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Hepatoprotective Effects of Ethanolic Extract of *Cnidoscolus aconitifolius* on Paracetamol-Induced Hepatic Damage in Rats

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Abstract: The study was designed to evaluate the possible hepatoprotective effect of *Cnidoscolus aconitifolius* on paracetamol poisoning in rats. Twenty five male Wistar rats were used in this study. They were divided into 5 groups of 5 rats. Groups I and II received normal saline (0.9% physiological saline). Animal in groups III-V were administered *Cnidoscolus aconitifolius* at 100, 500 and 1,000 mg kg⁻¹, respectively for 7 days. All animal in groups II-V were given paracetamol at 3 g kg⁻¹ by gastric gavage on days 8 and 9. Animals were sacrificed by cervical dislocation on day 10 after an overnight fast. Paracetamol overdose caused significant (p<0.05) increase in the plasma Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Blood Urea Nitrogen (BUN), triglycerides (TAG) with total cholesterol (TC) and Low Density Lipoprotein (LDL-cholesterol) and significant (p<0.05) decrease Total Protein (TP) and High Density Lipoprotein (HDL-cholesterol) in rats treated with paracetamol alone when compared with rats pre-treated with extract of *Cnidoscolus aconitifolius*. Pre-treatment with ethanolic extract of *Cnidoscolus aconitifolius* led to significant (p<0.05) decrease in serum ALT, ALP, AST, LDL and BUN when compared with the paracetamol treated rats in dose-dependent manner. The extract also similarly caused significant (p<0.05) increase in HDL values compared with paracetamol treated group. In conclusion, the results of this study demonstrated that *Cnidoscolus aconitifolius* can ameliorate paracetamol-induced hepatotoxicity. Significant hepato-protective activity was observed in rats treated with the dose of 1000 mg kg⁻¹ b.wt.

Key words: *Cnidoscolus aconitifolius*, clinical biochemistry, paracetamol poisoning, hepato-protection

INTRODUCTION

Recently, the use of medicinal plants to cure various forms of liver diseases and dysfunctions is becoming increasingly popular and has received wide acceptance. It has been reported that several hundred of plants have been examined for use in a wide variety of liver disorders but a handful has been fairly well researched (Luper, 1998). Liver diseases are the most serious ailments and are mainly caused by toxic chemicals (excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil (Maheswari *et al.*, 2008). Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue glutathione (GSH) levels. In addition, serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are also elevated in liver disease (Mascolo *et al.*, 1988).

Cnidoscolus aconitifolius is a perennial shrub belonging to the family *Euphorbiaceae*. It is commonly found in the tropic and sub tropical regions worldwide, including Africa, South of Sahara, North and South America, India, etc. It is commonly eaten as vegetable in soup (Ganiyu, 2005) in South Western Nigeria where it is called Iyana Ipaja. The edible parts of the plant provide important nutritional sources for protein, vitamin (A and C), minerals (calcium, iron and phosphorus), niacin, riboflavin and thiamine among populations that cannot afford expensive foods rich in these nutrients (Yang, 1979). *Cnidoscolus aconitifolius* shoots and leaves have been taken as laxative, diuretic, circulatory stimulant, to improve digestion, stimulate lactation and to harden the fingernails (Rowe, 1994). The high fibre content and antibacterial activities of this plant have been reported by various researchers including (Sarmiento-Franco *et al.*, 2002; Awoyinka *et al.*, 2007). Oladeinde *et al.* (2007) reported the anti-diabetic

properties of leaf extract of *Cnidioscolus aconitifolius* in inbred type 2 diabetic mice. Recently, the ameliorative effects of *Cnidioscolus aconitifolius* on anaemia and osmotic fragility induced by protein energy malnutrition has been reported by Oyagbemi *et al.* (2008).

