

## Hepatoprotective and Antioxidant Effects of the Flavonoid-rich Fraction of the Methanol Extract of *Jatropha tanjorensis* Leaves in CCl<sub>4</sub>-induced Liver Injury in Rats.

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**Abstract:** The leaves of *Jatropha tanjorensis* are edible and used in herbal medicine in the treatment of diseases associated with oxidative stress. The present study demonstrates the antioxidative effect of the flavonoid-rich fraction of the methanol extract of *Jatropha tanjorensis* leaves (FRJT) against CCl<sub>4</sub>-induced hepatotoxicity in rats. Hepatoprotective and antioxidant properties of FRJT were determined by serum biochemical enzymes; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), antioxidant enzymes (SOD, CAT and GPx), hematological parameters (PCV, Hb and WBC) and histology study. The results obtained showed a significant reduction ( $p < 0.05$ ) in the activities of liver marker enzymes across the pre-treated groups compared with the untreated rats. Assay of antioxidant enzymes showed that the extract significantly ( $p < 0.05$ ) enhanced SOD and GPx activities whereas CAT activity was non-significantly ( $p > 0.05$ ) increased when compared with the untreated animals. PCV, Hb and WBC levels were significantly ( $p < 0.05$ ) lower in the untreated group. However, supplementation with FRJT and Silymarin ameliorated the induced depletion of blood in the pre-treated animals. Histological examination of the liver tissue showed marked reduction in fatty degeneration across the pre-treated groups when compared with the untreated group. The results in this study indicate that FRJT exhibited varying levels of protection against CCl<sub>4</sub>-induced oxidative stress in rat models. These results also indicate that the flavonoid-rich fraction contains antioxidants, which mop up free radicals in the system and support its use in the treatment of diseases resulting from oxidative damage.

**Keywords:** Antioxidants, Carbondetrachloride, Flavonoid-rich Fraction, Hepatoprotective, *Jatropha tanjorensis*.

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### I. Introduction

Oxidative stress results from an imbalance between the production of reactive oxygen-containing molecules and biological antioxidants. Reactive oxygen species (ROS) and free radicals formed during oxidation have been reported to contribute to diseases such as cancer, diabetes and ageing<sup>(1)</sup>. Other diseases caused by oxidative stress include Alzheimer's and Parkinson's diseases, rheumatoid arthritis, and neurodegeneration in motor neuron diseases<sup>(2)</sup>. Hepatotoxic chemicals cause liver damage which is induced by lipid peroxidation and other oxidative damage<sup>(3)</sup>. Several compounds such as carbon tetrachloride (CCl<sub>4</sub>), acetaminophen, bromobenzene, ethanol and polycyclic aromatic hydrocarbons have been implicated in the etiology of liver diseases<sup>(4)</sup>. The biological system maintains complex mechanisms of antioxidants and enzymes such as catalase, superoxide dismutase, glutathione reductase, glutathione S-transferase and various peroxidases, which are compensatory mechanisms to deal with ROS and their effects. In recent years however, significant attention has been directed towards exploring plant-based natural antioxidants. Such natural antioxidants as previously reported, are associated with other health beneficial effects such as, lowering the incidence of aging, inflammation, cardiovascular diseases and certain cancers<sup>(5)</sup>. Flavonoid, a well known antioxidant of plant origin, is a water-soluble polyphenolic compound which possesses many health promoting effects.

*Jatropha tanjorensis* is a member of the *Euphorbiaceae* family, commonly called "hospital too far" or "Catholic vegetable" in southern Nigeria<sup>(6)</sup>. *Jatropha tanjorensis* is a native of Central America and has become naturalized in many tropical and subtropical countries, including Africa, India and North America<sup>(7)</sup>. *Jatropha tanjorensis* is predominantly grown in southern Nigeria and is primarily used for fencing<sup>(8)</sup>. The leaves of the plant are a source of edible leafy vegetable and taken as a tonic in herbal medicine, with the claim that it increases blood volume<sup>(9)</sup>. Traditionally, decoction of the leaves is used to treat anaemia (as a haematinic agent), diabetes, skin diseases, malaria, and cardiovascular diseases<sup>(10, 11)</sup>. *Jatropha tanjorensis* has been vastly studied due to its potential health benefits, availability and affordability. Phytochemical analysis of the leaves showed the presence of flavonoids, tannins, terpenoids, saponins and cardiac glycosides. These phytochemical ingredients have hypolipidemic and antioxidant properties and exhibited positive modulatory effects on serum lipid profile in albino rats<sup>(12)</sup>. Reports also showed that *J. tanjorensis* is rich in antioxidant nutrients like

