*, Liver, Wistar Rats.

The use of plants as source of medicine is the oldest form of health care known to mankind. From the early times, man not only uses plant materials as source of food but also as source of medicine in an unrefined form (Trease and Evans 2001). The early man while living in close relation with its environment was conscious of nature's gift of medicinal plants which he used to alleviate his problems and bringing succour to his community. These medicinal plants have contributed to research and development of new drugs (Bonati 1993).

Anchomanes difformis is a large herbaceous plant that is usually found in the forest of West Africa, with stout prickly stem (leaf-petiole), two metres (2m) bearing a huge much divided leaf. The plant also has some ethnomedical uses. The rhizome is everywhere eaten in time of scarcity but only after special preparation (Busson 1965; Dalziel 1937, Irvine, 1952, Morton 1961) but this requires prolonged washing and cooking. A phytochemical analysis carried out on the ethanolic extracts of the leaf, stem and tuber revealed that the extracts of these three parts of the plant were found to contain; saponins, tanins, and alkaloids, Oyetayo (2007). Adegoke et al, (1968) had earlier reported that *Anchomanes difformis* contain strong alkaloids which had been used by many for several ages as medicine. In recent studies, extracts of various parts of medicinal plants were found to have broad spectrum antimicrobial activities against pathogenic organisms (Sudhakar et al 2006). The result of this study confirms the local use of extract of tubers soaked in water in treatment of dysentery by herbal practitioners. The secondary metabolites have been proven to be medicinal in nature as they have various therapeutic effects essential to prevent diseases and maintaining the state of well being (Sujatha et al 1985). In Guinea the rhizomes are used to make rubefacients and vesicants for external application, and alternatives for internal medication, but care has to be exercised on account of the caustic nature of the sap (Dalziel 1937). In Ivory Coast the plant is considered to be a powerful purgative and is used to treat oedemas, difficult child-birth, as a poison antidote, and as a strong diuretic for treating urethral discharge, jaundice and kidney pains. For these, the root or the leaves and stems may be used (Bouquet and Debray 1974). Sap from the stem is used in Ghana as an eye-medicine (Dalziel 1937) and rhizomes from Ivory Coast have been reported to contain carbohydrates (77%), proteins (12%), fats

(0·6%), minerals (5%) etc., and a quantity of amino-acids (Busson 1965). A strong presence of alkaloids was found in Nigerian material (Adegoke et al 1968).

Its application in agriculture as a pesticide has also been investigated. It was evident in a research carried out by Akinkurolere (2006), when he investigated the efficacy of the crude stem extracts against the pulse beetle *Callosobruchus maculatus* (Fabricius). He found out that at the highest application rate, the plant extract provided good protection to grains stored for 90 days. Grain viability and water absorption capacity were also not affected by treatments with ethanol extracts of *Anchomanes difformis*.

The liver is the largest gland in the human body and it is present in vertebrates and some other animals. It plays a major role in metabolism and has numerous functions ranging from detoxification, protein synthesis, glycogen storage and decomposition of red blood cells. Considering the major role of liver in metabolism, this present study was designed to assess the histopathologic effects of the crude extract of *Anchomanes difformis* on the liver.

MATERIALS AND METHOD

Collection and harvesting of *Anchomanes difformis*

Fresh leaves of *Anchomanes difformis* were harvested from bush farms around the Benin City province in Edo state, Nigeria; and identified using a hand book on West African weeds (Akobundu and Agyakwa 1987). The leaves were sun-dried and the dried leaves were finally blended into powder in an electric rotor grinder.

Experimental Animals

Twenty-four adult wistar rats of both sexes weighing between 200-250g were used in this study. The School of Basic Medical Sciences, University of Benin granted approval before the work began. The animal care and use ethics was in compliance with the Animal Holdings protocol overseen by the head of department through the Animal Holding unit. The animals were domiciled at the Animal house of the Department of Anatomy, University of Benin, under normal laboratory conditions of temperature light and humidity and were fed with rat pellets (grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria) and tap water during acclimatization.

Treatment Regimen

The animals were randomly categorized into four experimental groups A, B, C and D; of six rats per group (n=6). Group A animals received 100% feed mash and water ad libitum and served as the control group. Treatment groups: B, C and D animals received a mixed diet of feed mash and the crude extract of *Anchomanes difformis* at 75%, 50% and 25% respectively. The weights of the animals were noted before and after the experiment. Treatment lasted for twenty one days duration and after which the animals were sacrificed and the liver excised and fixed in 10% buffered formal saline.