Paracetamol, which is also known as acetaminophen is widely used as anti-pyretic and analgesic and produces acute liver damage if overdose is used. Pharmacologically, paracetamol is mainly metabolized in liver to excrete glucuronide and sulphate conjugates (Jollow *et al.*, 1974; Wong *et al.*, 1981). However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic Cytochrome P-450 (Savides and Oehme, 1983) to a highly reactive metabolite N-Acetyl-P-benzoquinone Imine (NAPQI) (Vermeulen *et al.*, 1992). The NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid (Moore *et al.*, 1985). However, when the rate of formation of this toxic metabolite exceeds the rate of detoxification by intracellular GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. The medicinal use of *Cnidioscolus aconitifolius* for the treatment of jaundice has been reported by Mendieta and del-Amo (1981) and Diaz-Bolio (1975). Therefore, this study gave insight to study the possible hepatoprotective effects of *Cnidioscolus aconitifolius* leaf extract against paracetamol-induced hepatotoxicity in rats since, there is association between jaundice and liver injury.

MATERIALS AND METHODS

Collection of plant materials: Fresh matured leaves of *Cnidioscolus aconitifolius* were collected at the University Teaching Hospital, College of Medicine, Ibadan and were identified and authenticated at Department of Botany and Microbiology, University of Ibadan. The leaves were air-dried, reduced to powder and were kept separately in airtight containers until the time of use. This study was conducted at the University of Ibadan, Department of Biochemistry, College of Medicine, Oyo State, Nigeria in 2008.

Extraction of plant material: Air-dried powder (1 kg) of fresh matured *Cnidioscolus aconitifolius* were extracted by percolation at room temperature with 70% ethanol (EtOH). Leaf extract of *Cnidioscolus aconitifolius* was concentrated under reduced pressure (bath temp. 50°C) and finally defatted with n-hexane. The extract was evaporated to dryness. The dried mass yielded 62.5 g.

Drugs and chemicals: Paracetamol (Pfizer, United Kingdom) was used in this experiment and all other chemicals are of analytical grade.

Animals: Forty inbred male Wistar rats weighing 220- 250 g were used for the study. The animals were maintained on a 12 h light and dark cycle, at 30±5°C, fed *ad libitum* with standard rat chow (ladokun feeds, Nigeria) and had free access to water. The rats were acclimatized to laboratory condition for 2 weeks before commencement of the experiment.

Study protocol: The rats were randomized into 5 groups comprising 5 animals each. Group I served as normal control, which received 10 mL kg⁻¹ b.wt. of water as vehicle once a day orally for 9 days. Rats in Group II received paracetamol overdose at 3 g kg⁻¹ b.wt. by gastric gavage as described by Kumar *et al.* (2006) only on days 8 and 9. Group III-V rats were pre-treated with the extract of *Cnidioscolus aconitifolius* at 100, 500 and 1,00 mg kg⁻¹ b.wt. for 7 days and paracetamol on days 8 and 9. Preliminary pilot studies showed that the plant extract was not toxic up to 3,00 mg kg⁻¹; this therefore guided us in the dose administered. The animals were fasted overnight and sacrificed on day 10 by cervical dislocation. All of the animals received humane care according to the criteria outline in the guide for the care and the use of laboratory animals prepared by the National Academy Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments (PHS, 1996).

Blood sample collection and clinical chemistry: Blood was collected from the rats through the retro-orbital venous plexus into plain tubes. The rats were first anaesthetized with ether to make blood collection easier. Whole blood was separated with high speed macro-centrifuge at 3,500 rpm for 10 min and serum was separated by Pasteur pipette for analysis of the following biochemical assays; Alkaline phosphatase (ALP) as described by Tietz and Shuey (1986), aspartate aminotransferase (AST), (Bergmeyer *et al.*, 1985), alanine aminotransferase (ALT) (Klaue *et al.*, 1993) albumin (Tietz and Shuey, 1986) total protein (Koller, 1987) and total bilirubin (Schlebusch *et al.*, 1995). Total plasma triglycerides were assayed by the peroxidase-coupled method (Buccolo and David, 1973) total plasma cholesterol was measured by the Allain enzymatic method (Allain *et al.*, 1974). High Density

Lipoproteins (HDL) cholesterol was determined after precipitation of LDL, very low-density lipoproteins and chylomicrons using MgCl₂ and dextran sulphate. The LDL-cholesterol concentration was calculated from the above data using the Friedewald formula (Friedewald *et al.*, 1972). Plasma Sodium (Na⁺), Potassium (K⁺) and Phosphate (PO₄²⁻) ions were determined by flame photometry. The concentration of K⁺ was calculated using the standard calibration method (Tietz, 1995b). Bicarbonate (HCO₃⁻) and Chloride (Cl⁻) anions were measured as described, respectively by Tietz (1995a, b) and Schales and Schales (1971).

