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Defensive Influence of Methanol Fraction and Crude Extract of Cocoplum (*Chrysobalanus icaco* L.) on Nickel Toxicity in Male Wistar Rats

***STEPHEN-ONOJEDJE, OQ; ASAGBA, SO; KADIRI, HE**

Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Delta State, Nigeria

**Corresponding Author Email: obaroqueen2022@gmail.com; Tel: +2347036857003*
Co-Authors Email: asagbabch@yahoo.com; hekad@yahoo.com

ABSTRACT: The objective of this study was to investigate the defensive influence of methanol fraction and crude extract of cocoplum (*Chrysobalanus icaco* L.) on nickel toxicity in male Wistar rats. Biochemical analyses in the serum and tissues (liver and kidney) were carried out using standard procedures. Significant ($p < 0.05$) increase were observed in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, urea, fragmented deoxyribose nucleic acid (DNA), and decrease in albumin and total protein in serum and tissues of Group 2 when compared with Group 1. The oral administration of *C. icaco* methanol fraction and crude extract significantly decreased AST, ALT, ALP, creatinine, urea, fragmented DNA and increased albumin and total protein in the serum and tissues when compared with Group 2. The histology of various organs (liver and kidney) of Group 2 revealed inflammation of portal vein and degeneration of hepatocytes. The renal tubules showed inflammation of glomerulus and tubular cell. The administration of *C. icaco* extract markedly reduced the histological alterations of various organs studied. However, these efficacies were shown to be better in the methanol crude extract than the methanol fraction of *C. icaco*.

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Heavy metals are a noxious form of pollutants present in soil and water (De Oliveira and Tibbett, 2018; Osakwe and Okolie 2015). Heavy metals could build up in different body parts of humans where they cause disease conditions (Genchi, *et al.,* 2020; Asagba, 2010; Flora *et al.,* 2008; Asagba *et al.,* 2006; 2004). Nickel (Ni) metal has become of great interest because of its wide distribution in the environment. In other words, it has a wide variety of applications including metallurgical processes such as catalysis, coinage, foundry plating and electrical components (More *et al.* 2021; Kechrid and Bouhalit 2018; Luevano and Damodaran 2014; ATSDR 2005). Foods naturally high in Ni include chocolate, soybeans and nuts. High quantity of Ni has been reported to show various toxicities such as pulmonary, renal and cardiovascular effects (Schrenk *et al.,* 2020; Kang *et al.,* 2011). Carcinogenic and mutagenic effects of Ni has also been reported (Maha *et al.,* 2014; Sun *et al.,* 2016). The depletion of glutathione and other endogenous antioxidants may also contribute mainly for the development of nickel cytotoxicity threat (Gissi *et al.,* 2020; Chen *et al.,* 2003; Fidan and Dundar 2008). Antioxidants possess radical-scavenging properties; prevention of lipid peroxidation and other free radical facilitated processes (Ugwu *et al.,* 2021). Therefore, spices and herbs are regarded as valuable resources that may inhibit various disorders (Otuaga *et al.,* 2020a, 2020b), like hepatotoxicity (Achuba 2018; George *et al.,* 2015; Kadiri 2019 a, 2019b), lipid

abnormalities (Okonta *et al.,* 2021; Okpoghono *et al.,* 2024; Okpoghono *et al.,* 2018), malaria infection (George *et al.,* 2019) and erythrocyte osmotic fragility (Okpoghono *et al.,* 2021) other complications. Jamshidi-Kia *et al.* (2018) reported that medicinal plants are natural plant materials which are used at least or in the absence industrial processing for treatment of diseases. Those plants needed by pharmaceutical company's base on their active ingredients (Singh, 2015). *Chrysobalanus icaco* (*C. icaco*) is a spice commonly used by the Urhobo, Itsekiri, Izon and Ukwauni people in Niger Delta part of Nigeria. It occurs from Senegal to the Central African Republic and South to DR Congo and Tanzania. Vivien and Faure (1996) reported that it is found in South Maramagambo Forest. It probably also occurs in Uganda and it is found in mixed evergreen forest and riverine forest (Kadiri, 2019a; Davies and Zibokere, 2011). *C. icaco* is a medicinal herb used for different purposes such as therapeutic way to treat undesirable clinical conditions (Davies and Mohammed, 2011).The seeds can be used for the preparation of special soup for malaria fever and treatment of stomach disorder (Davies and Zibokere, 2011). Ndebia *et al.* (2007) reported that *C. icaco* is used in Cameroonian folk medicine for treating fever, diabetes, pain, skin diseases, sexual dysfunction and inflammation.

