

Hepatoprotective Potentials Of *Hibiscus Rosasinensis* Petal anthocyanin Extracts Against Carbon tetrachloride-Induced Acute Liver Damage in Wistar Rats.

Onyesom I*. Mordi J. Opajobi AO. and ^aEsume CO.

ABSTRACT

Carbon tetrachloride (CCl₄) is haloalkane that possesses a hepatotoxic effect.

Material and Methods: The impact of anthocyanin fractions obtained from *Hibiscus rosasinensis* petal on carbon tetrachloride (CCl₄) – induced acute liver damage in wistar rats was studied using a combination of alanine transferase (ALT) activity value and liver: body weight gain ratio as indices.

Results: CCl₄ treatment significantly increased both ALT value and the liver: body weight gain ratio at the 1% probability level when compared with the control values.

Conclusion: Pre-treatment with the anthocyanin fractions reduced the levels of these markers and hence, the degree of liver damage, though with varying potentials. The lead precipitated, non – slimy red fraction possessed the greatest protective property on the rat liver when compared with the other anthocyanin fractions so tested.

KEY WORDS: Anthocyanin, carbon tetrachloride, alanine transferase, *Hibiscus rosasinensis*, liver damage.

Carbon tetrachloride (CCl₄) is haloalkane that possesses a hepatotoxic effect. The hepatotoxicity has been extensively studied and its proposed mechanism involves initial reductive dechlorination of carbon tetrachloride (CCl₄) to trichloromethyl radical ([•]CCl₃) which subsequently precipitate membrane lipid peroxidation and hence liver damage¹. Investigations have demonstrated that the liver injury produced by CCl₄ could be prevented or greatly modified by pre-treating animals with various antioxidants².

Anthocyanin, a water-soluble glycoside of anthocyanidins, is a plant pigment. They are part of the C15 phenolics known collectively as flavonoids with the typical A-ring benzoyl and B-ring hydroxycinnamoyl system.

Anthocyanins are used in industries mostly as colourants and flavour enhancers. However, they serve protective functions in plants, hence speculated to have antioxidant properties³. This tendency may be due to their phenolic structure which shows antioxidant activity towards a variety of easily oxidizable substances and might be part of anthocyanin defense mechanisms against free radical mediated damage⁴.

This study reports the protective capacities of partially purified extracts of *H. rosasinensis* petal anthocyanin against carbon tetrachloride-induced liver damage.

Materials and Methods

Animals: Forty-nine (49) adult albino rats (Wistar strain) with an initial mean weight of 154±16g (135-170g) were used for the study. The rats were obtained from the Animal Unit, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

Collection of Hibiscus Flowers: Hibiscus flowers (*Hibiscus rosasinensis*) were collected from the environment in University of Benin, Ugbowo, Benin City.

* . Department of Medical Biochemistry,

a . Department of Pharmacology

Delta State University, Abraka, Nigeria.

Extraction and Purification of Anthocyanins:

Anthocyanins were extracted from flower petals and purified as previously described⁵. The extracts obtained from the stepwise purification techniques were respectively labelled: AN₁, AN₂, AN₃, AN₄ and AN₅. AN₁ is the crude anthocyanin extract and AN₂, the lead precipitated extract, while AN₃ is the 12 x 3.5cm sephadex G-50 column fraction. AN₄ and AN₅ are 50 x 10cm sephadex G-50 column fractions 1 and 2, respectively. AN₂ was obtained from crude, while the column fractions were obtained from AN₂.

Treatment of animals and anthocyanin administration:

The rats were allowed to acclimatize to the feed and laboratory condition for 7 days. Thereafter, they were divided into seven experimental groups with seven rats each. They were provided with feed (rat pellets) and clean water *ad libitum*.

Rats in groups 1 and 2 were given 0.25ml (5%) ethanol/100g body weight, 5 days a week for four (4) weeks. Rats in groups 3,4,5,6 and 7 received a solution of AN₁, AN₂, AN₃, AN₄ and AN₅ extracts respectively, in 5% ethanol (1:1v/v) at a dose of 0.25ml/100g body weight. All administrations were given orally by intubation.

Administration of Carbon Tetrachloide (CCl₄):

After the 4 – week anthocyanin treatment period, the rats in groups 2, 3, 4, 5, 6 and 7 received 0.6ml CCl₄ (in corn oil, 1:1v/v) per 100g body weight by subcutaneous injection after a 12-hour fast. The animals were then sacrificed after 18 hours of the CCl₄ treatment. The dosing regimen and the administration method were based on previous experience⁶.

Collection of Blood and Liver Samples:

Each rat was anaesthetised in chloroform saturated chamber and the abdominal and thoracic regions were surgically opened to expose the liver and the heart. Whole blood sample was then collected from the heart using 5ml hypodermic syringe and needle into lithium heparinized sample bottle. After leaving to stand on ice for few minutes, the sample was centrifuged at 1200 x g for about 5 min at room temperature in order to separate the plasma which was collected and stored frozen in bijoux bottle. Analysis was done within 48 hours of collection. The liver was also excised, washed and weighed fresh.

Analysis of specimens:

Plasma Alanine transferase (ALT) activity value was determined by a colorimetric method⁷, using commercially available reagent test kit supplied by Quimica Clinica Aplicada, S.A., Spain.

Statistics:

Analysis of variance (ANOVA) was used to compare group values, followed by Newman-Keuls post-hoc test⁸ to determine statistical significance between the groups. Differences were considered significant when P < 0.05.

Results:

The results obtained from the study were shown on Tables 1 and 2. Table 1 presents the changes in plasma alanine transferase (ALT) activity values for the various experimental groups. From Table 1, it can be observed that carbontetrachloride (CCL₄) treatment significantly (P < 0.01) increased ALT activity value by over 700% when compared with the control value.