Histology:

The tissues were dehydrated in ascending grades of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 5 microns thick were obtained using a rotary microtome. The thin sections were stained routinely with Haematoxylin and Eosin as adopted by Drury et al (1967).

Statistical Analysis

This was done using the Statistical Package for Social Sciences (SPPS). All data were expressed as Means + Standard Deviation of number of experiments. Test of significance was ascertained using Analysis of Variance (ANOVA).

RESULT

Body Weight

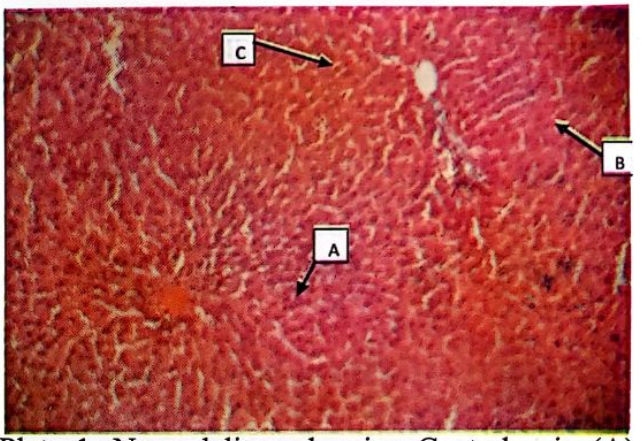
Weight loss noticed in animals appears to be dose dependent as group B and C animals significantly ($P<0.05$) experienced loss in weight, unlike the group D animals that had no significant difference in weight ($P>0.05$) after the experiment. Conversely, the control group A animals had weight gain at the end of the experiment.

Groups	Initial mean ± SEM (g)	Final mean ± SEM (g)
A	230.00 ±11.55	260.00 ± 7.07
B	255.00 ± 5.00	165.00 ± 11.90
C	225.00 ± 8.66	172.50 ± 7.50
D	225.00 ± 14.43	235.00 ± 20.61

Table1: Initial and final mean weight values between the groups of experimental animals and their standard errors. Data are an average of six (6) measurements.

Histologic Observations

Histological section of the liver of group A animals revealed normal liver with hepatocytes, and central vein. The sections of liver of the treated animals revealed distortions in the cyto-architecture of the liver tissue ranging from severe portal vein congestion, severe periportal fibrosis, interstitial periportal hemorrhage and chronic inflammations of the bile duct. The results also revealed these lesions to be dose dependent as the severity and distortions were noticed more in group B animals.Plate 1: Normal liver showing Central vein (A) Portal tract (B) and, Hepatocyte (C) [H&E x400].



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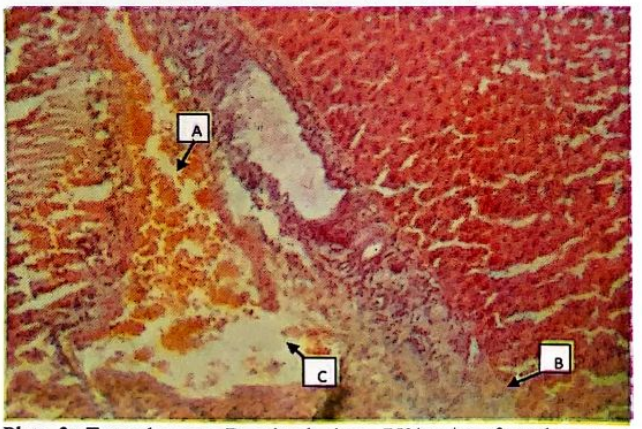

Plate 2: Treated group B animal given 75% w/w of crude extract showing liver with severe portal vein congestion (A), severe periportal fibrosis (B) and moderate inflammation (C) [H&E x400]