phosphorus, selenium, zinc and vitamins C<sup>(13)</sup>. It was previously reported that *J. tanjorensis* exhibited low antioxidant and very low hemagglutination titre value, the later indicating low toxicity on red blood cells<sup>(10)</sup>. Reports on *in vitro* and *in vivo* antioxidant properties of methanolic extracts of *J. tanjorensis* on nutritionally stressed rats (protein malnutrition) confirmed the local claims on the efficacy of the plant leaves in providing effective intervention for free radical mediated diseases<sup>(6)</sup>. Studies on the *in vitro* antioxidant properties of free and bound phenolic extracts of the leaves of *J. tanjorensis* showed that the extracts inhibited Fe<sup>2+</sup>-induced hepatic and cerebral lipid peroxidation process<sup>(14)</sup>. Antimicrobial studies of *J. tanjorensis* showed that the aqueous extract of the leaves inhibited gram +ve bacterium *Staphylococcus aureus* and gram -ve bacterium *Escherichia coli*<sup>(8)</sup>. Crude ethanolic extracts of the plant leaves exhibited relatively high antiplasmodial and low cytotoxic activities; this may be attributed to the presence of some inherent phytochemicals which might have conferred some protective/antioxidative effect against oxidative stress induced by the malaria parasite<sup>(9)</sup>. Studies on the aqueous extract of the leaves of *J. tanjorensis* showed a statistically significant increase on the PCV and Hemoglobin concentration of both male and female wistar rats, thereby justifying the local claim of the plant's use as a blood tonic<sup>(15)</sup>. Here, we report the antioxidant and hepatoprotective activities of the flavonoid-rich fraction of the methanol leaf extract of *J. tanjorensis*.

## II. Materials And Methods

### 2.1. Materials

The fresh leaves of *Jatropha tanjorensis* were collected from Ihiagwa, Owerri-North LGA, Imo State, Nigeria. The plant was identified at the herbarium unit of Bioresources Development and Conservation Programme (BDGP) Research Centre, Nsukka, Enugu State of Nigeria. The leaves were dried for two weeks, pulverized to fine powder and stored in air tight containers for subsequent use. The chemicals, reagents and kits used for this research were of analytical grade.

### 2.2 Extraction and Solvent-Solvent Partitioning of the Pulverized Leaves of *J. tanjorensis*

The extraction and solvent-solvent partitioning technique was done according the reported method<sup>(16)</sup>. Briefly, the ground leaves (1000 g) were macerated twice with 7.5 L of methanol and extracted at room temperature for 48hours with agitation. The filtrate was concentrated in *vacuo* (40 °C) to yield a dark green crude extract (50 g). The crude extract was reconstituted in 400ml of 10% methanol in water and the aqueous portion successively partitioned against n-hexane (250 ml x10) and ethylacetate (250 ml x10) to obtain n-hexane (HF, 5.50 g), EtOAc (8.50 g) and aqueous (1.20 g) soluble fractions respectively.

### 2.3 Animals

Twenty-five (25) adult male albino rats weighing 100-130g were obtained from the animal house of the Department of Zoology and Environmental Biology, University of Nigeria Nsukka. The rats were acclimatized for one week prior to commencement of the experiment. They were kept at room temperature, maintained *ad libitum* on standard growers mash rat pellets (Grand Cereals LTD, Enugu) and weighed before commencement of experiment. The guide for the care and use of laboratory animals' procedure was followed in this study.

### 2.4 Experimental Protocol

Twenty-five (25) adult male albino rats used in the experiment were divided into five groups of five rats (n = 5) each as summarized below:

**Group 1:** Normal control (received only the vehicle: 3% tween 80)

**Group 2:** Untreated (CCl<sub>4</sub>-induced without treatment)

**Group 3:** Induced with CCl<sub>4</sub> + 40mg/kg b.w of FRJT pre-treatment

**Group 4:** Induced with CCl<sub>4</sub> + 80mg/kg b.w of FRJT pre-treatment

**Group 5:** Standard control (Induced with CCl<sub>4</sub> + 25mg/kg b.w of Silymarin pre-treatment)

The experiment lasted for fourteen days. On days 13 and 14, the rats of groups 2 – 4 were given double oral doses of CCl<sub>4</sub> in olive oil (1:1) at 1ml/kg b.w 1hour after administration of FRJT, and group 5, 1hour after administration of Silymarin<sup>(17)</sup>. After 16-18hours of the last dose of CCl<sub>4</sub> induction, blood samples were collected by the orbital technique for biochemical parameters. Thereafter, animals were anesthetized and the liver dissected out and profused in 10% formal saline for histological studies.

