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Full Length Research Paper

Long term effects of aqueous stem bark extract of *Cissus populnea* (Guill. and Per.) on some biochemical parameters in normal rabbits

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***In vivo* clinical trials involving oral daily administration of crude aqueous extracts of *cissus populnea* to grouped sprague-dawley rabbits at two dose levels of 200 and 600 gkg^{-1} body weight over a 60 day study period revealed that continuous exposure of the plant extract had no damaging effects on the organs of xenobiotic metabolism (liver and kidney). results of levels of serum ast, alt, alkaline phosphatase, creatinine, bilirubin and triglycerides of the two groups were not significantly different ($p \leq 0.05$) at the end of the study from those of the control group.**

Key words: *Cissus populnea*, liver and kidney function, rabbits.

INTRODUCTION

Interest in medicinal plants as a re-emerging health-aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being, and the bioprospecting of new plant-derived drugs (Hoareau and Dasilva, 1999). A combination of this fact with the school of thought that believes that emergence/re-emergence of serious and costly infectious diseases might best be met with new anti-infective agents from traditional plant remedies (Nolan and Labbe, 2004) has greatly increased the awareness and use of herbals / traditional plant remedies. While medicinal plants and traditional medicine are integral parts of the health delivery system in developing societies like Nigeria where there is a heavy reliance on such, the developed countries too have in recent times turned to the use of traditional medicinal systems that involve the use of herbal drugs and remedies.

Many of our present medicines e.g. quinine, are derived directly or indirectly from higher plants. It is also incontrovertible that clinical plant-based research has made particularly rewarding progress in various significant fields of medicine, e.g. the use of taxoids and camptothecins in anticancer and artemisinin compounds in antimalarial therapies. In addition to purified plant-derived drugs, there is an enormous market for crude herbal medicines as dietary supplements, and for therapeutic purposes in both the developed and developing countries of the world (De Smet, 1997).

Cissus populnea is a plant associated with a myriad of medicinal uses in different parts of the world. Its extracts have been credited with antibacterial properties (Kone et al., 2004), as an anti-trypanosomal plant and a source of gum powder (Atawodi et al., 2002) and as a component of a herbal anti-sickling Nigerian formula (Moody et al., 2003). In Benin Republic, it is used for its diuretic properties while in Ghana it is used as a post-harvest ethnobotanical protectant (Belmain et al., 2000). The aqueous extract of its stem bark is associated with aphrodisiac / fertility potentials among the Yoruba-speaking people of South West Nigeria, where it is observed that men consume the aqueous and ethanolic extracts copiously and consistently for long periods of time either in mono or polyherbal formulations (Ojekale et al., 2006)). This use of various herbal remedies, including *C. populnea,* as an aphrodisiac and fertility enhancer amongst the males has been attributed to the declining fertility trend that has been established in this population over the years coupled with the attendant increasing levels of erectile dysfunction (Joint Report, 2004).

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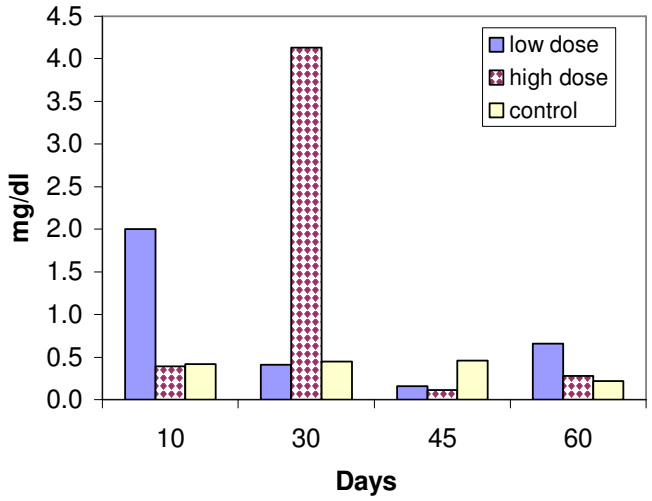

Figure 1. Serum levels of total bilirubin after long term exposure to aqueous extract of *Cissus populnea.*

There are relatively few reports in scientific literature that indicate potential or actual toxicity following the use of many herbs and where found, they are often single-case reports involving allergenic reactions or toxicity probably due to improper labeling, adulteration or an idiosyncratic reaction. There has however been a report (Geidam et al., 2004) of possible toxic effects of *C. populnea*, an otherwise promising herbal therapy.