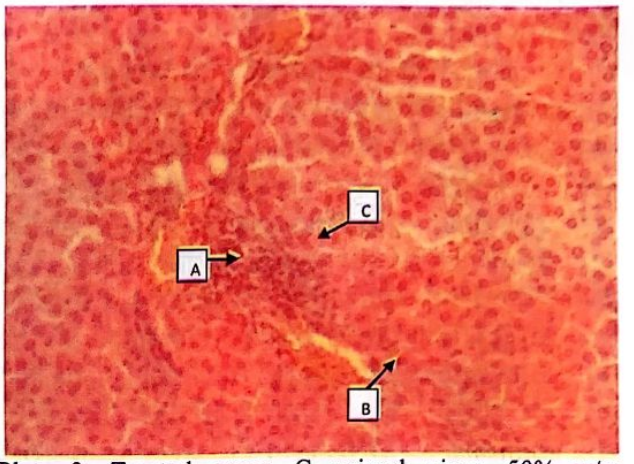

Plate 3: Treated group C animal given 50% w/w of crude extract showing liver with portal congestion (A) , interstitial periportal haemorrhage (B) and moderate chronic inflammation (C) [H&E x400]

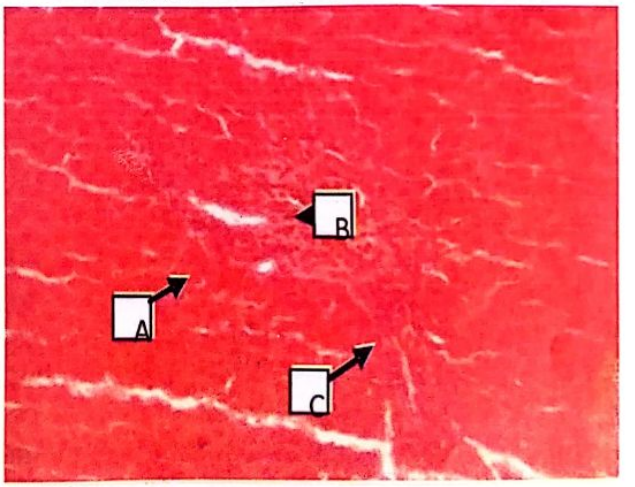

Plate 4: Treated group D animal given 25% w/w of crude extract showing liver with mild periportal fibrosis (A), portal congestion (B) and mild chronic inflammation (C) [H&E x400]

DISCUSSION

Treatment groups B and C experienced significant decrease in weight ($P<0.05$) when compared with their initial weights. However, the control group A animals had noticeable weight gain at the end of the experiment. Treatment group D animals experienced weight gain too after the experiment, though not significantly ($P>0.05$) as in group A. One might propound that the dose of the extract

75%, 50%, and 25% w/w for groups B, C and D respectively; could have altered the taste of the feed marsh and affected the appetite of the animals differently in a dose related manner. One cannot justifiably conclude that the herbal extract directly caused the weight differences as no previous study carried out, has suggested this. This further supports the possibility of the earlier submission concerning the effect of the extract on the appetite as being responsible for the weights of the animals noticed at the end of the experiment.

The liver is a very important organ in the body which serves in the detoxification of metabolic waste products, various drugs and toxins. It also destroys spent red cells and reclaims their constituents (Young and Heath 2004). Absorbed substances in the portal vein enter the liver sinusoids. If these substances are toxic, they may cause changes in the sinusoids and other parts of the liver (Kane and Kumar 2007). The results of the effects of *Anchomanes difformis* on the liver seen from the histological sections, suggests that the degenerative changes might be as a result of the increased metabolic demand on the liver by *Anchomanes difformis*. Thus, the histology of the liver sections from rats in the different treatment groups that were characterized by degenerative/necrotic and inflammatory changes might be indicative of onset of toxic process/stimulus in the liver (Butler 1996).

The results also revealed dose dependent degenerative changes as the treatment groups B and C showed the greatest severity of damage when compared with that of group D animals which received the lowest dose. This dose dependent toxicity explains why indiscriminate use of *Anchomanes difformis* should be discouraged. The fact that *Anchomanes difformis* could cause this in animals does not preclude its possibility of causing the same in humans because Range et al (1995) posits that toxic effects caused by a drug is similar in man and other animals; and that toxic effects can range from negligible to so severe as to preclude further development of the compound. Increased concentrations of active compounds in plant extracts are not always beneficial and can even promote adverse biological effect (Pepato et al., 2001). However it is noteworthy that natural medical products especially that of plant origin represents indispensible tools for the development of new drugs both in developing and industrialized nations (Bonati, 1993). This demands that toxicity testing in animals is highly valuable. Sofowora, 1993 reported that such testing is done to determine the upper limits of administration of such potential drugs. It is in-line with the fact that not all contents of plants' extract usually have such medicinal property (Kim et al., 1999) that this study recommends that *Anchomanes difformis* should be used with caution.

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