The toxicity of Ni to organs and tissues is a serious problem which have been reported (Kechrid and Bouhalit 2018). People may be exposed to Ni by smoking tobacco and wearing jewelry that contains Ni (ATSDR, 2005, Cempel and Nikel 2006). In some people, wearing jewelry that contains nickel produces skin irritation. Exposure to high levels of Ni compounds that dissolve easily in water (soluble) may also result in cancer (Cempel and Nikel 2006). In addition, although many studies have been done on the effects of Ni on the liver kidney and brain, the search for natural substances that can ameliorate such toxic effects is scarce in literature. The reality is that, orthodox medicine has not been able to fully manage metal induced organ damage (Sytar *et al.*, 2016). Thus, the search for natural products to curb metal toxicity is still on as researchers evaluate different herbs for their potential antioxidant properties in ameliorating toxicity of different environmental toxicants in experimental animals. This current study provides new insights into biochemical properties of *C. icaco* fractionated extract that could be of benefit in Ni toxicity. The findings of this study may help in the management of Ni toxicity in Wistar rats and human subjects that will be exposed to Ni toxicity knowingly or unknowingly. Tobacco smokers, inhabitants of industrial areas, occupational settings (for example, welding or soldering) in Nigeria and other parts of the world with significant source of Ni exposure will be enlightened on the protective properties or benefits of the intake of antioxidant enrich medicinal plant supplement in their food. Therefore, the objective of this study was to investigate the defensive influence of methanol fraction and crude extract of cocoplum (*Chrysobalanus icaco* L.) on nickel toxicity in male Wistar rats

MATERIALS AND METHODS

Chrysobalanus icaco: The fruits of *C. icaco* 'Gbofilo' were purchased from Igbudu main market Warri, Delta State, Nigeria.

Authentication of the plant: The plant *C. icaco* was authenticated in Department of Plant Biology and Biotechnology, University of Benin with herbarium number of UBH-C437.

Extraction of C. icaco: A portion of four hundred grams (400 g) of the pulverized Gbofilo kernels was suspended in 2 L of methanol for 48 hours in large amber bottles with intermittent shaking. The crude methanol extract was filtered using a muslin cloth and then concentrated in a water bath upheld at 45^0C. The crude was subjected to fractionation.

Fractionation of crude extract of C. icaco: The crude extract of *C. icaco* was subjected to liquid-liquid partition separation to separate the extract into different fractions.

The crude extract of 50 g was reconstituted with 250 mL of methanol, n-hexane, ethylacetate, n-butanol solvents and 250 mL of water 1:1 (v/v) separately in a separating funnel and then rocked vigorously. The sample was allowed to stand for 30 minutes for each of the solvents on the separator funnel until a fine separation line appear clearly indicating the supernatant from the sediment before it was eluted sequentially. The process was recurrent three times in order to get a satisfactory quantity for each fraction. The methanol, n-hexane, ethyl acetate, n-butanol and the aqueous residue fractions were evaporated to dryness in a water bath to obtain five fractions in (grams) respectively. Dimethyl sulfoxide (DMSO) was used as a solvent for the preparation of doses of various fractions.

Induction of nickel toxicity: Nickel was subcutaneously injected in the experimental rats at a dose of 20 mg/kg body weight using nickel sulphate

on alternate days (three time a week for four weeks) (Bordes and Papillion 1983).

Experimental Design: Thirty-five male rats were divided into five groups and each group was having seven rats and this were treated as follows:

Group 1: Control
Group 2: Nickel control
Group 3: Nickel plus DMSO
Group 4: Nickel plus 400 mg/kg body weight of methanol fraction
Group 5: Nickel plus 400 mg/kg body weight crude extract

The oral administration of *C. icaco* methanol fraction and crude extract to the nickel administered rats were carried out daily for a period of 28 days.

The dose of 400 mg/kg body weight extract was adopted because it is below the LD_{50} and was tolerable to the mice.