The objectives of this study were therefore guided by the need to take a holistic look at this apparently useful plant by assessing the possible implications (if any) of a prolonged exposure of the physiological system to the aqueous extract using rabbits as model. Specifically, this study was designed to investigate possible resultant dysfunction of rabbit kidney and/or liver following an oral administration of aqueous stem bark extract of the plant.

MATERIALS AND METHODS

Plant material collection and extraction

Fresh stem bark samples of *C. populnea* were obtained from a commissioned local herbal practitioner in Oyingbo, Lagos State, South-Western Nigeria. The samples were authenticated at the Pharmacognosy Department of the College of Medicine, University of Lagos, Nigeria (Voucher number PCGLH-370). The bark samples were chopped into tiny bits, rinsed thoroughly and then blotted. The fresh, blotted, weighed samples were steeped in sterile distilled water at a concentration of 23 g/100ml for 72 h with constant stirring and then filtered. The resulting crude extract was refrigerated (4°C) until needed.

Study design

Thirty-six Sprague-Dawley rabbits with an average weight of 1.2 ± 0.4 kg were obtained from the animal house of the College of Medicine of the University of Lagos, Lagos, Nigeria. They were housed in plastic cages in a 12 h light/dark cycle and allowed to acclimatize prior to commencement of studies. Access to food and water was *ad libitum.* The animals were randomly arranged into 3 groups of 12 animals each and then labeled and treated as follows:

Group 1 (low dose group): Extract was administered (p.o using an oesophageal catheter) daily for the duration of the study at a dose of 200 mg kg^{-1} body weight.

Group II (high dose group): Extract was administered (p.o using an oesophageal catheter) daily for the duration of the study (60 days) at a dose of 600 mg kg^{-1} body weight.

Group III (control group): No extract was administered to this group throughout the study. The group had distilled water instead.

The individual weights of the rabbits in each group were monitored every five (5) days. Three rabbits randomly selected from each group for each stage of analyses were fasted overnight before being sacrificed and serum samples collected. Samples were collected on days 10, 30, 45 and 60. Sacrifice was achieved via chloroform anaesthesia as permitted by the University Ethical Committee.

Biochemical analyses

Blood obtained from each sacrificed animal by carotid bleeding into a centrifuge tube was allowed to clot. Serum samples were then obtained by centrifuging the clotted blood samples at 3000 rpm using a Wisperfuge 1384 Centrifuge for 10 min (Ojiako and Nwanjo, 2006a). These serum samples were then used for biochemical analyses. *Creatinine and bilirubin* were determined using the modified method of Henry (1974). *Triglyceride* and proteins were determined according to the methods of Tietz (1990). Aminotransferases (ALT and AST) and alkaline phosphatase were assayed using Randox kits and methods (1993).

Data analyses

Data values used for plotting graphs are means for all groups while graphs were plotted using MS Excel for Windows (XP Version). ANOVA was used in the determination of the differences in the levels of the different parameters measured using SPSS (Version 11). Differences at $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

The findings emanating from this study do not indicate the likelihood of the liver or kidney being under extreme stress (Rees et al., 2001). Creatinine levels were not significantly affected by the exposure of the animals to the extract (Figure 3), suggesting minimal or no wastage in muscle mass. This position is also corroborated by the fairly stable levels of blood proteins (Figure 4). The bilirubin (total and direct) levels (Figures 1 and 2) even indicate a protective effect of the extract on the liver.