Statistical analysis: One-way ANOVA with Dunnett's post test was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Values of p<0.05 were taken to be significant.

RESULTS

The results of Table 1 shows that administration of paracetamol caused a significant (p<0.05) increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) 48 h after paracetamol intoxication in comparison with the control. However, animals pre-treated with aqueous extract of *Cnidoscopus aconitifolius* significantly restored

the enzymes below levels that can cause hepatic damage. Similarly, serum albumin in paracetamol intoxicated animals was significantly reduced compared with the control and animals that were pre-treated with extract of *Cnidoscopus aconitifolius* (Table 1). Also, there was increase in serum total bilirubin of paracetamol treated group which was not significantly animals pre-treated with the extract (Table 1). Table 2 shows a significant (p<0.05) increase in total cholesterol, triglycerides and low density lipoprotein-cholesterol in paracetamol treated group compared with the control. Animals pre-treated with the extract significantly reduced the elevated lipid profiles in dose-dependent manner. Also, there was reduction in serum high density lipoprotein-cholesterol in paracetamol treated group compared with the control. Similarly, animals pre-treated with the extract significantly (p<0.05) reduced the elevated lipid profiles in dose-dependent manner. In Table 3, there was no significant difference (p>0.05) in serum potassium ions (K⁺), chloride anions (Cl⁻), bicarbonate (HCO₃⁻) and creatinine in paracetamol treated group compared with the control respectively. However, there was significant (p<0.05) increase in sodium ions (Na⁺) and blood urea nitrogen in paracetamol treated group compared with the control (Table 3). Elevated levels of these electrolytes and metabolites were reduced to near normal by the extract of *Cnidoscopus aconitifolius* in dose-dependent manner (Table 3).

Table 1: The effects of *Cnidoscopus aconitifolius* on biomarkers of hepatic damage

Groups and design of treatment	ALT	AST	ALP	TP	T.BIL	Albumin
	----- (IU L ⁻¹) -----				(mg dL ⁻¹)	(g dL ⁻¹)
Group I, control, 0.9% NaCl (5 mL kg ⁻¹)	62.4±3.40	115.00±5.23	152.40±5.17	8.18±0.65	0.68±0.22	4.30±0.48
Group II, PCM control (3 g kg ⁻¹)	144.33±2.35 ^a	216.50±6.76 ^a	368.20±8.48 ^a	5.74±1.84 ^a	0.82±0.34	2.62±0.41 ^a
Group III, 100 mg of CA+PCM (3 g kg ⁻¹)	85.00±2.53 ^b	172.30±4.61 ^b	262.00±8.30 ^b	6.70±1.19	0.74±0.30	3.44±0.47
Group IV, 500 mg of CA+PCM (3 g kg ⁻¹)	30.00±8.03 ^b	187.00±3.93 ^b	209.80±12.31 ^b	8.43±0.38	0.63±0.10	4.93±0.55
Group V, 1,000 mg of CA+PCM (3 g kg ⁻¹)	31.30±5.54 ^b	130.67±2.37 ^b	136.60±7.15 ^b	8.16±1.20	0.58±0.32	4.20±0.38

Values are Mean±SD. 5 animals in each group (n = 5). p<0.05 value are considered statistically significant. ^ap<0.05 paracetamol (PCM) compared with control and ^bp<0.05 rats treated with extract compared with paracetamol alone