Blood collection and preparation of tissue homogenate: The rats were sacrificed after overnight fasting on the 29 day. The blood was collected by cardiac puncture using hypodermic syringe and needle and then transferred to an anticoagulant free test tube. After wards the clotted blood was centrifuged at 2,500 g for 15 minutes to separate the serum. One gram of various tissues were homogenized using 9 ml of normal saline under cold condition then centrifuged at 2,500 g for 15 minutes to obtain the supernatant which was stored in the refrigerator for further biochemical analysis.

Biochemical analysis: The following parameters are estimated as organ function markers:

Assay for Alkaline phosphatase (ALP) (EC.3.1.3.1): Alkaline phosphatase activities were assay for using the method of Kaplan and Righetti (1955). Steps 1, zero point five millilitres (0.5 ml) of alkaline phosphatase substrate (10 mmol/L of p-nitrophenylphosphate) was added in labelled test tubes and equilibrate to 37 ºC for three minutes. Step 2, at time interval 0.05 ml of each standard, control and sample was added to respective test tubes and mixed gently. Deionized water was use as sample blank and this was incubated for exactly ten minutes at 37 ºC. Following the same sequence in step 2, two point five millilitres (2.5 ml) alkaline phosphatase colour developer was added at time interval and mixed well. Absorbance was read against reagent blank spectrophotomerically at a wavelength of 580 nm.

Determination of Aspartate Aminotransferase (AST, EC 2.6.1.1): Aspartate aminotransferase activities were determined by the method of Reitman and Frankel (1957). Reagents were prepared according to standard procedures using commercially available diagnostic kits supplied by Randox Laboratories Limited, England. Into two test tubes labelled sample and blank were dispensed 250 µl of reagent 1 (100 mmol/L, pH 7.4 phosphate buffer, L-alanine 200mmol/L, and 2.0 mmol/L alpha-oxoglutarate). Into sample test tubes, 50 µl of samples were added and 50 µl of distilled water into blank then mixed and incubated for 60 minutes at 37 °C. Into each test tube, 250 µl of reagent 2 was added, mixed and incubated for 20 minutes at 20 – 25 °C. Into each test tubes, 2.5 ml of working NaOH reagent was added, mixed well and allowed to stand for 10 minutes. The absorbance were read against reagent blank at a wavelength of 540 nm.

Assay of Alanine Aminotransferase (ALT, EC2.6.1.2): Alanine aminotransferase were determined by the method of Reitman and Frankel (1957). Reagents were prepared according to standard procedures and instructions using commercially available diagnostic kits supplied by Randox Laboratories Limited, England. Into 2 test tubes labelled sample and blank were dispensed 250 µl of reagent 1 (100 mmol/L, pH 7.4 phosphate buffer, L-alanine 200mmol/L, and 2.0 mmol/L alpha-oxoglutarate). Into sample test tubes, 50 µl of sample was added and 50 µl of distilled water into blank, then mixed and incubated for 30 minutes at 37 °C. Into each test tubes, 250 µl of reagent 2(2,4-dinirophenylhydrazine) was added, mixed and incubated for 20 minutes at 20 – 25 °C. Into each test tubes, 2.5 ml of working NaOH reagent was added, mixed well and allowed to stand for 10 minutes. Absorbance was read against reagent blank at a wavelength of 540nm.

Determination of Albumin: The albumin in the serum and liver were estimated using the method of Doumas *et al.* (1971).Three milliliters (3 ml) of BCG regent was dispensed in test tube labeled blank, standard and sample. Ten microliters of sample were transfer to tubes. Spectrophotometry absorbance was at 580 nm after five minutes.

Determination of Total Protein: The total protein in the serum and liver were estimated using the method of Tietz (1976). One millilitres (1 ml) regent 1 (Biuret reagent: 100 mmol/L sodium hydroxide, 16 mmol/L Na-K-tartrate, 15 mmol/L potassium iodide and 6 mmol/L cupric sulphate) was added to test tube labelled blank, standard and sample, then 20 µl of

sample, blank reagent (100 mmol/L sodium hydroxide, 16 mmol/L Na-K-tartrate) and standard reagent (protein) was transferred to respective tubes and mixed well and allowed to stand at room temperature for 30 minutes. The absorbance was read at 546 nm against reagent blank.

