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Ameliorative Effects of Turmeric Extract against CCL_4 Induced Liver and Kidney Injury in Adult Wistar Rat

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Authors' contributions

This work was carried out in collaboration between both authors. Author EUE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EUE and EA managed the analyses of the study. Author EA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Objectives: To examine and ascertain the toxic effects of CCL_4 on the histology of the liver and kidney and to investigate the ameliorative of turmeric against his injury.
Materials: Thirty (30) male adult Wistar rats weighing 150 ± 2.6 g - 317 ± 3.5 g were procured from the animal house of the Department of Pharmacology, College of Health Sciences Niger Delta University, Bayelsa state. Nigeria, assigned into five (5) major groups with five (5) animals in each group after the period of acclimatization: a control group "A" and three test groups (C, D and E) except group B with ten (10) rats. Animals in Group A (Control**):** received pelleted growers mash (feed) and water .Group B (Positive Control): Received CCl_4 only (0.5 mL/100 g). Group C**:** received CCL_4 (0.5 mL/100 g) and turmeric extract 200 mg/kg**. Group D:** Received 200 mg/kg of turmeric extract and then treated with CCL_4 (0.5 ml/100 g) **Group E:** Received 200 mg/kg of turmeric extract only, at the end of the treatment, the liver and the kidney of each sacrificed rat were processed for paraffin sectioning and stained with Harris hematoxylin and eosin.

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Result: Photomicrograph of Groups B, C and D show moderate inflammatory cells and fat infiltration which are features of hepatic injury but the result further shows that there were no noticeable histopathological changes observed in the histology of the kidney of all animal groups as compared with the control group.
Conclusion: The study demonstrates that turmeric extract has no ameliorative effect against CCl_4-induced liver damage in rats.

Keywords: Liver; kidney; turmeric; carbon tetrachloride; oral; ameliorative.

1. INTRODUCTION

Carbon tetrachloride (CCl_4) is one of the most potent toxins, which is widely used in scientific research to produce experimental model that mimic the oxidative stress in many pathophysiological situation [1]. CCl_4 is a possible carcinogen; inhalation or ingestion can cause damage to the brain, liver, kidneys, and can even cause death. CCl4 also contributes to ozone layer depletion [2].

Carbon tetrachloride (CCl_4) is a toxin that was used extensively to induce liver, renal and cardiac toxicity. CCl_4 administered to rats induces histologically proven severe hepato-pathology, nephropathy, cardiopathy and an increase of serum concentrations of urea, creatinine and liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), that are indicators of renal and liver tissue damage [3]. The biochemical mechanisms to CCl_4 exposure is induction of an increase in lipid peroxide and free peroxide radical concentrations that are highly reactive and cause injury or necrosis [4]. CCl4 is metabolized by P450 cytochrome to CCl_3 radical that induces the peroxidation of membrane lipids and disturbs Ca^{2+} homeostasis thus inducing liver tissue damage [5]. It has been reported that CCl_4 administration increases the silver-stained nucleolar organizer region, alters its size, morphology or spreading in the nucleus, which may be utilized as an indicator of genotoxicity, neoplasia and hyperplasia to complement other histological procedures [6].

Turmeric (Cu*rcuma longa*) is the rhizome or underground stem of ginger like plant. Turmeric has also been used for centuries in Ayurvedic medicine, which integrates the medicinal properties of herbs with food. This extraordinary herb has found its way into the spotlight in the west and rest of globe, because of its wide range of medicinal benefits. It is extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases [7]. Turmeric is a potent blood purifier and helps to create new blood. Turmeric also protects liver from toxins and pathogens. It is known to destroy major hepato toxins, like aflatoxin and to rebuild the liver. Turmeric has a protective effect against kidney and liver toxicity caused by certain medications. In addition, it is used as herbal remedy due to the prevalent belief that the plant has medical properties. In folk medicine, the rhizome juice from *C. longa* is used in the treatment of many diseases such as anthelmintic, asthma, gonorrhea, and its essential oil is used in the treatment of carminative, stomachic and tonic [8]. In traditional medicine, several plants and herbs have been used experimentally to treat liver and kidney disorders, including liver cirrhosis and Chronic Kidney Disease. Turmeric possesses antioxidant, anti-tumor, antimicrobial, anti-inflammatory; wound healing, and gastro-protective activities. Several studies have shown that the aqueous extract of turmeric has hepatoprotective and nephroprotective activity against carbon tetrachloride toxicity (Suzy et al. 2013).