Triglyceride levels which are independent risk factors for cardiovascular problems (Wierzbicki and Mikhailidis, 2002) were fairly stable in both the low- and high-dose groups throughout the study period (Figure 5). Though not clearly significant, the triglyceride level decreased as a consequence of the drug administered, confirming a hypolipaemic effect of the aqueous extract. Our observa-

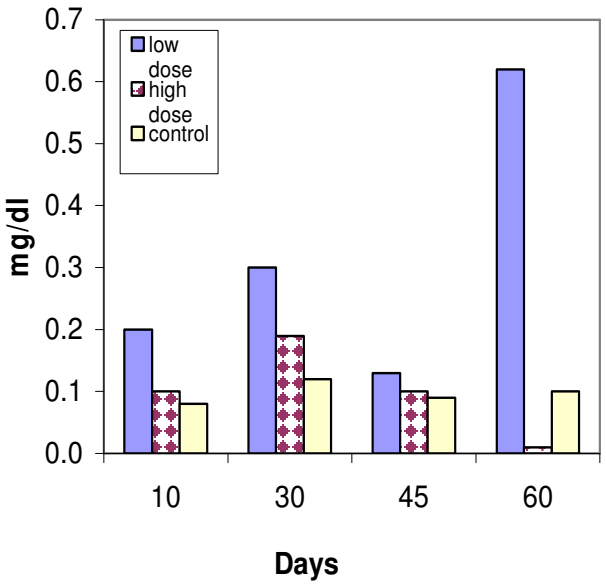

Figure 2. Serum levels of direct bilirubin after long term exposure to aqueous extract of *Cissus populnea*.

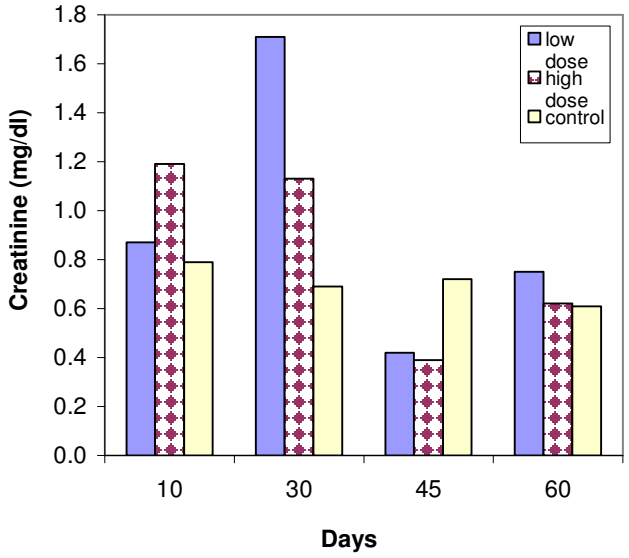

Figure 3. Serum levels of creatinine after long term exposure to aqueous extract of *Cissus populnea*.

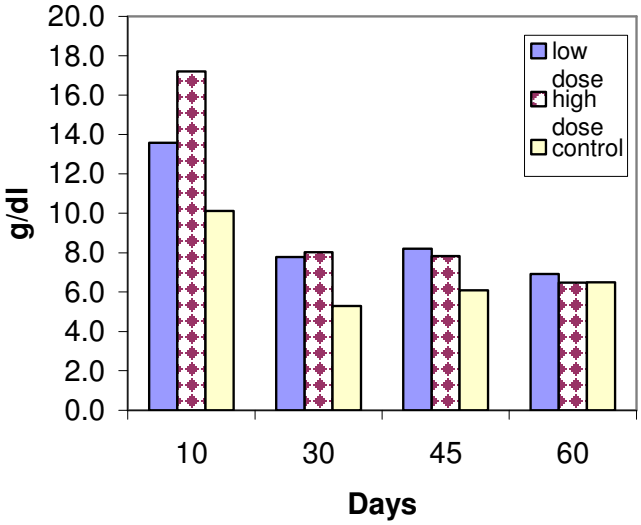

Figure 4. Bar chart showing blood levels of Protein after long term exposure to aqueous extract of *Cissus populnea*