### 2.5 Blood collection and preparation

Blood samples for biochemical tests were collected by puncturing the retro-bulbar plexus with a microcapillary tube, which was carefully inserted into the medial canthus of the eye of the rats, enabling 2 ml of blood into a clean glass test tube. The blood sample was kept at room temperature for about 30 minutes to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3,000 rpm for 10 minutes to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was then carefully aspirated with syringe and stored in a clean sample bottle for biochemical tests.

### 2.6 Assay for Hepatoprotective activity of FRJT

Commercial kits obtained from Randox Laboratories, United Kingdom were used for the biochemical evaluation of ALT, AST and ALP as previously reported<sup>(18, 19)</sup>.

### 2.7 Assay for Antioxidant activity of FRJT

Antioxidant enzymes; Glutathione peroxidase (GPx), Catalase (CAT) and Superoxide dismutase (SOD) were determined according to previous reports<sup>(20, 21, 22)</sup>.

### 2.8 Determination of haematological Parameters

Blood samples for the determination of haematological parameters were collected by puncturing the retro-bulbar plexus of the medial canthus of the eye, thus enabling outflow of blood into a clean sample bottle containing ethylene-diamine-tetra-acetic acid (EDTA). The sample bottle was shaken gently to mix the blood with EDTA and prevent clotting. Haemoglobin (Hb) concentration was determined with a haemoglobin test kit (Smar Test Diagnostic, Isreal) using the cyanomethaemoglobin method, packed cell volume (PCV) was determined by the Microhaematocrit method, while total WBCs were determined using an improved Neubauer Haemocytometre as previously reported<sup>(23)</sup>. All haematological parameters were determined at room temperature (27±0.5 °C).

### 2.9 Histological examination of liver tissues

Histological examination of liver tissues was carried out according to previous report<sup>(24)</sup>. The liver tissues were fixed in paraffin. Thin sections (5 µM) were cut and stained with hematoxylin and eosin (H & E) for photo-microscopic assessment with the purpose of determining histological alterations in the liver tissues.

### 3.0 Statistical Analysis

The results were expressed as mean ± SD and test of statistical significance was employed using one-way analysis of variance (ANOVA). The results obtained were analyzed using Statistical Product and Service Solutions (SPSS), version 20 and p values < 0.05 were considered significant.

## III. Results

### 3.1 Biochemical and haematological parameters

Effects of the flavonoid-rich fraction of the methanol extract of *J. tanjorensis* leaves (FRJT) on CCl<sub>4</sub>-induced liver injury in rats, with reference to serum biochemical changes and haematology are shown in Tables 1 - 3. It was observed that the CCl<sub>4</sub>-induced (untreated) group showed a significant (p < 0.05) elevation in the activities of ALT, AST and ALP compared with the normal control group, indicating liver injury caused by CCl<sub>4</sub> induction. Animals pre-treated with FRJT and Silymarin showed a significant (p < 0.05) reduction in the activities of these liver enzymes compared with the untreated (Table 1). Antioxidant enzymes (GPx and CAT) were significantly (p < 0.05) depleted while SOD was non-significantly (p > 0.05) depleted in the untreated animals, compared with the normal control. In contrast, GPx activity was found to be significantly (p < 0.05) higher across the pre-treated groups, CAT activity was non-significantly (p > 0.05) higher in groups 3 and 4 whereas SOD activity was found to be significantly (p < 0.05) higher in groups 4 and 5 compared with the untreated group (Table 2). PCV, Hb and WBC levels were significantly (p < 0.05) lower in the untreated animals compared with the normal control. However, supplementation with FRJT and Silymarin ameliorated the induced depletion of blood in the pre-treated animals (Table 3).

**Table 1:** Effect Of The Flavonoid-Rich Fraction Of *J. Tanjorensis* (FRJT) Leaves On Liver Function Parameters in CCl<sub>4</sub>-Induced Hepatotoxicity in Rats.

