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**Effect of Flavonoid Rich Fraction of *Irvingia gabonensis* Seed Extract
 on *Tetrachloromethane* (CCl₄) – Induced Liver Damage in Mice**

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ABSTRACT

Herbal medicine has been consistent in the traditional treatment of diseases since the time of early man. Traditional medications have been used all around the world in the treatment of different human and animal diseases. Different types of plant have been utilized as traditional drugs. These drugs are widely used in most parts of the world, especially in Africa and Asia. According to the World Health Organization, above 80% of people living in Asia, Africa and some parts of America still depend on traditional medicine in the treatment of diseases. In this study, we investigated the hepato-curative effect of the flavonoid rich fraction of *Irvingia gabonensis* seed extract on *tetrachloromethane* (CCl₄) induced liver damage in mice for duration of two weeks and four weeks of extract administration. The study discovered a significant increase ($p \leq 0.05$) in serum ALT, AST, and ALP levels from groups IV to VI, as well as a slight significant decrease after two weeks of extract administration. Groups VII to IX showed declined in the level of biomarkers after four weeks of extract administration. This remarkable normalization in the mice liver enzymes (AST, ALT and ALP) in this study reveals a dose and time dependent curative medicinal potential of the flavonoid-rich fraction of *Irvingia gabonensis* seed extract in CCl₄ induced liver damage.

Keywords: Phytochemicals, Flavonoid, Medicinal, Liver, Hepato-curative

1.0 INTRODUCTION

The medicinal potentials of plants used for treating different diseases cannot be overemphasized. Plants' medicinal properties have long been studied and advocated for use in conventional drug development. Medicinal plants have been an integral part of the African healthcare system since time immemorial (Palmer, 2002). Interest and utilization of traditional medicine can be explained by the fact that it is a fundamental part of the culture of the people who use it and also due to the economic challenges

In Africa, the traditional use of plants in the treatment of illness has long been practice since prehistoric times. In the use of medicinal plants for disease treatment, several plant parts such as leaves, bark, berries, flowers, roots, seeds, and/or their extracts have been used, including whole plants in human therapeutics (Sofowara, 1993). These plant parts used are believed to contain some active ingredients responsible for their therapeutic potentials in disease treatment (Jamshidi *et al.*, 2018). The chemicals present in

plants, serving as bioactive ingredients in disease treatment are called phytochemicals. These phytochemicals are thought to be responsible for the plant's therapeutic potential. This medicinal potential includes scavenging activity, antibacterial, antimalaria, antifungal, anticancer, and so on. Some of the essential phytochemicals present in plants include flavonoids, alkaloids, tannins, steroids, terpenoids, glycosides and carotenoids (Sofowara, 1993). According to Palmer (2002), the World Health Organization reported that at least 80% of the population in Asia, Africa, and Latin America still rely on medicinal plants to meet their primary health care needs. This can be attributed to its accessibility, low cost, affordability, acceptability, availability, and apparently low toxicity (Jamshidi *et al.*, 2018).

Irvingia gabonensis is a plant that is widely distributed in Africa, specifically in West Africa. The plant is very important to the endogenous people of Africa as a source of food, medicine and fuel. Studies have been reported on the medicinal potential of the plant in the treatment

of hernia, yellow fever, diarrhea, dysentery, liver disease, sterility, and urethral discharge (Ayuk *et al.*, 1999, Shiembo *et al.*, 1996). Further study by Franzel *et al.*, (1996) stated the use of the plant in nutritional therapy for underweight babies. The plant has also been used in obesity moderation and normalizing lipidemia (Ngondi *et al.*, 2005). The antioxidant capacity of the plant has further been studied by Agbor *et al.* (2005). Etta *et al.* (2014) investigated the effect of the plant seed on the liver and gonads of male albino rats, and Ewera *et al.* (2021) investigated the plant's hypolipidemic therapeutic potential, all with promising results.