The main aim of this research is to investigate the curative, hepatoprotective and nephro-protective effect of turmeric extract on Albino Wistar Rats.

2. MATERIALS AND METHODS

2.1 Location of Study

This study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Science, Niger Delta University, Wilberforce Island Amassoma, Bayelsa State of Nigeria.

2.2 Procurement of Turmeric Extract

Commercially prepared turmeric extract (5 g) was purchased from Loba Chem Pvt Ltd (India) was used for this studies.

2.3 Animal Housing

Thirty (30) male albino Wistar rats weighing between 150 ± 2.6 g-317 ± 3.5 g were procured from the animal house of the Department of Pharmacology, College of Health Sciences Niger Delta University, Amassoma Bayelsa state and moved to the animal house of the department of Medical Laboratory Science Niger Delta University Amassoma, Bayelsa State, Nigeria, where they were housed under standard condition of temperature (27 ± 2°C) with twelve hours light/dark periodicity in plastic cages. The rats were allowed to acclimatize for two weeks and were fed ad libitum during this period, with water and grower mesh feed. Animals where handled throughout study according to institutions guidelines for an experiment involving the use of laboratory animals.

2.4 Experimental Design

The animals were weighed and assigned into five (5) major groups with five (5)animals in each group after the period of acclimatization: A control group "A" and three test groups (C, D and E) except group B with ten (10) rats.

2.5 Substance Administration

Group A (Control): Rats were administered orally with pelleted growers mash (feed) and water throughout the experiment (21 days).

Group B (Positive Control): Rats were intraperitoneally injected with of CCl_4 only (0.5 mL/100 g body weight)and given pelleted growers mashes (feed) and water for 2 days, five (5) animals sacrificed on the 14^{th} day and the remaining five were given pelleted growers (feed) for 21 days.

Group C: Rats were injected intraperitoneally with CCL_4 (0.5 mL/100 g body weight) for 2 days, and then given orally with turmeric extract 200 mg/kg body weight, pelleted growers mash (feed) and water throughout the experiment (21 days).

Group D: Rats were administered orally with 200 mg/kg of turmeric extract for 21 days and injected intraperitoneally with CCL_4 (0.5 ml/100 g body weight) for 2 days, and they were also given pelleted growers mash (feed) and water throughout the experiment (21 days).

Group E: Rats were administered orally with 200 mg/kg of turmeric extract and given pelleted growers mash and water for twenty days (21 days).

2.6 Collection of Sample

At the end of three weeks of administration, the rats were sacrificed by administering chloroform as anesthesia. The rats were then dissected to harvest the liver and the kidney which were then fixed immediately in 10% formalin.

2.7 Histopathological Processing

The excised organs (liver and kidney) were cut in slabs of about 0.5 µm thick and fixed in 10% formal saline. Routine Tissue Processing was carried out using Automatic Tissue Processor-Histokinette (LEICA TP 1020).The tissues were embedded in paraffin wax in tissue Embedder (LEICA EG 1160) and sectioned in a Rotary Microtome (Heitz 150 Rotary Microtome) at 5 microns and stained in Hematoxylin and Eosin using the method for general tissue architecture [9]. The stained slides were examined using the compound light microscope at ×100 and x400 magnifications.

2.8 Microscopy and Photomicrography

The sections were examined using Olympus binocular microscope with in-built lighting system. The sections were then photomicrograph using a digital microscope camera (Samsung Model SS850) attached to an Olympus trinocular microscope.