Parameters (iu/L)	Normal control (Group 1)	Untreated CCl <sub>4</sub> only (Group 2)	CCl <sub>4</sub> + 40mg/kg b.w FRJT (Group 3)	CCl <sub>4</sub> + 80mg/kg b.w FRJT (Group 4)	Standard control CCl <sub>4</sub> + 25mg/kg b.w Silymarin (Group 5)
ALT	16.20 ± 1.92	23.60 ± 2.07 <sup>a</sup>	14.60 ± 2.07 <sup>b</sup>	14.20 ± 1.30 <sup>bc</sup>	16.80 ± 1.92 <sup>b</sup>
AST	25.40 ± 1.82	108.80 ± 10.23 <sup>a</sup>	43.80 ± 8.38 <sup>abc</sup>	41.20 ± 3.77 <sup>abc</sup>	89.80 ± 7.19 <sup>ab</sup>
ALP	32.00 ± 4.00	57.40 ± 3.05 <sup>a</sup>	35.20 ± 5.63 <sup>b</sup>	36.20 ± 4.15 <sup>b</sup>	32.20 ± 5.22 <sup>b</sup>

<sup>a</sup> Indicates significant difference from the normal control value at p < 0.05; <sup>b</sup> indicates significant difference between pre-treated groups and the untreated group, while <sup>c</sup> indicates significant difference between FRJT pre-treated groups and the standard control. Data are expressed as means ± SD, n = 5.

**Table 2:** Effect Of The Flavonoid-Rich Fraction Of *J. Tanjorensis* (FRJT) Leaves On Antioxidant Enzymes in CCl<sub>4</sub>-Induced Hepatotoxicity in Rats.

Parameters (U/L)	Normal control (Group 1)	Untreated CCl <sub>4</sub> only (Group 2)	CCl <sub>4</sub> + 40mg/kg b.w FRJT (Group 3)	CCl <sub>4</sub> 80mg/kg b.w + FRJT (Group 4)	Standard control CCl <sub>4</sub> + 25mg/kg b.w Silymarin (Group 5)
GPx	0.62 ± 0.12	0.16 ± 0.03 <sup>a</sup>	0.67 ± 0.13 <sup>b</sup>	0.86 ± 0.14 <sup>ab</sup>	0.83 ± 0.14 <sup>ab</sup>
Catalase	2.69 ± 1.39	1.10 ± 0.47 <sup>a</sup>	2.01 ± 0.45	1.98 ± 0.54	2.15 ± 0.43 <sup>b</sup>
SOD	11.38 ± 0.07	11.29 ± 0.11	11.28 ± 0.11 <sup>c</sup>	11.42 ± 0.08 <sup>b</sup>	11.41 ± 0.04 <sup>b</sup>

<sup>a</sup> Indicates significant difference from the normal control value at p < 0.05; <sup>b</sup> indicates significant difference between pre-treated groups and the untreated group, while <sup>c</sup> indicates significant difference between FRJT pre-treated groups and the standard control. Data are expressed as means ± SD, n = 5.

**Table 3:** Effect Of The Flavonoid-Rich Fraction Of *J. Tanjorensis* (FRJT) Leaves On Hematological Parameters in CCl<sub>4</sub>-Induced Hepatotoxicity in Rats.