Estimation of Urea: Urea was determined using the method of Henry (1974). The Urea Enzyme Reagent was reconstituted according to the manufacturer instructions and Urea Enzyme Reagent (1.5 mL) was pipetted into labelled test tubes, sample, blank and standard then allowed to equilibrate to room temperature. About 0.010mL (10µl) of each sample and standard reagent was introduced in respective tube. Water was used as the sample reagent blank. All tubes were incubated for five minutes at 37C. About 1.5 mL Urea colour developer (120mmol/L phenol and 27 mmol/L sodium hypochlorite) was added and mixed gently and then incubated for five minutes (5) at 37°C. The spectrophotometer was zeroed with the reagent blank at 630 nm. The absorbance of samples of all tubes were read and recorded.

Determination of Creatinine: Creatinine was determined using the method of Henry (1974)**.** The combined equal volumes of creatinine picric acid reagent and creatinine buffer reagent, was mixed well, and placed in respective test tubes which were labelled reagent blank, standard, and sample. Then 3.0 mL of working reagent was pipetted into the test tubes and 0.1 mL (100 µl) of sample and standard reagent were transferred to the respective tubes. Distilled water was added to reagent blank and mixed. The test tubes were transferred to heating bath at 37°C for fifteen (15) minutes. The wavelength of the spectrophotometer was then set at 510 nm and the instrument was zeroed with the reagent blank. The absorbance of all tubes was read and recorded.

Statistical analysis: All data were analysed using analysis of variance and the results were expressed in mean ±SD. Significant difference between means were determined at $p < 0.05$ confidence level using least significant difference.

RESULTS AND DISCUSSION

The results of body weight and weight gain of rats administered methanol fraction and crude extract of *C. icaco* for a period of 28 days are shown in Table 1 and 2.The significant ($p<0.05$) decrease in weight gain in the Ni control when compared with control, may be associated with considerable organ damage caused by Ni toxicity, which is in line with the study of Fadairo and Otite-Douglas (2015). The authors also stated that alterations in body weight gain are usually seen as toxicity indices. The results of the study also aligns with the reports of Timbell (1991).The work of Horiguchi *et al.* (1996) and also the work of Asagba *et al.*(2019), showed how Cd toxicity led to significant reduction in organs/ body weight ratio in rats. The increase in body weight gain of Ni administered rats treated with crude extract having the highest weight gain followed by the treatment with methanol fraction when compared with the Ni control, may likely indicates the ameliorating effects of the *C. icaco.*

Table 1. Body weight of rats administered methanol fraction and crude extract of *C. icaco* for 28 days.

Group	Day 1	Day 7	Day 14	Day 21	Day 28
1:Control	130.00 ± 19.10^a	132.00 ± 16.43^a	133.00 ± 15.65^a	136.60 ± 16.81^a	141.40 ± 18.88^b
2: Nickel control	135.50 ± 12.42^a	134.00 ± 5.47^a	135.08 ± 31.64^a	130.60 ± 30.81^a	127.40 ± 37.72^a
3: Nickel plus DMSO	138.00± IL92^a	136.00± 8.94^a	136.20 ± 9.I7^a	138.20 ± 7.56^a	14I.00 ± 8.66^b
4: Nickel plus methanol fraction	I30.00±2I.23^a	I32.00± I0.95^a	I3I.08± I0.77^a	I40.20 ± 30.49^a	I47.40 ± 28.52^c
5: Nickel plus crude extract.	121.00 ± 12.00^a	120.00 ± 14.00^a	121.08 ± 2.04^a	136.40 ± 4.15^b	138.60 ± 4.09^b

Values are represented in mean ± SD. n=5. Different superscript letter of mean values in the same horizontal row differ significantly at $p < 0.05$.

Table 2. Weight gain of rats administered methanol fraction and crude extract of *C. icaco* for 28 days.

Group	Initial body weight (g)	Final body weight (g)	Weight gain (g)
1:Control	130.00±I 9. I0^a	14I.40 ±I8.88^b	11 .40±2.05^a
2: Nickel control	135.50±I2.42^a	127.40±37.72^b	-8.10±0.03^b
3: Nickel plus DMSO	138.00± 11.92^a	141.00± 8'66^b	3.00± 0.23^d
4: Nickel plus methanol fraction	130.00 ±21.23^a	147.40± 28.52^c	17.40 ± 3.85^c
5: Nickel plus crude extract.	I2I.00±2.00^a	138.60±4.09^b	17.60±2.34^c

Values are represented in mean ± SD. n = 5. Different superscript letter of mean values in the same horizontal row differs significantly at $p< 0.05$