Table 2: The effects of *Cnidoscopus aconitifolius* on lipid profiles

Groups and design of treatment	Total cholesterol	Triglycerides	HDL-cholesterol	LDL-cholesterol
	----- (mg dL ⁻¹) -----			
Group I, control, 0.9% NaCl (5 mL kg ⁻¹)	93.60±5.67 ^a	63.40±4.46	63.40±4.46	12.33±2.52
Group II, PCM control (3 g kg ⁻¹)	123.67±5.11 ^b	89.00±6.00 ^a	17.60±3.67 ^a	64.60±7.78 ^b
Group III, 100 mg of CA+PCM (3 g kg ⁻¹)	101.60±8.18 ^b	58.40±9.98 ^b	25.20±2.56 ^b	64.60±3.21
Group IV, 500 mg of CA+PCM (3 g kg ⁻¹)	98.00±4.27 ^b	54.80±3.10 ^b	25.60±4.09 ^b	33.00±7.38 ^b
Group V, 1,000 mg of CA+PCM (3 g kg ⁻¹)	61.20±4.36 ^b	52.00±4.31 ^b	23.00±4.68 ^b	12.33±2.52

Values are Mean±SD. 5 animals in each group (n = 5). p<0.05 values are considered statistically significant. ^ap<0.05 paracetamol (PCM) treated group compared with animals in control group. p<0.05 rats treated with the extract of *Cnidoscopus aconitifolius* compared with paracetamol treated group

Table 3: Effects of *Cnidoscopus aconitifolius* on serum electrolytes

Groups and design of treatment	Na ⁺	K ⁺	Cl ⁻	HCO ₃ ⁻	BUN	Creatinine
	----- (mmol L ⁻¹) -----				(mg dL ⁻¹)	(mmol L ⁻¹)
Group I, control, 0.9% NaCl (5 mL kg ⁻¹)	137.00±4.53	4.14±0.61	102.80±2.59	19.20±2.28	58.80±4.97	1.46±0.09
Group II, PCM control (3 g kg ⁻¹)	134.50±2.38 ^a	4.35±0.39	103.50±1.29	19.00±1.16	78.40±5.82 ^a	1.58±0.47
Group III, 100 mg of CA+PCM (3 g kg ⁻¹)	128.60±4.93 ^b	3.74±0.63	97.40±4.5 ^b	16.00±1.00 ^b	41.20±7.48 ^b	1.60±0.22
Group IV, 500 mg of CA+PCM (3 g kg ⁻¹)	131.20±1.30 ^b	3.90±0.70	103.60±2.19	21.20±2.17	32.40±3.72 ^b	1.52±0.16
Group V, 1,000 mg of CA+PCM (3 g kg ⁻¹)	113.22±5.54 ^b	3.89±0.54	102.00±4.95	19.00±2.35	16.00±1.39 ^b	1.34±0.44

Values are Mean±SD. 5 animals in each group (n = 5). p<0.05 values are considered statistically significant. ^ap<0.05 paracetamol (PCM) treated group compared with control. ^bp<0.05 rats treated with the extract of *Cnidoscopus aconitifolius* compared with paracetamol treated rats

DISCUSSION

Paracetamol is commonly and widely used analgesic- antipyretic agent. It is metabolized in the liver to an active metabolite, N-acetyl-p-benzoquinone imine (NAPQI), by the cytochrome-P-450 microsomal enzyme system with this resultant oxidative stress producing liver glutathione and glycogen depletion and hepatic necrosis (Hong *et al.*, 1992; Mitra *et al.*, 2000). Paracetamol is relatively safe when taken at prescribed therapeutic doses. Paracetamol-induced liver injury is commonly used as models for investigation into the efficacy of hepatoprotective drugs (Dixon *et al.*, 1971; Kumar *et al.*, 2006). The elevated serum liver enzymes such as ALT, AST and ALP in intoxicated rats can be attributed to the damage in the histostructural integrity of the liver cells (hepatocytes) (Kaplowitz, 2001).

It has been documented that covalent binding of N-acetyl-p-benzoquinone imine, an oxidation product of paracetamol, to sulphhydryl groups of protein resulted in cell necrosis and lipid peroxidation with concomitant decrease in glutathione levels in the liver (Jolow *et al.*, 1973; Handa and Sharma, 1990). In the assessment of liver damage by paracetamol, the determination of enzyme marker levels such as ALT and AST is often used. In necrosis or membrane damage, the enzymes are released into circulation and it can be therefore measured in serum as markers of hepatic damage. High levels of AST indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. The ALT catalysis the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury (Moss and Butterworth, 1974). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978). Similarly, serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis; in presence of increasing biliary pressure has been reported by Muriel *et al.* (1992).