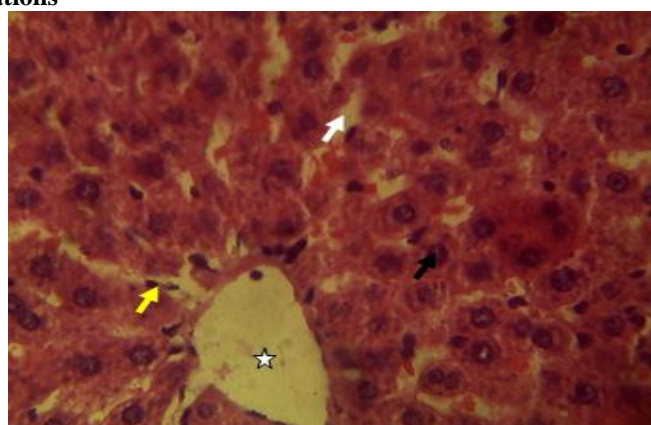
Parameters	Normal control (Group 1)	Untreated CCl <sub>4</sub> only (Group 2)	CCl <sub>4</sub> + 40mg/kg b.w FRJT (Group 3)	CCl <sub>4</sub> + 80mg/kg b.w FRJT (Group 4)	Standard control CCl <sub>4</sub> + 25mg/kg b.w Silymarin (Group 5)
Hb (g/dL)	17.16 ± 1.49	13.46 ± 2.34 <sup>a</sup>	13.86 ± 0.79 <sup>a</sup>	16.32 ± 1.18 <sup>b</sup>	13.38 ± 0.83 <sup>a</sup>
PCV (%)	47.60 ± 1.67	41.00 ± 2.24 <sup>a</sup>	46.80 ± 4.82 <sup>b</sup>	43.60 ± 3.85	45.40 ± 3.44
tWBC (mm <sup>-3</sup> )	7920.00±1493.99	5760.00±726.64 <sup>a</sup>	7440.00±167.33 <sup>bc</sup>	6640.00±517.69 <sup>a</sup>	5760.00 ± 792.47 <sup>a</sup>

<sup>a</sup> Indicates significant difference from the normal control value at p < 0.05; <sup>b</sup> indicates significant difference between pre-treated groups and the untreated group, while <sup>c</sup> indicates significant difference between FRJT pre-treated groups and the standard control. Data are expressed as means ± SD, n = 5.

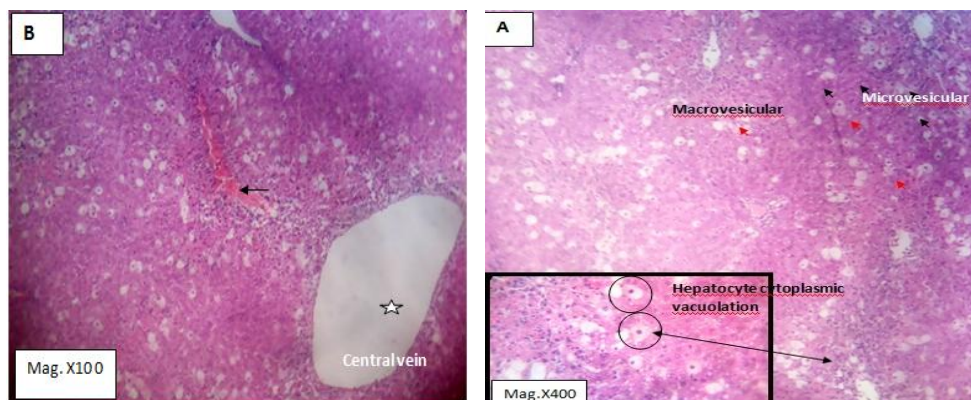
**Table 4:** Summary Of Histopathological Alterations In CCl<sub>4</sub>-Induced Hepatotoxic Rats.

Experimental groups	Degree of tissue changes (DTC)					
	Sinusoid dilation	Pyknosis	Fibrosis	Hepatic vacuolation	Liver steatosis	Congestion
Group 1 (Normal control)	-	-	-	-	-	-
Group 2 (CCl <sub>4</sub> + Untreated)	-	-	-	+++	+++	+
Group 3 (CCl <sub>4</sub> + 40mg/kg b.w FRJT)	+++	-	+++	-	-	-
Group 4 (CCl <sub>4</sub> + 80mg/kg b.w FRJT)	-	++	-	-	+++	++
Group 5 (CCl <sub>4</sub> +25mg/kg b.w silymarin)	-	-	-	+	-	+++