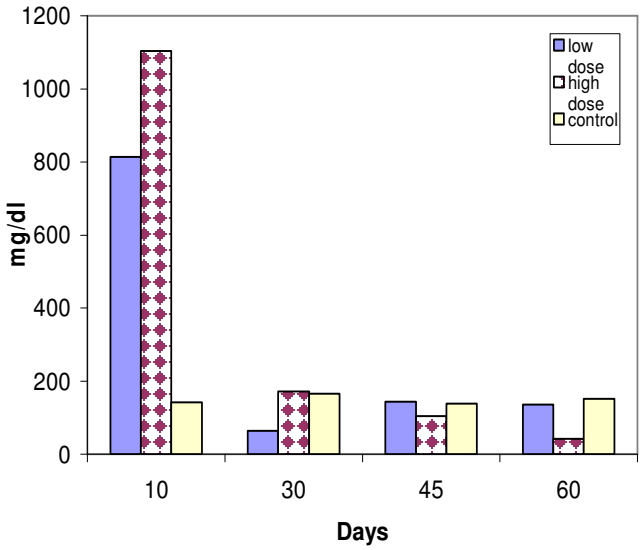

Figure 5. Serum levels of triglycerides after long-term exposure to aqueous extract of *Cissus populnea*.

tion is supported by another report (Viana et al., 2004) that a closely-related species *Cissus sicyoides* has both hypoglycaemic and antilipaemic effects. It is also supported by the findings of another research group (Oben et al., 2006) that statistically significant net reductions in weight and central obesity, as well as in fasting blood glucose, total cholesterol, LDL-cholesterol, triglycerides, and C-reactive protein were observed in participants who received a formulation containing *Cissus quadrangularis*, another closely related species.

The results of the diagnostic enzyme levels further support our observation that long-term exposure of the extract is safe especially with regards to the liver and kidney. The levels of alkaline phosphatase in the low-dose group throughout the duration of the study was fairly stable relative to the control while except on day 45; a similar observation was made even in the high-dose group (Figure 6). This finding does not agree with the conclusion by Geidam et al. (2004) that the aqueous extract of this same plant from the northern part of Nigeria elevated alkaline phosphatase levels in both normal and diabetic rats. A close look though, shows that their results properly interpreted, support our present finding because the enzyme level was significantly lower

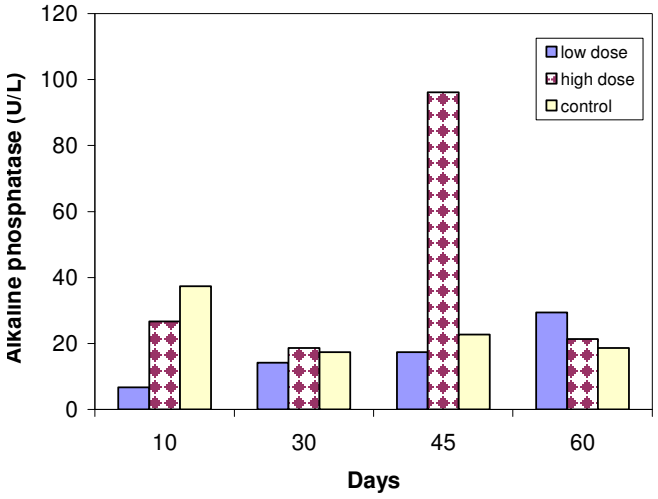

Figure 6. Levels of alkaline phosphatase in Rabbits after long-term exposure to aqueous extract of *Cissus populnea.*