Table 3 shows the results of serum, liver and kidney AST, ALT and ALP of Ni administered rats treated with *C. icaco* methanol fraction and crude extract.The findings of this study demonstrated that Ni induces severe liver damage which is detected by increase in the serum levels of AST, ALT, and ALP. This was

seen as evidence demonstrated by the liver histology of Ni control and Ni plus DMSO rats showing inflammation (I) of portal vein (PV) (Fig. 1). Damage to tissues (such as liver and kidney cells) changes their functional integrity, and leads to the leakage of enzymes into extracellular space (Das *et al.,* 2018; Guo *et al.,* 2016; Kadiri 2019b; Okpoghono *et al.,* 2018). Treatment with *C. icaco* (methanol fraction and crude extract) largely modulated the severity of Ni-induced liver damage. Enzymes activities return to near-normal levels in treated rats which shows that *C. icaco* extracts can stabilize liver cell membranes and prevent the leakage of enzymes. However, the values, reduced most in the methanol crude extract in comparison with the methanol fraction. This may be due to loss of bioactive compounds during fractionation.

It is well known that albumin is among plasma protein synthesized by the liver. These results obtained for level of albumin and total protein in the serum, liver and kidney are in line with previous study illustrating decrease in serum total protein and albumin in rats due to Ni toxicity (Samir and Zine 2013; Bhardwaj *et al.,* 2021). The significant decrease in total protein and albumin level in Ni administered rats indicated that Ni toxicity had an effect on the synthetic function of the liver. This is in line with Singh *et al.* (2019) who stated that the decrease in albumin and total protein in the liver of Ni intoxicated rats suggest a possible liver cell damage or bile duct damage.

Table 3. AST, ALT and ALP activity in the Serum, liver and kidney of Ni induced toxicity in rats treated with *C. icaco* methanol fraction and crude extract.

Group		Serum			Liver			Kidney	
	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)
1:Control	40.90 ± 1.34^{a}	34.80 ± 3.99^{a}	140.42 ± 55.31^{a}	180.56 ± 8.39^{a}	62.80 ± 5.06^{a}	50.80 ± 3.27^{a}	162.62 ± 14.93^{a}	44.52 ± 3.94^{a}	31.90 ± 5.43^{a}
2: Nickel control	68.20 ± 4.80^{b}	55.90 ± 16.16^{b}	210.39 ± 17.56^{b}	255.26 ± 12.20^{b}	92.00 ± 7.50^{b}	76.92 ± 11.46^{b}	220.54 ± 14.19^{b}	71.00 ± 6.49^{b}	57.10 ± 5.77^{b}
3: Nickel plus DMSO	70.60 ± 9.81^{b}	52.80 ± 6.18^{b}	209.95 ± 36.65^{b}	255.65 ± 29.78^{b}	95.00 ± 4.06^{b}	77.60 ± 6.72^{b}	221.44 ± 18.80^{b}	69.80 ± 9.65^{b}	57.50 ± 4.48^{b}
4: Nickel plus methanol fraction	54.50 ± 4.98^{c}	38.80 ± 7.19^{a}	178.19 ± 4.29^{c}	230.58 ± 22.52^{c}	72.90 ± 5.94^{c}	56.10 ± 6.40^{a}	216.13 ± 5.32^{c}	59.80 ± 7.08^{c}	51.42 ± 5.84^{c}
5: Nickel plus crude extract.	39.40 ± 6.22^{a}	26.20 ± 2.86^{c}	160.70 ± 6.55^{d}	208.40 ± 11.47^{d}	55.10 ± 5.12^{d}	42.10 ± 7.55^{c}	158.01 ± 31.05^{a}	44.20 ± 5.31^{a}	40.80 ± 5.06^{d}

Values are given in mean ± SD. n=5. Mean values in the same column with different alphabet (a-d) differ at p<0.05.