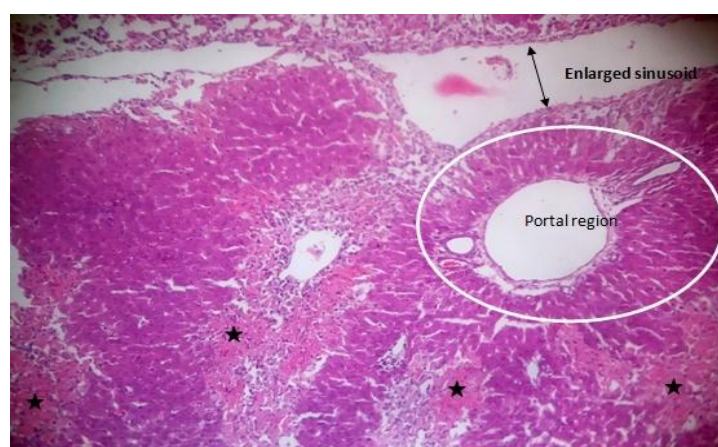
### 3.2 Histological observations



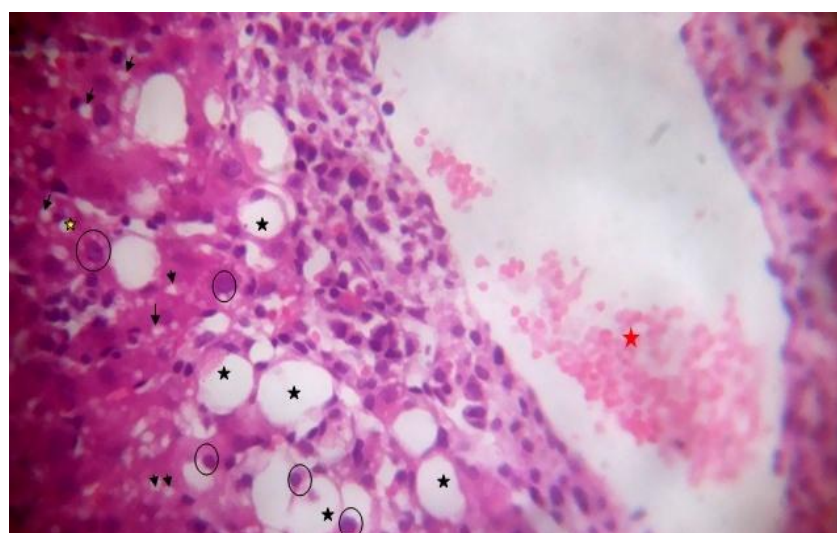
**Fig. 1:** Photomicrograph of the liver tissue of group 1 (normal control); Showing intact liver architecture; hepatocytes (black arrow), central vein (star) and kupffer cells (spindleoid cells) (yellow arrow) lining the sinusoids (white arrow). H & E. Mag x100.



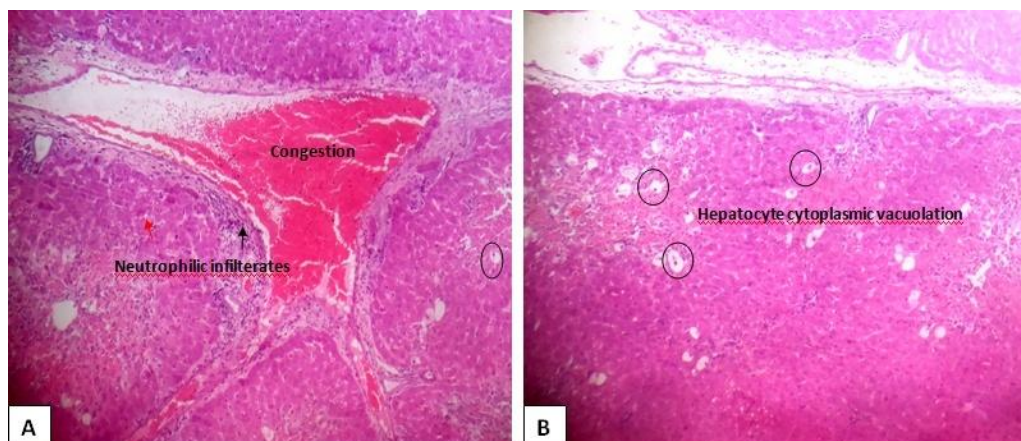
**Fig. 2:** Photomicrograph of the liver tissue of group 2 (CCl<sub>4</sub>-induced without treatment). (A). Showing hepatocyte cytoplasmic vacuolation (arrow head) (B). Similar hepatocyte cytoplasmic vacuolation and fatty overload was also seen but there was minor passive congestion (black arrow) surrounded by neutrophilic infiltrates. Central vein was also observed (star). H&E. Mag X100, X400.



**Fig. 3:** Photomicrograph of the liver tissue of group 3 (CCl<sub>4</sub>-induced + 40mg/kg b.w of FRJT); showing fibrosis (pericellular fibrosis) which surrounds hepatic lobules adjacent to portal areas (stars). Sinusoidal dilatation was evident (double arrowhead). Normal portal region showing bile duct, veins and arteries were observed. (white circle). H&E. Mag. X400.