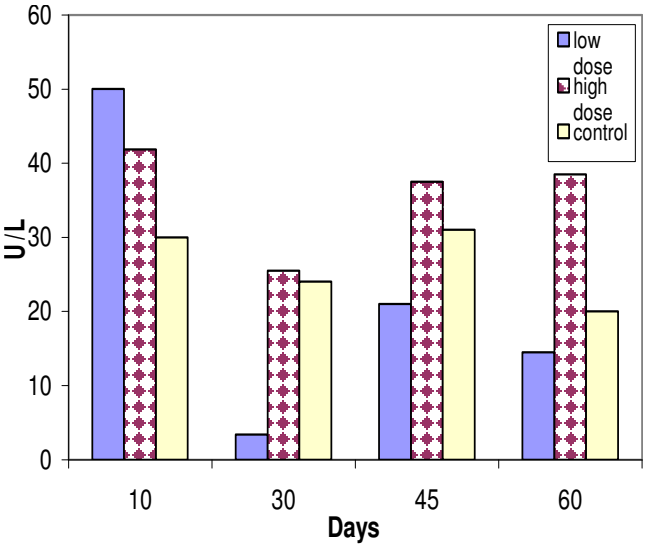

Figure 7. Serum levels of alanine aminotransferse (ALT) after long term exposure to aqueous extract of *Cissus populneas*

in the control animals that took the extract relative to the group that did not. So also was the case for the diabetic group that took the extract relative to the group that did not.

The levels of the aminotransferase enzymes ALT and AST (Figures 7 and 8) observed in the study corroborate the stand that the liver is not under stress especially with regards to the low-dose group. AST levels significantly ($p \leq 0.05$) lowered relative to the control group as time progressed. For ALT, though there was no clear pattern of decrease with time, the levels of the enzyme in the low-dose group from the 30th day to the end of study were significantly lower than those of the control group. The extract can therefore be said to be hepatoprotective and

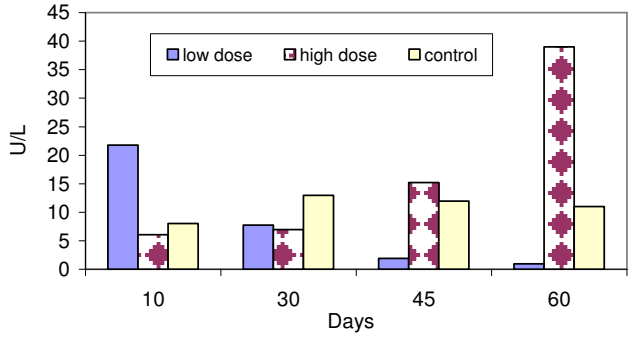

Figure 8. Serum levels of Aspartate aminotransferase (AST) after long term exposure to aqueous extract of *Cissus populnea*.

not hepatotoxic at low doses. This disagrees with the position that the extract is toxic (Geidam et al., 2004) and that it elevates the activities of the aminotransferases. The results of Geidam and colleagues properly interpreted, show that at least for ALT, there was a significant reduction in the level of the enzyme in the diabetic animals that took the extract relative to the diabetic group that did not and there was no difference in the levels of the enzyme between a control group that fed on the extract and another group that did not. Hepatotoxity could therefore not have been established from such a finding. The elevated levels of the enzymes in the high-dose animal groups rather suggest a physiological dysfunction arising from an overdosage. A common Nigerian vegetable *Vernonia amygdalina* with proven hepatoprotective effects has been shown to be hepatotoxic at very high doses (Ojiako and Nwanjo, 2006b).

It is an established fact that we have extremely limited knowledge about the ingredients in herbal medicines and their effects in humans (Chan, 1997), especially in polyherbal formulations, which are more in use than monoherbal ones. This is further compounded by the little understood synergism/mode of action attributable to herbal formulations that are known to be efficacious. The exact compounds responsible for the observed salutary effects of this plant extract are not yet clearly understood. Phytochemistry of the stem bark of the plant had earlier revealed such secondary metabolites as tannins, flavonoids, saponins and steroids (Ojekale et al., 2006). Some of these have been associated with functions related to fertility enhancement potentials (Das et al., 2004; Malini and Vanithakumari, 1991; Barry, 1985). Some of these may also be responsible for the observed effects but this will require further investigations.

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