Table 4. Effect of Ni induced toxicity on albumin and total protein levels in the serum and liver of rats treated with methanol fraction and crude extract of *C. icaco.*

Group	Serum Urea (mg/dL)	Kidney Urea (mg/dL)	Serum Creatinine (mg/dL)	Kidney Creatinine (mg/dL)
1:Control	9.91 ± 6.09^{a}	20.41 ± 3.25^{a}	2.07 ± .08^{a}	5.49 ± 2.65^{a}
2: Nickel control	22.00 ± 3.80^{b}	30.42 ± 4.60^{b}	8.62 ± 2.71^{b}	14.39 ± 3.68^{b}
3: Nickel plus DMSO	21.35 ± 3.79^{b}	30.60 ± 4.34^{b}	9.53 ± 1.27^{b}	13.75 ± 2.00^{b}
4: Nickel plus methanol fraction	15.66 ± 3.93^{a,c}	25.31 ± 3.80^{a}	5.30 ± 1.89^{c}	9.34 ± 3.68^{c}
5: Nickel plus crude extract.	11.30 ± 3.93^{a}	21.22 ± 2.02^{a}	3.54 ± .60^{a}	5.31 ± 1.56^{a}

Values are given in mean ± SD. n=5. Mean values in the same column with different letter differ at p<0.05.

Table 5. Effect of *C. icaco* extracts on the Creatinine levels in the serum and kidney of Nickel administered rats.

Group	Serum ALB (g/dl)	Liver ALB(g/dl)	Serum TP(g/dl)	Liver TP (g/dl)
1:Control	14.57 ± 4.06^{a}	20.00 ± 3.37^{a}	26.44 ± 3.46^{a}	33.62 ± 10.79^{a}
2: Nickel control	7.31 ± 1.53^{b}	8.73 ± 4.40^{b}	13.48 ± 2.80^{b}	17.08 ± 2.55^{b}
3: Nickel plus DMSO	7.49 ± 3.54^{b}	10.31 ± 1.53^{b}	14.16 ± 2.25^{b}	16.40 ± 1.97^{b}
4: Nickel plus methanol fraction	9.69 ± 3.04^{a,b}	13.22 ± 2.39^{a,b}	16.15 ± 3.50^{a,b}	21.33 ± 1.54^{a,b}
5: Nickel plus crude extract.	13.07 ± 2.36^{a}	19.35 ± 3.76^{a}	25.11 ± 2.87^{a}	28.85 ± 1.36^{a}

Values are given in mean ± SD. n=5. Mean values in the same column with different letter differ at p<0.05.

