TWIGS OF *Andrographis paniculata* (Burn. F) NEES ATTENUATES CARBON TETRACHLORIDE (CCl₄) INDUCED LIVER DAMAGE IN WISTAR ALBINO RATS

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ABSTRACT

This research's primary focus was to evaluate the hepatoprotective potentials of *Andrographis paniculata* in carbon tetrachloride-induced hepatotoxicity in Wistar rats. A total of thirty-six (36) Wistar rats were used for this study. The animals were shared equally into six (6) groups. Three of the six groups (1, 3, and 5) served as control and were given distilled water (1 ml/kg), Silymarin (50 mg/kg), and *Andrographis paniculata* (500 mg/kg) group. The administration duration was 28 days, after which they were given olive oil and normal saline (1 ml/kg). The other groups (2, 4, and 6) were treated similarly as 1, 3, and 5 for 28 days; after that, they were administered a single dose of carbon tetrachloride in olive oil on the 28th day. All animals were sacrificed on the 29th day after an overnight fast. Animal weights were assessed once a week during the research. Biochemical parameters were measured using the spectrophotometry method. Significant changes ($p < 0.05$) in the activities of hepatic markers (Alanine transaminase, Aspartate transaminase, Alkaline phosphatase), and concentrations of triglyceride and high-density lipoprotein in the carbon tetrachloride-induced group treated with *Andrographis paniculata* in comparison with group 2 (negative control) were observed. The outcomes demonstrated that the ethanolic extract of *Andrographis paniculata* has hepatoprotective properties against carbon tetrachloride-induced liver injury.

Keywords: *Andrographis paniculata*, Carbon Tetrachloride (CCl₄), Hepatoprotective, Liver Damage, Ethanol Extract

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INTRODUCTION

Carbon Tetrachloride (CCl₄) is a common hepatotoxicant used to study free-radical induced liver injury in animal models. It is utilized as a laundry agent, refrigerant synthesis precursor, lava lamps, vaporized jars propellant, fire quenching fluid, degreasing agent, and fumigating plants.¹ Its modern use has been to a great extent surrendered because of overall recorded unfavorable wellbeing impacts. CCl₄ is bio-transformed by cytochrome enzymes (CYP2E1, CYP2B1, or CYP2B2, and perhaps CYP3A) to the profoundly responsive trichloromethyl radical (CCl₃·), which results in hepatic damage through lipid peroxidation.¹⁻³ CCl₃· radical can bind to cellular biomolecules such as nucleic acid, protein, and lipid, damaging critical cellular processes such as lipid metabolism, with the possible effect of fatty degeneration (steatosis).¹ Liver zonation, which is the spatial separation of a large spectrum of different metabolic pathways along the Porto-central axis of the liver lobule, is essential for its function. Activated

CCl_4 radical ($\text{CCl}_3\cdot$) by the cytochrome enzymes induces a zonal expression causing a pericentral cell necrosis pattern.^{4,5} Significant hepatocyte necrosis prompts elevated ALT levels in the plasma within 60 minutes to activities higher than 10,000 U/L in less than two days after mice exposure to CCl_4 .⁶ Three days following CCl_4 introduction; plasma ALT action is decreased > 90% comparative with the 36 hours' level. Free of necrosis, evidence abounds that programmed cell death significantly induces hepatocyte death following CCl_4 introduction,⁶ but this mechanism contributes passively to CCl_4 -mediated cell death. *Andrographis paniculata* (AP) (Burm. F.) Nees (Family: *Acanthaceae*), popularly identified as King of Bitters, is a yearly herb having massive health benefits (the most part utilized parts are roots, leaves, and aerial part of the matured twig)⁷. The species is additionally revealed as a perennial shrub.⁸ This plant is commonly found in the tropics. The species is very much investigated medicinally and successfully utilized as an immunostimulant and for asthma, gonorrhoea, heaps, loose bowels and dyspepsia, blood cleansing, flu, gastric grumblings, looseness of the bowels, pharyngotonsillitis, fever, persistent cold, hair loss around the scalp, myocardial ischemia, diabetes, diseases of respiratory organs, jaundice among others.⁹ The species likewise has antiulcerogenic, antityphoid, antiplatelet accumulation, anti-HIV, antimalarial, antifertility, anti-inflammatory, and antihyperglycemic properties.¹⁰ Its secondary metabolites enhance the plant's importance in medicine, with the most prominent being Andrographolide.⁹ The plant extract has been used extensively in Asian traditional medicine due to its potency in treating various ailments and ameliorating the effects of venoms and poisons.⁷ Snakebites, cough, and insect stings, amongst others, are previous applications of this extract. Thus, the study was intended to bridge the gap in our continued efforts to establish the effects of local medications (*A. paniculata*) on CCl_4 -induced liver damage.