Table 1. Treatment dose for studied sample

Group A (Control)	Group B	Group C	Group D	Group E
Feed and Water Only	CCL_4 (0.5 ml/100 g bw) + Feed and Water.	CCL_4 (0.5 ml/100 g bw) first + turmeric extract (200 mg/kg) + Feed and Water.	Turmeric extract (200 mg/kg) first + CCL_4 (0.5 ml/100 g bw) + Feed and Water.	Turmeric extract (200 mg/kg) first + Feed and Water.

3. RESULTS

Photomicrograph of kidney sections stained with hematoxylin and Eosin X400. Sections shows normal Bowman's capsule (BC), the renal tubules (RT) all are consistent with normal kidney histology.

Photomicrograph of liver section stained with hematoxylin and Eosin x 400. Sections shows portal vein (PV), central vein (CV) and sinusoids (S) consistent of liver histology. Plate B shows fat infiltration (F) indication fatty liver degeneration. Plate C and D show occlusion (O) of sinusoidal spaces with enlargement of hepatocytes.

Fig. 1 shows the histology of the Kidney stained with Hematoxylin and Eosin. Slide A (Control) shows normal kidney stroma with Bowman's capsule (BC) with glomerulus (renal corpuscle) and renal tubules (RT). Slide B, C and D is similar to the control.

Fig. 2 shows the histology of the liver stained with Hematoxylin and Eosin. Slide A (Control) shows normal hepatic stroma with portal vein (PV), central vein (CV) and sinusoids (S) consistent of liver histology. Slide B shows fat infiltration (F) indication fatty liver degeneration. Slide C and D show occlusion (O) of sinusoidal spaces with enlargement of hepatocytes. Slide F shows similar morphology with the control.

4. DISCUSSION

CCl_4 is a well-known hepatotoxin that is widely used to induce toxic liver injury in a range of laboratory animals. CCl_4-induced hepatotoxicity is believed to involve two phases. The initial phase involves the metabolism of CCl_4 by cytochrome P450 to the trichloromethyl radicals, which lead to membrane lipid peroxidation and finally to cell necrosis. The second phase of CCl_4-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators [10]. Turmeric therapeutic properties including antioxidant effects, anti-inflammatory effects, anti-cancer and anti-microbial effects, hepato-protective effects, reno-protective effects, thrombo inhibitory effects, cardio-protective effects, an anti-inflammatory effects on the rheumatoid arthritis, has been confirmed by modern and advanced researches.