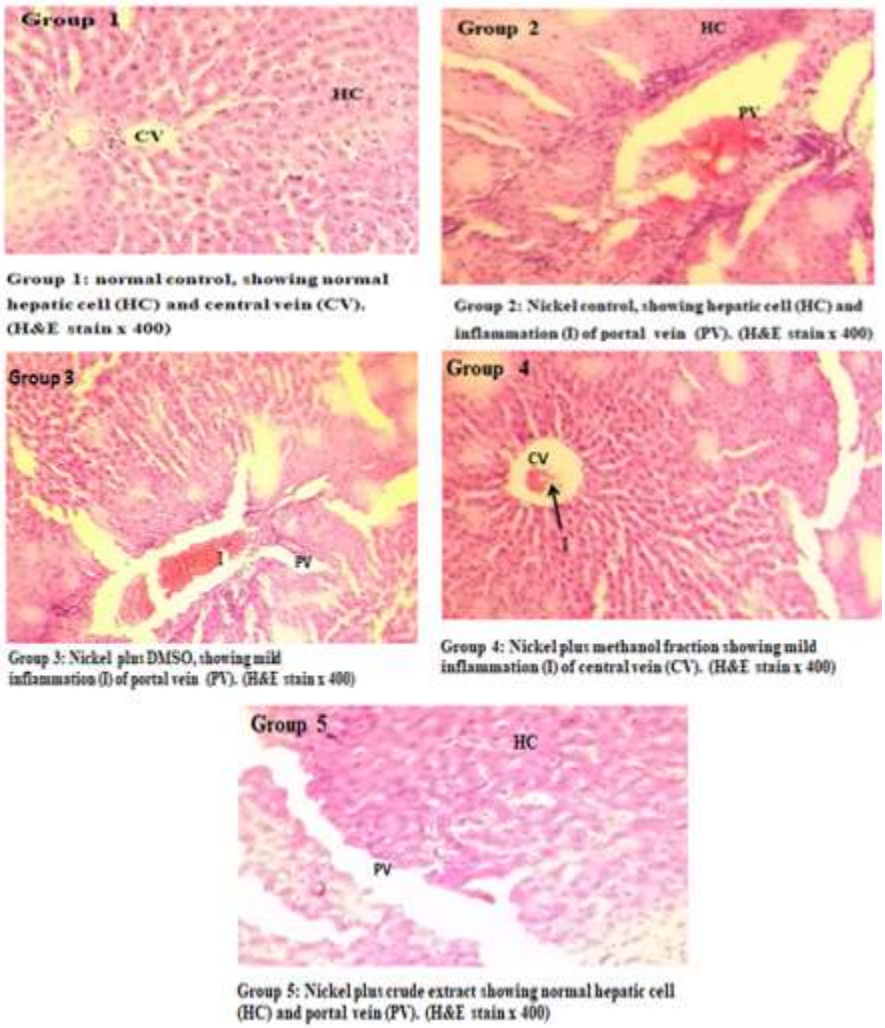

Fig. 1. Liver histology of rats administered with Ni treated with methanol fraction and crude extract of C. *icaco.*

The increase in albumin and total protein level after the treatment with methanol fraction and *C. icaco* crude extract in Table 4, indicates that the extracts can prevent liver damage probably through stabilizing endoplasmic reticulum and resynthesizing protein or through neutralizing ROS by scavenger compounds. This can also be as a result of antioxidative compounds presentt in *C. icaco* (Stephen-Onojedje *et al.,* 2023). Many dietary constituents and phytochemicals of medicinal plants are being investigated as antioxidants because of their protective effects (Guaadaoui *et al.,* 2014; Asagba *et al.,* 2019). Creatinine is a breakdown product of phosphocreatine in muscle, and is usually produced in amount proportional to muscle mass. Serum creatinine is a great significant indicator of renal health. Creatine is normally synthesized primarily in the liver and also in the kidney. Urea, on the other hand, is formed as means to rid body of nitrogenous waste from protein degradation. It is formed in the liver and excreted by the kidney in urine. Elevated plasma urea level has been linked to reduced renal function. The results in Table 5 of this study indicated significant ($p<0.05$) increase in urea and creatinine level in the serum and kidney of rats in Ni control and Ni plus DMSO compared to Ni plus methanol fraction, Ni plus crude extract and the control. However, the marked improvement detected in renal function after treatment with *C. icaco* supports the protective effect of *C. icaco* against Ni nephrotoxicity. Our results agreed with Seif et al. (2019) who reported hepato-renal protective effects of Egyptian *Purslane* extract against cadmium exposure. In our study, histological analysis in Fig. 2 revealed significant renal impairments such as inflammation of glomerulus (G) and inflammation of tubular cell in response to Ni intoxication. These results are in agreement with those of previous studies (Adi *et al.,* 2016, Wongmekiat *et al.,* 2018). Furthermore, Rafati *et al.* (2015) discovered Cd-induced glomeruli structural abnormalities, increases in the mesangial

matrix, and glomeruli inflammation with larger urine gaps. Cd treatment caused tubular malfunction as well as nuclear membrane damage in rat glomerular epithelial cells (Adi *et al.*, 2016). However, Gobe and Crane (2010) attributed the relationship between metals-intoxication and renal cell injury to the sensitivity of the proximal tubular epithelium to oxidative stress. Similarly, increased nitric oxide and ROS generation is related to renal injury and induces the progression to renal failure (Wongmekiat *et al.*, 2018). However, the decrease observed in creatinine and urea level in rats given methanol fraction and crude extract of *C. Icaco* may likely be due to the antioxidant compounds present in the *C. icaco.* Antioxidant maintain the normal architecture of the glomerulus and protect the kidneys of rats from nephrotoxicity (Yousuf and Vellaichamy, 2015).