In the present study, we have demonstrated the effectiveness of *Cnidoscolus aconitifolius* by administration of paracetamol over dosage in rats, which is a known model for both hepatic injury and GSH depletion. Present results using the model of paracetamol-induced hepatotoxicity in the rats demonstrated that *Cnidoscolus aconitifolius* at the different doses caused significant inhibition of ALT and AST levels. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary

pressure (Moss and Butterworth, 1974). Present results using the model of paracetamol-induced hepatotoxicity in rats demonstrated that *Cnidoscolus aconitifolius* at different doses caused significant inhibition of serum ALP and bilirubin levels. Effective control of bilirubin level and alkaline phosphatase activity has been described to point towards an early improvement in the secretory mechanism of the hepatic cell. The abnormal high level of serum ALT, AST, ALP and bilirubin observed in our study (Table 1) are the consequences of paracetamol induced liver dysfunction and denoted the damage to the hepatic cells. Treatment with *Cnidoscolus aconitifolius* reduced the enhanced level of serum ALT, AST, ALP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells. A reduction in Total Serum Protein (TSP) observed in the paracetamol treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and consequently decrease in the liver weight. However, when *Cnidoscolus aconitifolius* was given along with paracetamol, there was increase in TSP, though not significant indicating the hepatoprotective activity of extract and also accounting for the increase in the liver weight most probably through the hepatic cell regeneration.

Acute administration of paracetamol produced a marked elevation of the serum levels of SGOT, SGPT, ALP, total proteins and significant reduction in serum albumin in treated animals (Group II-V) when compared with control group (Group I). Treatment with *Cnidoscolus aconitifolius* at a dose of 100, 500 and 1000 mg kg⁻¹ significantly reduced the elevated levels of the enzymes. Treatment with *Cnidoscolus aconitifolius* decreased the serum levels of ALT, AST towards near normal values which is an indication of stabilization of plasma membrane as well as repair of hepatic tissue. Likewise, serum total cholesterol, triglycerides and low density lipoprotein levels were significantly increased in paracetamol treated group. This biochemical aberration was corrected in animals that were administered both paracetamol and ethanolic extract of *Cnidoscolus aconitifolius*. Further work is therefore needed to elucidate the precise mechanism of action of the plant on fat breakdown. Similarly, the significant reduction observed in serum values of High Density Lipoproteins (HDL-cholesterol) in paracetamol treated rats was elevated in experimental animals that received both the extract and overdose of paracetamol. The alteration in lipid profiles might result from accumulation of triglycerides, inhibition of bile acid synthesis from cholesterol which is synthesized in liver or derived from plasma lipids, leading to increase in

cholesterol levels (Recknagel, 1967). Therefore, suppression of cholesterol levels by the extract suggests that the bile acid synthesis was reversed. Paracetamol overdose also significantly altered serum electrolytes and metabolites. The alteration in serum electrolytes was not striking enough. This might be due to the time frame of the experiment. Blood Urea Nitrogen (BUN) was however abnormally higher in paracetamol treated group compared with control. This is indicative of injury to the nephrons (kidney cells). Interestingly, animals pre-treated with the plant extract significantly lowered the elevated BUN pointing to the nephroprotective effect of the plant extract. The protective effects of the extract was dose-dependent with the highest dose (1000 mg kg⁻¹) being more effective. To our knowledge, we are reporting for the first time the hepato-protective effect of *Cnidoscolus aconitifolius* on paracetamol poisoning in experimental animal models.

CONCLUSION

The results of this study demonstrated that *Cnidoscolus aconitifolius* has significant action on paracetamol induced hepato-toxicity and kidney damage. Moderate hepatoprotective activity was revealed in rats treated with of ethanolic extract of *Cnidoscolus aconitifolius* at the dose 1000 mg kg⁻¹ b.wt. This therefore, suggests that the hepatoprotective effects of *Cnidoscolus aconitifolius* may be due to its antioxidant and free radical scavenging properties. Detailed studies on the mechanism of action and phytochemical analysis are in progress at our laboratory for active constituents responsible for its hepatoprotective effect.

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