Table 1: Changes in mean plasma alanine transferase (ALT) activity values for the different experimental groups.

Group	Treatment	ALT Activity (IU/L)	Percentage mean increase compared with G1	Percentage mean decrease compared with G2
G1	Control	61.89±1.51 ⁺	-	88.08
G2	CCl ₄	519.23±54.34*	738.95	-
G3	AN ₁ + CCl ₄	190.00±15.97 ⁺	207.00	63.41
G4	AN ₂ + CCl ₄	161.30±59.52 ⁺	160.62	68.93
G5	AN ₃ + CCl ₄	225.40±50.07	264.19	56.96
G6	AN ₄ + CCl ₄	226.40±51.01*	265.81	56.91
G7	AN ₅ + CCl ₄	327.54±65.60*	425.23	36.92

ALT activity values are expressed as mean±SD of seven rats per group.

*Significantly different from the control value ($P < 0.01$)

⁺Significantly different from the CCl₄ – treated (G2) value ($P < 0.01$)

AN₁ – AN₅ represents the various fractions of the anthocyanin extracts:

AN₁: Crude (slimmy red) extract

AN₂: Lead precipitated (non – slimmy red) extract

AN₃: 12 x 3.5cm Sephadex G-50 column fraction.

AN₄: 50 x 10cm Sephadex G – 50 column (single red pigment) first fraction.

AN₅: 50 x 10cm Sephadex G-50 column (blue-violet pigment) second fraction.

Table 2: Mean Liver: Body weight gain Ratio

Group	Treatment	Liver weight (g)	Initial body weight (g)	Final body weight (g)	Weight gained (g)	Liver: Body weight gain ratio
G1	Control	3.44±0.99	156.00±21.92	175.00±12.91	19	0.1811
G2	CCl ₄	4.42±0.34	165.50±1.59	165.50±1.41	0.5 ⁺	8.8400*
G3	AN ₁ + CCl ₄	3.76±0.99	149.30±10.73	173.10±9.04 ⁺	23.8	0.1580
G4	AN ₂ + CCl ₄	3.59±0.14	162.50±18.65	189.00±13.25	20.5	0.1751
G5	AN ₃ + CCl ₄	3.77±0.23	136.00±47.71	176.20±26.65	40.2*	0.0938 ⁺
G6	AN ₄ + CCl ₄	4.03±0.22	154.00±4.42	181.70±5.13	27.7	0.1455
G7	AN ₅ + CCl ₄	3.82±0.91	155.80±7.79	188.90±5.44*	33.1	0.0202 ⁺

Weight values are expressed as mean±SD of seven rats per group.

* Significantly ($P < 0.01$) higher than the control value.

⁺Significantly ($P < 0.01$) lower than the control value.

This large increase suggests hepatic injury. However, rats that were challenged by the anthocyanin extracts had between 160-425% increments in ALT activity value, an evidence of a measure of hepatoprotection. The lead precipitated extract (AN₂) possessed the highest degree of protection and the 50x10cm Sephadex G-50 column second extract (AN₅) the lowest, as judged by their ALT values.

Table 2 shows the liver weight: body weight gain ratio. The group of rats challenged with

12x3.5cm Sephadex G-50 column fraction (AN₃) gained the highest weight followed by the group given the 50x10cm Sephadex G-50 column second fraction (AN₅). The liver weight: body weight gain ratio for the CCl₄ treated group had the highest value, indicating that CCl₄ increased liver weight – an evidence of inflammation, with very minimal gain in body weight. The liver weight: body weight gain ratio for the rats that received

AN₂ compares well with the control value. AN₂ extract appears to have the highest potential of protecting the liver against CCl₄-induced toxicity.

DISCUSSION

Measurement of plasma ALT activity value has been used as an index of acute liver damage induced by CCl₄ exposure. Koji, *et al.*⁶ observed a significant (P<0.01) increase in serum ALT activity value (from 37 IU/L to 553 IU/L), 24 hours after administration (subcutaneous injection) of 0.6ml/kg body weight of a mixture of carbon-tetrachloride (CCl₄) in olive oil (1:1 v/v) to 12-hours fasted rats. They attributed such outrageous increase to acute liver damage occasioned by CCl₄ toxicity. In this present study, the significant (P<0.01) increase (738.95%) in ALT activity value (519.23 IU/L) for the CCl₄-treated rats (Table 1) further confirms the hepatotoxic potential of CCl₄. But, pre-treatment with anthocyanin extracts reduced the significant increase in plasma ALT activity induced by CCl₄ administration. However, the various fractions of the anthocyanin extract differ in their ability to protect the liver from CCl₄ acute damage as evidenced by the ALT activity values (Table 1). The AN₂-lead precipitated, non-slimmy red extract possessed the greatest protective property, while AN₅ – the sephadex second fraction, showed the least protecting ability. Similarly, *Ficus carica* leaf extract has been observed to possess a measure of hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity in rats⁹.

The liver: body weight gain ratio for the CCl₄-treated group was statistically demonstrated to be the highest (P<0.01), suggesting that CCl₄ toxicity increased liver weight (size) but significantly reduced the gain in body weight by mechanism(s) that have not been clearly established.

Carbon tetrachloride could damage the liver via injuries caused by free radical (·CCl₃) - mediated lipid peroxidation¹⁰. From the available data, it appears that the lead

precipitated, non-slimmy red extract of anthocyanin, AN₂ possesses the highest antioxidant potential. The AN₂ fraction should be characterized and studied for its potent antioxidant property against acute liver damage caused especially by CCl₄ and allied substance(s).

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