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Citrus Tangerina pre-treatment mitigates carbon tetrachloride induced hepatotoxicity on Wistar rats

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ABSTRACT

Phytochemical constituents of medicinal plants are being used in the management of liver disease and in various pathological states. In this present study, we evaluated the mitigating effect of peels of fruit of *C. tangerina* against CCl4-induced hepatotoxicity in pre-treated Wistar rats. Rats (180 – 220 g) were randomly placed into five groups of five animals each. Group 1 served as normal control, group 2 received CCl4, group 3 was silymarin-treated (standard), while groups 4 and 5 received *Citrus tangerina* peels extract (CTP) at 200 mg/kg and 400 mg/kg. CTP and silymarin were administered orally for six days while CCl4 was given subcutaneously on the 7th day only. Following euthanasia, blood samples were collected used for the estimation of biochemical parameters namely superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), **a**lkaline phosphatase (ALP), aspartate aminotransefrase (AST), and alanine transaminase (ALT). The liver tissues harvested were subjected to histopathology. Statistical analysis was done and $P<0.05$ were considered significant. CTP significantly ($P<0.05$) reduced elevated AST caused by CCl4 intoxication. CCl4 induced decrease in antioxidant enzymes SOD and CAT were elevated by both doses of CTP as much as silymarin. Histopathological observation of the liver tissue supported biochemical findings of this study. Methanol extracts of *C. tangerina* fruit peels possess potential protective activity on the liver in CCl4-induced hepatotoxicity.

Keyword: Antioxidant, *Citrus tangerina,* hepatic cell protection, methanol extract.

1. INTRODUCTION

Hepatic damage is a growing global concern and efforts are constantly been targeted at both protecting the liver from damage and treatments which improve its integrity following damage (Abou Seif, 2016; Sing *et al*., 2016). The liver plays significant role in regulating basic physiological process of the human body, most importantly, the detoxification of toxic substances (Abou Seif, 2016). Exposure of the liver to toxic substance such as (alcohol, carbon tetrachloride – CCl4, thioacetamide), drugs (analgesics, antimalarials, antitubercular agents, antidepressants), as well as various infections and some autoimmune disorders are stimulate hepatic damage, leading to hepatitis and liver cirrhosis (Okon et al. 2020). The hepatotoxic action of the substances has been pointed to the raised oxidative stress resulting from reactive oxygen species they generate and depletion of the antioxidant defense system of the liver (Abou Seif, 2016).

Phytochemical constituents of medicinal plants are being used in the management of liver disease and in various pathological states (Abou Seif, 2016; Sing *et al*., 2016; Gillensson and Schmidt, 2020). This practice of herbal medicine has grown over the years particularly as their usage is considered safe, cost-effective, and readily available (Ekor, 2014; Moke *et al*., 2021). A number of medicinal plants have been shown to reduce hepatic damage by the antioxidant defense mechanisms, inhibiting hepatic stellate cells (HSC) and reducing extracellular matrix (ECM) deposition (Abdelazizi and Ali, 2014; Duval *et al*., 2014; Sing *et al*., 2016; Moke *et al*., 2020). Amongst these plants is *Citrus tangerina* (family, Rutaceae), commonly known as tangerine, whose plant parts have been reported to have numerous biological effects such as anti-inflammatory and anti-tumor activity (Karimi *et al*., 2012; Lv *et al*., 2015). Robust antioxidant action of *C. tangerina* effect via its free radical scavenging activity have been reported (Oikeh *et al*., 2016; Rafiq *et al*., 2018).

In this present study, we evaluated the mitigating effect of peels of fruit of *C. tangerina* against CCl4-induced hepatotoxicity in pre-treated Wistar rats.

2. MATERIALS AND METHODS

2.1. Plant material and preparation of extract

Fruits of *Citrus tangerina* were purchased locally from the Abraka main market in Nigeria and were authenticated at the Department of Botany, Faculty of Sciences, Delta State University, Abraka. The peels of the fruits were properly rinsed with water, air-dried, and powdered. About 1.67 kg of powdered peel of *Citrus tangerina* was extracted exhaustively in 3,200 ml of methanol (70%) using Soxhlet evaporator at 25-35 0C, thereafter, the filtrate was concentrated to dryness with the aid of a water bath set at 40 0C. The final extract was refrigerated prior to commencing the study.