EXPERIMENTAL

Plant

A. paniculata twigs were collected at farmland in Ibadan, Nigeria, in July 2015. The botanical identification was carried out by Dr. J.O. Popoola, Biological Sciences Department, Covenant University, Ota, Ogun State, Nigeria. Authentication was done in the Department of Botany, Covenant University, Ota, Ogun State, Nigeria. The twigs (204.54 g) were air-dried, grounded, and soaked in ethanol (4000 ml, 80v/v) for 72 hours and filtered. The filtrate was concentrated using a rotary evaporator at 40 °C.

Experimental Animals

Experimental animals between the ages of 4-6 weeks old were obtained from the animal holding facility of Bells University, Ota, Ogun state. The animals were allowed to acclimatize to the animal house of Covenant University, Ota, for two weeks with free access to feed and water *ad libitum*. The animals were of an average weight of 150 g, and they were housed under standard experimental conditions, including 12-hour light and darkness. The animals' care and handling were by standard protocols.

Research Design

This research was designed to study the hepatoprotective effects of *A. paniculata* (AP) on CCl_4 induced toxicity. A modification of the hepatoprotective impact on rodents as portrayed by certain researchers was adopted.^{11,12} A total of 36 Wistar rats were used for the study. The animals were shared equally into six groups. Three of the six groups (1, 3, and 5) served as control groups and were given water (1 ml/kg), Silymarin (50 mg/kg), and AP (500 mg/kg), respectively. Consequently, the remaining three groups (2, 4, and 6) served as the treatment groups and were treated similarly as groups 1, 3, and 5. The duration of administration for all the groups was 28 days, after which groups 1, 2, and 3 were given normal saline in olive oil (1 ml/kg). In contrast, the other groups were administered a single dose of CCl_4 in olive oil on the 28th day. All animals were sacrificed on the 29th day after an overnight fast by the method previously reported by Ogunlana and colleagues.¹³⁻¹⁵

Biochemical Analysis

Blood samples were collected after sacrifice, and plasma samples were obtained using standard methods. Liver function biomarkers including alanine aminotransferase (ALT), aspartate aminotransferase (AST),

alkaline phosphatase (ALP), and lipid profile markers such as triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and cholesterol were carried out according to manufacturer's instructions (Randox Laboratories, UK). Total protein of the samples was assayed using the Lowry method as described by Noeman and others.¹⁶

Statistical Analysis

One-way analysis of variance (ANOVA) with SPSS 15 was used for the study. Data were represented as the mean $\pm$ standard error of the mean (SEM). The least significant difference (LSD) was utilized to contrast the difference between the methods for the groups of the *A. paniculata* treated rats at various doses with negative and positive controls.

RESULTS AND DISCUSSION

The weight of the animals increased progressively well throughout the experiment. Table-1 showed the relative organ weights of all experimental animals. AP did not have a significant effect on relative organ (liver and kidney) weights in all groups. Figure-1 showed the hepatoprotective effect of AP and Silymarin on liver function enzymes with ALP, ALT, and AST. All liver function enzymes considered were significantly increased in group 2 on the administration of CCl₄ (Fig.-1). The negative effect of CCl₄ was ameliorated by the hepatoprotective ability of the administered AP and Silymarin. There was a significant ($p < 0.05$) decrease in all liver function enzymes in groups administered with CCl₄ and treated with AP and Silymarin in comparison with negative control administered with CCl₄ untreated. However, there was no significant ($p > 0.05$) change in the groups' total protein concentration.

Table-1: Effects of *A. paniculata* (AP) And Silymarin on Organ Weight of CCl₄ Induced Hepatotoxicity

Group	Kidney	Liver
Control	0.086 $\pm$ 0.04	3.49 $\pm$ 0.10
CCl ₄	0.084 $\pm$ 0.04	4.08 $\pm$ 0.17
Silymarin (50 mg/kg)	0.092 $\pm$ 0.04	3.41 $\pm$ 0.14
Silymarin + CCl ₄	0.092 $\pm$ 0.02	3.28 $\pm$ 0.06
AP	0.084 $\pm$ 0.04	4.37 $\pm$ 0.20
AP + CCl ₄	0.083 $\pm$ 0.03	3.54 $\pm$ 0.17

Values are presented as mean $\pm$ standard error of mean (Mean $\pm$ SEM). Control, CCl₄, Silymarin, Silymarin+CCl₄, AP, and AP+CCl₄ represent groups (1 – 6) designated as Normal Control; administered with CCl₄ untreated (Negative control); treated with Silymarin (50 mg/Kg); administered with CCl₄ and treated with Silymarin; treated with AP; and administered with CCl₄ and treated with AP respectively.