**Fig. 4:** Photomicrograph of the liver of group 4 (CCl<sub>4</sub>-induced + 80mg/kg b.w of FRJT); showing cells appearing pyknotic (circles) with microvesicular (black arrow) and macrovesicular (star) fatty changes. RBCs (red star) were observed in the central vein and infiltrates of inflammatory cells mainly neutrophils were also observed. H&E. Mag. X400.



**Fig. 5:** Photomicrograph of the liver tissue of group 5 (CCl<sub>4</sub>-induced + 25mg/kg b.w of Silymarin). **(A).** Showing chronic non-passive congestion (centrolobular congestion) in between the lobular regions of the liver, together with mixed infiltrates mainly neutrophils. There was reduced hepatocyte cytoplasmic vacuolation (circles) and fatty changes mainly microvesicular (red arrow). **(B).** There was marked reduction of fatty changes in the liver, with reduced inflammation. H&E. Mag. X100.

#### IV. Discussion

The liver is very susceptible to damage by xenobiotic and non-xenobiotic induced oxidative stress. It is the major target organ of CCl<sub>4</sub>-induced toxicity owing to its high content of cytochrome P<sub>450</sub> enzyme. Cytochrome P<sub>450</sub> enzymes are believed to metabolize CCl<sub>4</sub> to trichloromethyl radicals that can initiate peroxidation of unsaturated fatty acid and initiate chain reactions of lipid peroxidation<sup>(25)</sup>. Most experiments involving CCl<sub>4</sub> induction is usually accompanied by elevation of liver enzyme markers<sup>(26)</sup>. This is because CCl<sub>4</sub> has been known to produce hepatic damage by generation of highly reactive trichloromethyl (CCl<sub>3</sub>·) and trichloromethylperoxy (CCl<sub>3</sub>OO·) radicals when metabolized by cytochrome P<sub>450</sub><sup>(27)</sup>. In the present study, there was significant elevation ( $p < 0.05$ ) in the activities of ALT, AST and ALP of untreated animals (Group 2) compared to the normal control (Group 1), indicating CCl<sub>4</sub> induced liver damage (see Table 1). ALT is a cytoplasmic enzyme found in very high concentrations in the liver and an increase in the serum of this specific enzyme indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function<sup>(28)</sup>. High level of ALP is an indication of obstructive jaundice and intra-hepatic cholestasis<sup>(26)</sup>. Elevated levels of these biochemical parameters are direct reflection of alterations in the hepatic structural integrity<sup>(29)</sup>. A previous research recorded a significant ( $p < 0.05$ ) elevation in ALT and AST as well as a non-significant ( $p > 0.05$ ) elevation in ALP activities of rats administered with methanolic extracts of *J. tanjorensis* which implied a negative effect on the liver<sup>(12)</sup>. However, in the present study, a significant reduction ( $p < 0.05$ ) was observed in the pre-treated groups compared with the untreated group. Also, no significant ( $p > 0.05$ ) difference was observed between these pre-treated groups and the normal control, indicating hepatoprotective/antioxidative effect of the flavonoid-rich fraction.

Natural antioxidant cell defenses include enzymes such as glutathione peroxidase, catalase and superoxide dismutase. When the balance between ROS production and antioxidant defense is lost, oxidative stress results, which through a series of events deregulates cellular functions leading to various pathological conditions<sup>(30)</sup>. Result of antioxidant enzyme assay in the present study showed that the activities of GPx and CAT were significantly ( $p < 0.05$ ) depleted while that of SOD was non-significantly ( $p > 0.05$ ) depleted consequent to CCl<sub>4</sub> induction in the untreated animals compared with the normal control. Depletion of these endogenous enzymes indicated CCl<sub>4</sub>-induced oxidative stress in the untreated group of rats. In contrast, SOD activity was found to be significantly ( $p < 0.05$ ) higher in groups 4 & 5 when compared to the untreated animals. GPx activity was significantly ( $p < 0.05$ ) higher in groups 3 and 4 while CAT activity was non-significantly ( $p > 0.05$ ) higher in groups 3 and 4 when compared to the untreated group. The effect of the flavonoid-rich fraction on these antioxidant enzymes within the FRJT pre-treated groups was also comparable to that of the standard drug (see Table 2). It was previously reported that *J. tanjorensis* is capable of inhibiting protein denaturation<sup>(31)</sup>. Also, supplementation of methanolic extracts of *J. tanjorensis* leaves in nutritionally-stressed rats resulted in significantly ( $p < 0.05$ ) higher levels of SOD and CAT in previous report<sup>(6)</sup>.