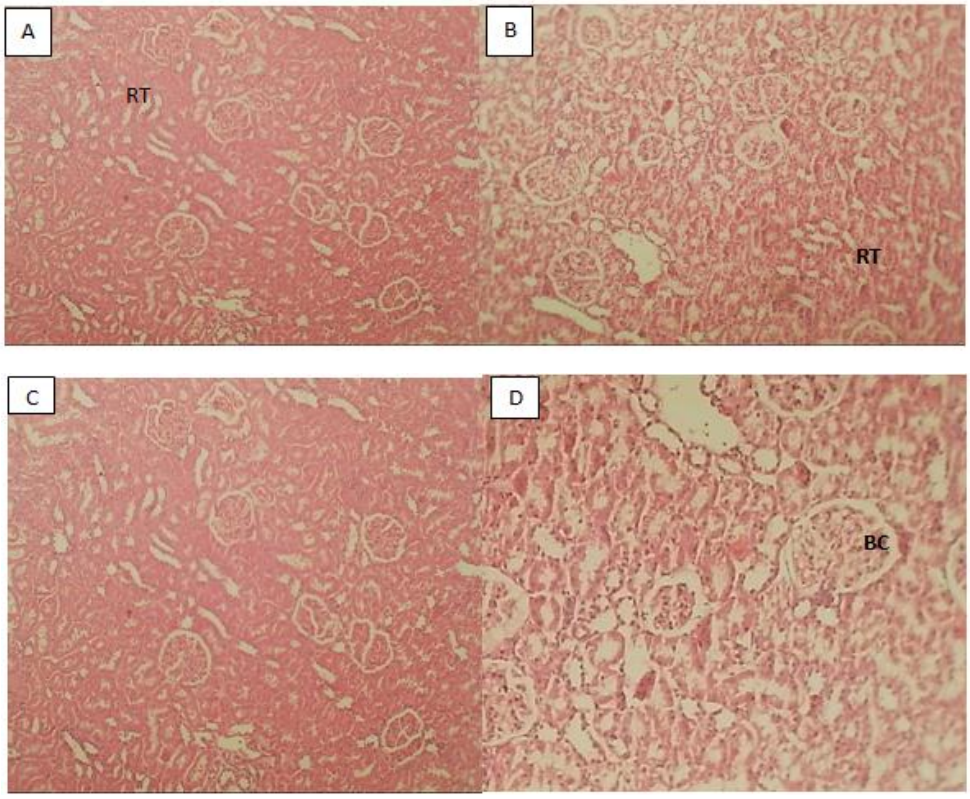

Fig. 1. Shows the Histology of the Kidney stained with Hematoxylin and Eosin, X400

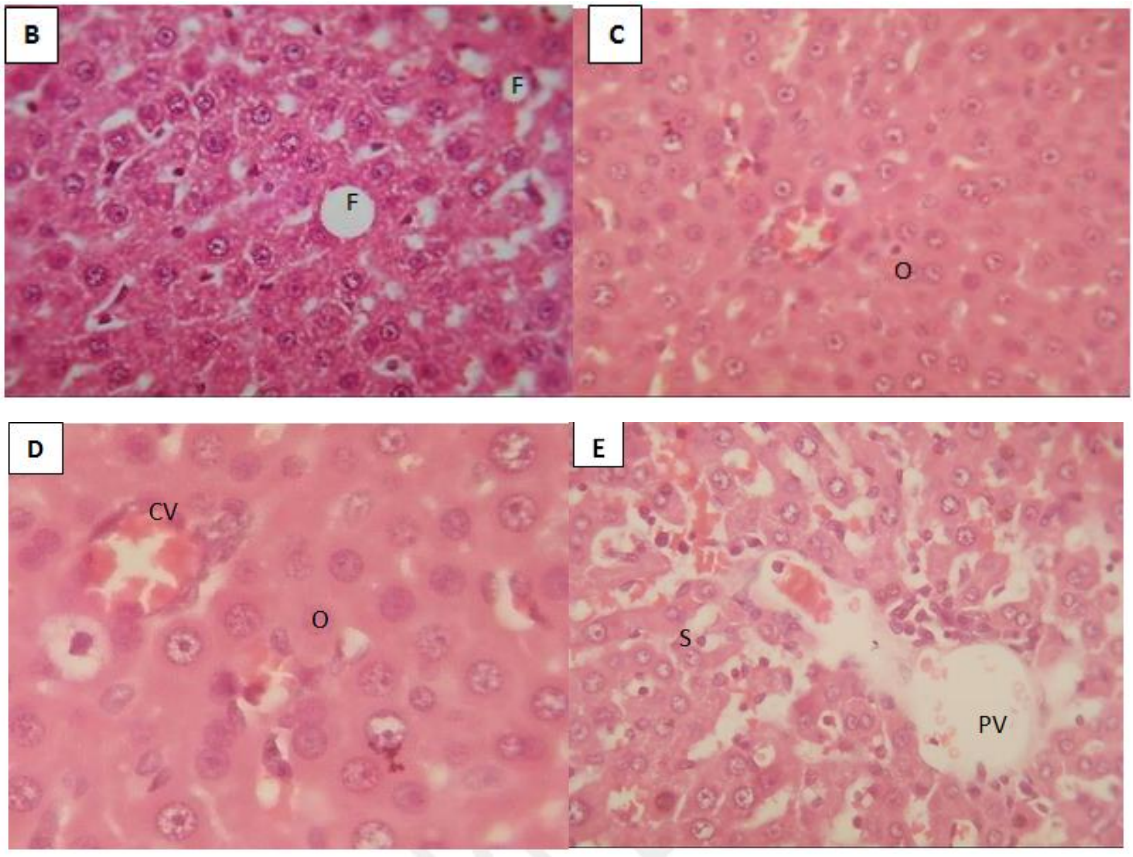

Fig. 2. Shows the histology of the Liver stained with Hematoxylin and Eosin, X400