2.2. Animals

Wistar rats (180-220 g) were procured from Animal House, Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria. The rats were allowed access to clean drinking water and fed with standard animal pellets (Chikun Feed® Grower Pellet, Nigeria). They were acclimatized for two weeks before starting the study. Handling of the animals were in accordance with guidelines of the global standard adopted by the Ethical Committee of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria (FBS/CT/091720).

2.3. Carbon tetrachloride (CCl4) – induced hepatotoxicity

The rats were randomly placed into five groups of five animals each. Group 1 (normal control) received normal saline (2 ml/kg) once daily for 6 days, group 2 (CCl4 control) received CCl4 (1 ml/kg in olive oil) (Issa *et al*., 2018) single dose on the 7th day, group 3 (Silymarin) received standard group treatment of silymarin 100 mg/kg once daily for 6 days + CCl4 (1 m/kg) on the 7th day, group 4 (CTP 200 + CCl4) received *Citrus tangerina* peels 200 mg/kg once daily for 6 days + CCl4 (1 m/kg) on the 7th day, while group 5 (CTP 400 + CCl4) received *Citrus tangerina* peels 400 mg/kg once daily for 6 days + CCl4 (1 m/kg) on the 7th day of the experiment. The extracts (CTP) and silymarin were administered orally for six days while CCl4 was given subcutaneously on the 7th day only, afterwards, animals were observed for 24 hours prior to euthanasia.

Blood samples were collected under chloroform anaesthesia by cardiac thoracic puncture into plain sample bottles, allowed to coagulate and centrifuged at 2500 rpm for 10 minutes. The serum was collected and used for the estimation of biochemical parameters namely superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), alkaline phosphatase (ALP), aspartate aminotransefrase (AST), and alanine transaminase (ALT) (Reitman and Frankel, 1957; Roy, 1970; Misra and Fridovich, 1972; Sinha, 1972; Gutteridge and Wilkins, 1982). The liver tissues harvested were subjected to histopathology (Galighter and Koyloff, 1971).

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2.4. Statistical Analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test processed on GraphPad Prism software version 7. Results are presented as the mean ± standard error of the mean (SEM). P-values < 0.05 were taken as significant.

3. RESULTS

Pre-treated with silymarin (100 mg/kg) and methanol extracts of fruit peels of *Citrus tangerina* (CTP 200 and CTP 400) significantly (P<0.05) reduced serum AST as compared to CCl4 control group but produced non-significant (P>0.05) decrease in ALT and ALP as compared to CCl4 control group (Table 1). Animals treated with CCl4 significantly (P<0.05) decreased the level of SOD and CAT as compared to the normal control group (Table 2). CTP 200, CTP 400, and silymarin showed significantly (P<0.05) increase in SOD and CAT as compared to CCl4 control group. CCl4 significantly (P<0.05) increase MDA level as compared to normal control group. Both doses of the extract showed non-significant (P>0.05) decrease in MDA as compared to CCl4 control group, although, silymarin-treated rats significantly (P<0.05) reduced MDA as compared to CCl4 control group. Photomicrographs on the liver sections illustrating the effects of CCl4 and silymarin/extracts are shown in Figure 1.

Table 1: Effect of *Citrus tangerina* fruit peel on liver function parameters in CCl4 induced hepatotoxicity in rats

	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Control	42.41 ± 1.51	10.53 ± 1.79	31.23 ± 3.27
CCl4 Control	54.50 ± 1.16*	15.20 ± 2.55*	38.09 ± 3.13
Silymarin	45.34 ± 0.48**	10.66 ± 4.72**	29.95 ± 5.20
CTP 200	46.29 ± 1.15**	14.53 ± 1.99	31.35 ± 5.70
CTP 400	45.51 ± 1.68**	14.84 ± 3.19	31.49 ± 2.87

*All values are expressed as mean ± standard error of mean (SEM); n=5. * P<0.05 significant as compared to normal control; ** P<0.05 significant as compared to CCl4 control group*