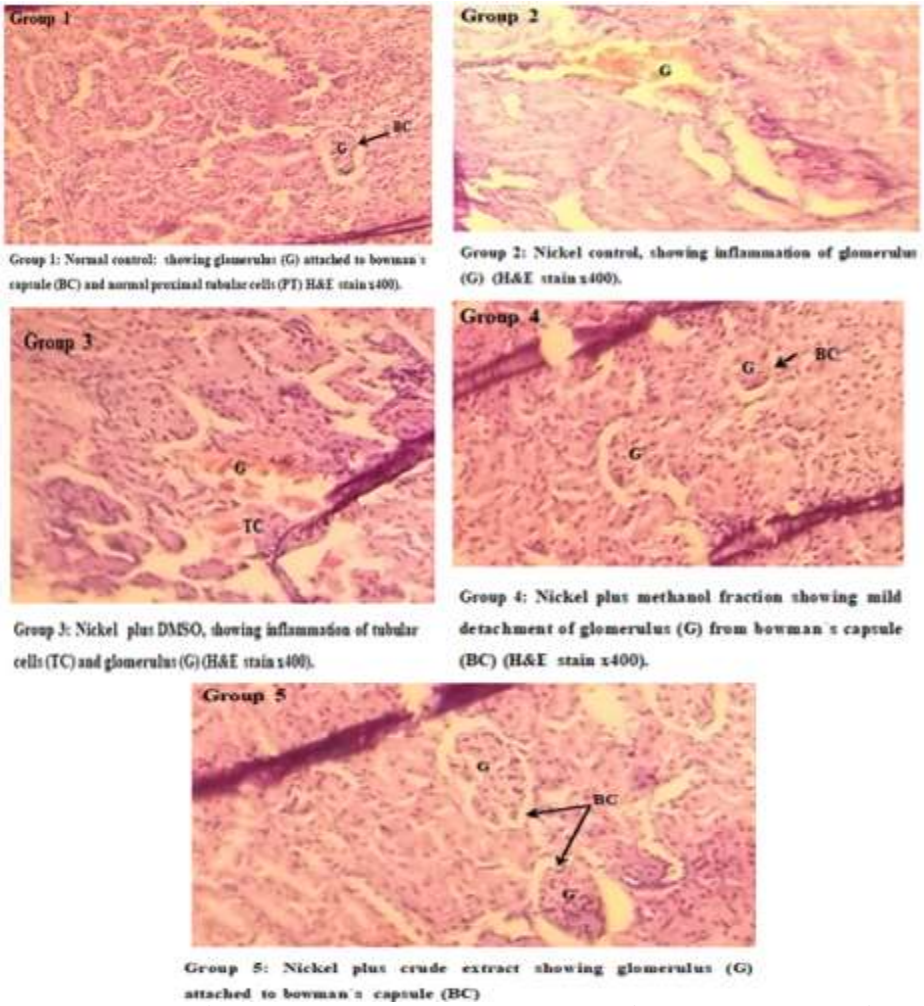

Fig. 2. Kidney histology of rats administered with Ni treated with methanol fraction and crude extract of *C. icaco.*

Table 6. Percentage of Fragmented DNA in the liver and kidney of Ni induced rats treated with methanol fraction and crude extract of *C. icaco*

Group	% Fragmented DNA	
	Liver	Kidney
1:Control	2.32 ± 0.74 ^{a}	2.28± 0.34^{a}
2: Nickel control	24.35 ± 2.82 ^{b}	23.40 ± 6.11^{b}
3: Nickel plus DMSO	23.63 ± 4.64 ^{b}	22.47 ± 4.54^{b}
4: Nickel plus methanol fraction	13.29 ± 5.84 ^{c}	10.31± 3.84^{c}
5: Nickel plus crude extract.	6.19 ± 2.56 ^{a}	4.50 ± 1.66^{a}

Values are represented in mean ± SD. n=5. Mean values with different superscript letter in the same column differ significantly at $p<0.05$.

In Table 6, the significant ($P < 0.05$) increase in fragmented DNA in the liver and kidney of Ni administered rats when compared with the control indicated in the present study could result indifferent forms of biological damage. Ni is highly toxic reactive substance that may cause oxidative damage to DNA **(**Guo *et al.,* 2019; Guo *et al.,* 2016; Das *et al.,* 2018; Dumala *et al.,* 2017**).** The cell damage observed in rats administered Ni in the present work might have resulted from these oxidative processes. Treatment with the methanol crude extract and fraction resulted in reduction of the generated electrophilic compounds, which is in association with DNA molecule sites that may cause several lesions during Ni toxicity. The crude extract had more effect when compared to methanol fraction, probably decrease of high concentration of bioactive compounds.

Conclusion: In conclusion, the outcome of the present investigation clearly demonstrated that Ni-induced toxicity may generate free radicals *in-vivo* and this could have led to alterations of enzymes, proteins and DNA molecule. This study has proved that methanol fraction and crude extract of *C. icaco* are beneficial in the amendment of Ni prompted damage. The defence against Ni-induced toxicity may be due to the scavenging of free radicals by methanol fraction and crude extract of *C. icaco*. However, these efficacies were shown to be better in the methanol crude extract than the methanol fraction of *C. icaco.*

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