The effect of AP extract on the lipid profile of CCl₄ induced hepatotoxicity is shown in Fig.-2. From the figure, there is a significant reduction in HDL-C of group 2 when compared with the normal control group and other groups. The treatment with Silymarin and AP significantly ($p < 0.05$) increases the HDL-C concentration in normal control and the treated groups. Also, CCl₄ induced-hepatotoxicity caused a significant ($p < 0.05$) increase in triglyceride concentration in group 2 compared to normal control and other treated groups. Only group 5 animals, not exposed to CCl₄ but treated with AP showed a significant decrease in the concentrations of LDL-C in comparison with negative control group 2. While groups 6 and 5 animals showed a significant change in the concentration of CHOL in comparison with both negative and normal control groups 1 and 2.

Carbon tetrachloride (CCl₄) is the most suited system of xenobiotic-induced hepatotoxicity and is frequently employed in animal models to study the hepatoprotective activity of drugs. It is metabolized in the body to a highly reactive trichloromethyl radical, which attacked the cell membrane by inducing a phospholipid-stimulating lipid peroxidation which results in cell lysis hence, resulting in organ dysfunction.¹² The effective functioning of the liver could be impaired since the metabolic organ is responsible for metabolizing various chemicals exposed to the system. Protecting the integrity of the liver from chemically driven liver injury is thus of importance. The use of herbs and plant products in treating various diseases has been on the rise due to part of such medicinal plants with little or no reported side effects.

Fig.-1: Effect of *A. paniculata* (AP) and Silymarin on liver function enzymes ALP, ALT, AST and TP of CCl₄-induced hepatotoxicity. Superscript 'b' indicates values significantly ($p < 0.05$) different from negative control group.

Control, CCl₄, Silymarin, Silymarin+CCl₄, AP, and AP+CCl₄ represent groups (1 – 6) designated as Normal Control; administered with CCl₄ untreated (Negative control); treated with Silymarin (50 mg/Kg); administered with CCl₄ and treated with Silymarin; treated with AP; and administered with CCl₄ and treated with AP respectively.

Fig.-2: Effect of the plant extract *A. paniculata* (AP) and Silymarin on lipid profile (HDL, LDL, TRIG, CHOL) of CCl₄-induced hepatotoxicity. Superscripts 'a and b' indicate values significantly ($p < 0.05$) different from normal and negative control groups respectively. Control, CCl₄, Silymarin, Silymarin+CCl₄, AP, and AP+CCl₄ represent groups (1 – 6) designated as Normal Control; administered with CCl₄ untreated (Negative control); treated with Silymarin (50 mg/Kg); administered with CCl₄ and treated with Silymarin; treated with AP; and administered with CCl₄ and treated with AP respectively.

The hepatoprotective property of *A. paniculata* leaf extract has been previously reported with the major component of the extract, Andrographolide is reported to have played a role even though it does not solely perform this function.¹⁷ CCl₄ induced hepatotoxicity from this investigation highlighted a significant elevation in liver function enzymes, thus clearly indicating liver injury. This increase is attributed to highly significant leakage of the liver function enzymes into circulation as a result of the injury. *A. paniculata* twigs significantly reduced the concentration of these enzymes highlighting their importance in ameliorating hepatotoxicity. The result obtained is similar to that of normal control and could confirm the hepatoprotective property of AP. Serum levels of ALT, AST, and ALP were significantly reduced as seen in previous studies.^{18,19} This could be as a result of prevention of hepatic damage, hepatic repair, and restoration of cellular permeability thus reverting hepatic injury.²⁰ Besides, the potent component present in the plant, Andrographolide, and other phytochemicals present could also be of importance in this reduction in levels of the enzymes.²¹

Also, significant changes in lipid profile in particular that of high-density lipoprotein (HDL) and triglyceride concentrations further confirm the hepatoprotective ability of the extract. As recorded in this study, hepatotoxicity resulted in a significant elevation in LDL, TRIG, and CHOL with a significant reduction of HDL. Lipid metabolism largely occurs in the liver. The liver plays a significant role in lipid metabolism. It is the main organ involved in the synthesis and active oxidation of fatty acids as well as lipid dissemination through lipoprotein synthesis.²² Lipid droplet aggregation into hepatocytes can bring about hepatic steatosis, which might be because of numerous hepatic dysfunctions. Hence, the functionality of the liver can therefore be assessed based on concentrations of lipoproteins and triglyceride. The significant changes observed in the concentrations of triglyceride, and HDL were in line with previous studies by reducing the levels of lipid markers such as low-density lipoprotein (LDL), triglycerides, and cholesterol.^{23,24} Ethanol extracts of *A. paniculata* twigs reduced lipids in CCl₄ induced hyperlipidemia, especially by reducing the blood levels of triglycerides and increasing the level of high-density lipoprotein.^{23,24}

CONCLUSION

This investigation showed that twigs of *A. paniculata* have hepatoprotective properties, which could enhance and justify its folkloric use as a hepatoprotective agent. However, there is a need to further highlight and underscore the molecular dynamics of the antilipidemic properties of *A. paniculata*.

Compliance with Ethical Standards

Ethical Approval: The authors carried out all procedures in the study involving animals according to the ethical standards of our institution.

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