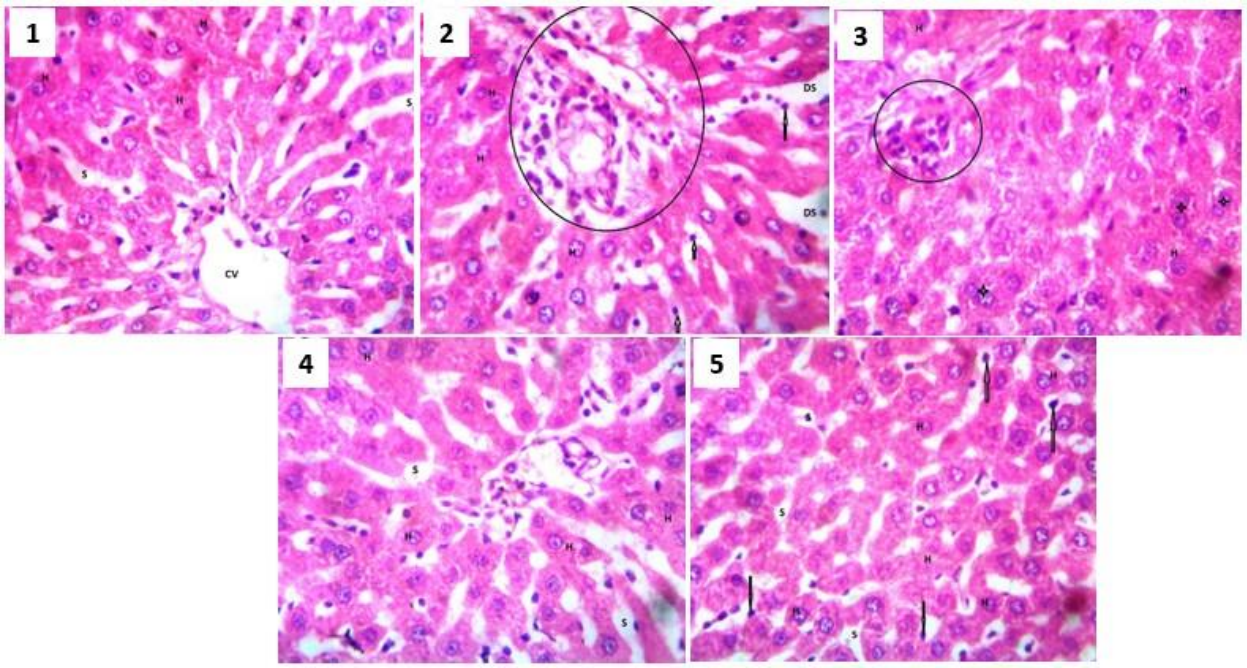

Figure 1: Photomicrographs of liver section: (1) Normal control group; (2) CCl4 control group; (3) Silymarin group; (4) CTP2+CCl4; (5) CTP4+CCl4. *(CV–central vein; 'circle' illustrates infiltrated by inflammatory cells) (H&E staining; × 400 magnification)*

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Table 2: Effect of *Citrus tangerina* fruit peel on antioxidant activity in CCl4 induced hepatotoxicity in rats

	SOD (IU/L)	CAT (IU/L)	MDA (IU/L)
Normal Control	0.38 ± 0.03	1.09 ± 0.12	0.42 ± 0.06
CCl4 Control	0.23 ± 0.03*	0.61 ± 0.08*	0.89 ± 0.06*
Silymarin	0.41 ± 0.01**	0.91 ± 0.38**	0.46 ± 0.07**
CTP 200	0.42 ± 0.02**	1.04 ± 0.56**	0.75 ± 0.04
CTP 400	0.50 ± 0.09**	0.91 ± 0.40**	0.77 ± 0.09

*All values are expressed as mean ± standard error of mean (SEM); n=5. * P<0.05 significant as compared to normal control; ** P<0.05 significant as compared to CCl4 control group*

4. DISCUSSION

Currently, liver disease is a global health challenge of which most recent researches are targeted at establishing a beneficial curative approach. The use of herbal products in the treatment of ailments associated with hepatic diseases has increased over time (Singh *et al*., 2016; Moke *et al*., 2021). Numerous plants are being used as hepatoprotective agents in traditional therapy (Bedi *et al*., 2016; Moke *et al*., 2019). The present study evaluated the prophylactic potential of methanol fruit peel extract of *C. tangerina* against hepatotoxicity in CCl4 treated rats.