Previous study on the effects of CCl<sub>4</sub> on haematological parameters showed that acute CCl<sub>4</sub> toxicity led to transient decrease in Hb concentration as well as PCV and RBC counts<sup>(32)</sup> which is similar to the observation in the present study. CCl<sub>4</sub> induction also causes lymphopenia (a condition characterized by an abnormally low level of lymphocytes in the blood) in rats as reported<sup>(33)</sup>. Lymphocyte is a white blood cell with important functions in the immune system. In the present study, PCV, Hb and WBC levels were found to be significantly

( $p < 0.05$ ) lower in the untreated group when compared to the normal control. However supplementation with flavonoid-rich fraction of *J. tanjorensis* ameliorated the induced depletion of blood in the test animals as shown in Table 3. It was previously reported that *J. tanjorensis* is rich in iron (Fe) and this increases the amount of iron available for erythropoiesis<sup>(34)</sup>. Studies on the aqueous extract of the leaves of *J. tanjorensis* showed a statistically significant ( $p < 0.05$ ) elevation in the PCV and Hemoglobin concentrations of both male and female wistar rats, thereby justifying the local claim of the plant's use as a blood tonic<sup>(15)</sup>. The flavonoid-rich fraction of *J. tanjorensis* may, therefore, be used in physiological conditions such as pregnancy and menstruation when there is a drop in Hb and PCV. It may also be employed in stress-induced depletion of blood as a hematinic.

The result of the histological studies provided supportive evidence for the biochemical analysis. Histology of the liver section of the normal control group showed an intact liver architecture with well defined hepatocytes, central vein and kupffer cells or spindle cells lining the sinusoids (see Fig. 1). In contrast, the histology of the untreated group showed hepatocyte cytoplasmic vacuolation which appeared to be consistent with glycogen and macro vesicular fatty overload; but there was minor passive congestion surrounded by neutrophilic infiltrates. At the same time, liver steatosis (steatohepatitis) was also evident due to proliferated/or multiple micro and macro vesicular fatty change as a result of inability of cells to metabolise fat (see Fig. 2). The clear vacuoles contained lipids which are abnormal retention of lipids within a cell. The presence of fatty degeneration is associated with possible toxins due to the fact that the liver is the primary organ of lipid metabolism which is mostly associated with steatosis. Liver histology of group 3 (40 mg/kg b.w FRJT) showed a normal portal region with bile duct, veins and arteries which are normal parts seen in liver architecture. However, pericellular fibrosis surrounded hepatic lobules adjacent to portal areas, characterized by the formation of excess fibrous connective tissues which usually represent a reparative or reactive process. Hepatocyte cytoplasmic vacuolation was not observed in group 3 compared with the untreated group, suggesting that the flavonoid-rich fraction of *J. tanjorensis* leaves may have contributed to the prevention of severe damages to the hepatocytes. Liver histology of group 4 (80 mg/kg b.w FRJT) showed cells appearing pyknotic characterized with the development of micro and macrovesicular fatty changes (see Fig. 4). Pyknosis is a stage in the process of liver necrosis in which nuclear shrinkage transpires<sup>(35)</sup>. This is an indication that pre-treatment with the flavonoid-rich fraction may have prevented complete necrosis of the hepatocytes. Figure 5 showed that there was a marked reduction of fatty changes, showing reduced inflammation in group 5 (25 mg/kg b.w Sylimarin), compared with the untreated group. The summary of the degree of tissue changes is shown in Table 4.

## V. Conclusion

The flavonoid-rich fraction of *Jatropha tanjorensis* leaves exhibited convincing antioxidant effects in the present study by restoring liver enzymes, supplementing endogenous antioxidant defense and ameliorating depleted blood volume. Traditional claims suggested that decoctions of *J. tanjorensis* leaves were used to treat anaemia (as a haematinic agent), diabetes, skin diseases, malaria, arthritis and various cardiovascular diseases. The present study supports these local claims and suggests that these effects may be attributed to the presence of flavonoids/polyphenolic compounds in the plant extract, which makes it a potential supplement for the prevention of diseases associated with oxidative stress.

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