The livers of the rats from control group is consistent with normal liver histology (Fig. 1A). Histopathological changes such as fat infiltration (F) were consistently observed in liver of all the rats from CCl_4-treated group (Fig. 1B). Also, predominant changes such as occlusion (O) of sinusoidal spaces with enlargement of hepatocytes was observed in livers of all rats treated with a combination of CCL_4 and turmeric extract (Fig. 1C & D). However, the histological appearance of the liver in the turmeric extract group had no morphological change (Fig. 1E). Administration of turmeric extract had no ameliorative effect on the changes induced by CCl4 in liver. In other studies, carried out by Saad et al. (2017) on hepatoprotective effect of aqueous extracts of some medicinal plant mixtures on CCl4-Induced Liver Toxicity, administering 1.5 ml/kg of CCL_4 for 10 days. It reported that CCL4 induces severe histological changes such as extensive hepatocellular degeneration and necrosis, fatty changes, inflammatory cell infiltration, congestion, and sinusoidal dilatation in the hepatic tissues. He further observed that treatment with turmeric extract effectively prevented the necrosis and the other histopathological changes induced by CCl_4 toxicity.

The result further shows that there were no noticeable histopathological changes observed in the histology of the kidney of all animal groups as compared with the control group (Fig. 1A-1D). This may be due to the duration of administration (twice a week for one week) of 0.5 ml/kg CCL4. Other study conducted by Naima et al. [11] reported that CCL_4 induced nephrotoxicity in rats in the duration of administration of 0.5 ml/kg (twice a week for six weeks) and 5 ml/kg (once a week for six weeks) of CCL_4 respectively. Also curcumin prevented the decrease in the following enzymatic activities: aconitase, antioxidant enzymes and mitochondrial respiratory complexes I, II, II–III and V [12]. Rosita et al. [13] also reported that C. mangga extract was able to inhibit the increase of creatinine level and showed a significantly different from negative control ($p < 0.05$). The result was supported by histopathology examination which did not show any cell damage, in a similar Study carried out by

Alireza and Daryoush [14] on preventive effects of turmeric *(Curcuma longa* linn) on renal ischemia-reperfusion injury in rats showed that renal structure is normal in the control group and there were not pathologic changes. In the group 2, degenerative changes of tubular cells, acute tubular necrosis, edema, hyperemia and sever hemorrhage were more prevalent.

5. CONCLUSION

Although turmeric has also been used for centuries in Ayurvedic medicine, which integrates the medicinal properties of herbs with food. This extraordinary herb has found its way into the spotlight in the west and rest of globe, because of its wide range of medicinal benefits. It is extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases [15]. Turmeric is a potent blood purifier and helps to create new blood. Turmeric also protects liver from toxins and pathogens. It is known to destroy major hepatoxins, like aflatoxin and to rebuild the liver. Turmeric has a protective effect against kidney and liver toxicity caused by certain medications. In addition, it is used as herbal remedy due to the prevalent belief that the plant has medical properties. In folk medicine, the rhizome juice from *C. longa* is used in the treatment of many diseases such as anthelmintic, asthma, gonorrhea, and its essential oil is used in the treatment of carminative, stomachic and tonic [16]. Tumeric possesses antioxidant, anti-tumor, antimicrobial, anti-inflammatory; wound healing, and gastroprotective activities [17]. However this study demonstrates that turmeric extract had no had no ameliorative effect against CCl4-induced hepatic damage in rats at the dose and duration of administration.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethics committee approval has been taken and preserved by the author for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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