Carbon tetrachloride (CCl4) has been established is a potent hepatotoxic compound, and often used as an animal model of experimental hepatotoxicity (Cheng *et al*., 2013; Gillesson and Schmidt, 2020). It is frequently used to evaluate hepatoprotective activity of plant agents (Gillesson and Schmidt, 2020). Oxidative stress is well associated in the pathophysiology of CCl4-induced hepatotoxicity (Hafez *et al*., 2014).

Silymarin is a well established drug treatment for liver damage, hence, used in the evaluation of hepatoprotective action of medicinal plant products (Shaker *et al*., 2010; Gillesson and Schmidt, 2020). As an antioxidant compound, it scavenges free radicals that are destructive to cell, while promoting hepatic cell regeneration and antioxidant enzymes in the liver (Shaker *et al*., 2010).

Carbon tetrachloride (CCl4) compromises hepatic cell function and damages liver cells characterized by leakage of transaminase enzymes (AST and ALT) from cells and an increase in serum ALP (Bera *et al*., 2011; Fu *et al*., 2020). Results from this study reveal that pre-treatment with *C. tangerina* fruit peel extract (CTP) reduced the CCl4-induced elevated serum liver enzymes, particularly aspartate transaminase (AST) level which was much effect with dose at 400 mg/kg. Silymarin showed an improved effect in reducing the enzymes. Return towards normal of the liver enzymes is typical of hepatic healing from injury (Jeschke *et al*., 2009; Osadebe *et al*., 2012). This infers that CTP protects against CCl4 damage to liver tissue.

Pre-treatment with CTP at 200 and 400 mg/kg, as well as silymarin, prior to hepatotoxicity induced by CCl4 led to increase in enzymatic antioxidant markers (SOD and CAT) which was depreciated following treatment with carbon tetrachloride as evident in CCl4 treated control animals. Increased lipid peroxidation status induced by CCl4 was reduced only by silymarin treatment but not in CTP-treated animals. This point out the fact is that CTP improves the hepatic antioxidant defense system via increasing levels of superoxide dismutase and catalase, but has little or no effect on reducing lipid peroxidation. Thus, CTP possesses free radical scavenging activity against generated reactive oxygen species which is implicated in cell damage repair (Rafiq *et al*., 2018).

Histopathological observation (Figure 1) strongly supported biochemical findings in this study. Normal liver histoarchitecture with central vein and cords of hepatocytes was seen in the normal control group, while liver section of CCl4–treated control group revealed marked hepatocellular degeneration with infiltration of inflammatory cells evident of oxidative stress induced by CCl4 (Yang *et al*., 2013; Jahan *et al*., 2021). *Citurs tangerina*-treated rats at both doses revealed mild histoarchitectural changes as also seen in silymarin-treated rats. This is an indication that the fruit peel of *C. tangerina* possesses mitigating effect as silymarin against CCl4 -induced hepatotoxicity. Silymarin has been reported to have anti-inflammatory activities and protect membrane permeability as it act as a free radical scavenger (Baradaran *et al*., 2019; Gillesson and Schmidt, 2020). The mitigating effect of fruit peel extract of *C. tangerina* against hepatotoxicity could be related to their intrinsic antioxidant properties as *C. tangerina* has been reported to be rich in flavonoids and phenolic compounds (Rafiq *et al*., 2018; Falcinelli *et al*., 2020).

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5. CONCLUSION

Pre-treatment with methanol extracts of *C. tangerina* fruit peels exhibits potential protective activity on the liver in CCl4-induced hepatotoxicity, as indicated by results of improved metabolic activity and cellular stability. Antioxidant action of *C. tangerina* is the most probable mechanism.

Ethical approval

Animal ethical approval adopted by the Ethical Committee of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria (FBS/CT/091720).

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

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