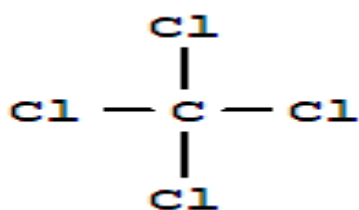


Figure 1: Structure of tetrachloromethane

1.1 Tetrachloromethane-Induced Liver damage

Tetrachloromethane is a chemical compound that causes severe liver damage by inducing hepatocellular destructive oxidative stress metabolic reaction. Tetrachloromethane binds to hepatocellular triacylglycerols and phospholipids through subcellular segments, resulting in lipid peroxidation in the liver parenchymal cells as reported by Starkel and Leclercq (2011). This is why it is regarded as the most widely used hepatic toxin for experimental induction of liver injury in laboratory animals. CCl_4 directly impairs

hepatocytes by altering the permeability of the cellular membrane, lysosomal and mitochondrial membranes (Constandinou *et al.*, 2005). Its metabolite, toxic trichloromethyl radical produced by CYP2E1 in liver cells, binds with oxygen to induce lipid peroxidation and membrane damage, causing severe centrilobular necrosis as seen from figure 1 (Starkel and Leclercq, 2011, Wu and Norton, 1996). Hepatocellular damage develops progressively, starting from pericentral hepatocytes, followed by severe bridging fibrosis and finally leading to cirrhosis (Domenicali *et al.*, 2009, and Frezza *et al.*, 1994). The degree of hepatocellular damage varies depending on species, strain, dose, route and frequency of administration. *The tetrachloromethane* model has been used extensively to study the pathogenesis of different liver conditions, including liver cirrhosis and fibrosis (Liu *et al.*, 2013).

Regardless of the intrinsic medicinal potentials of many plants or their parts, the efficacy of a single photochemical extract in disease treatment is yet to be fully explored in scientific research. The possibilities of generating beneficial effects from a single phytochemical extract of a plant can be overwhelming. In spite of the combined fundamental medicinal potentials of many plants, due to different phytochemical compounds, the efficacy of a flavonoid-rich extract in disease treatment is yet to be fully explored in drug design. Therefore, this study will investigate the medicinal properties of the flavonoid-rich fraction of *Irvingia gabonensis* seed extract on *tetrachloromethane* induced liver damage in mice.

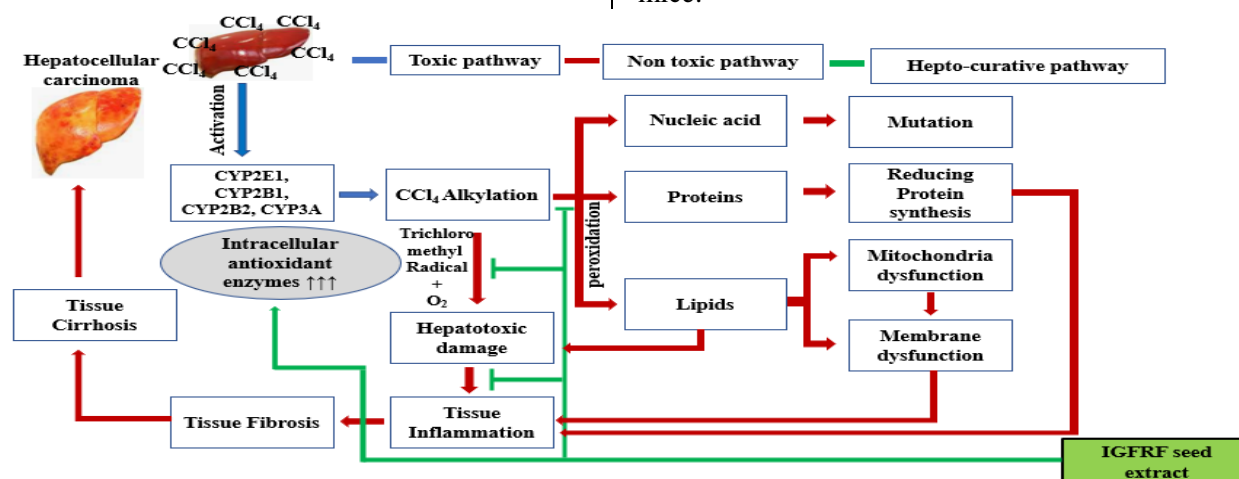


Figure 2: Mechanism of CCl_4 induced liver damage and hypothetical medicinal pathway of *Irvingia gabonensis* (IG) flavonoid-rich fraction.

2.0 MATERIALS AND METHODS

2.1 Defatting and Extraction of Plant Material

The plant seeds of *Irvingia gabonensis* were rinsed with water and shade-dried by spreading on sacks for two weeks. After drying, the plant seeds were pulverized with mortar and pestle after which they were weighed. They were kept in a sealed plastic bag duly labeled as described by Laghari *et al.* (2011). Defatting of the seed powder was accomplished using a non-polar solvent (n-Hexane) in a Soxhlet extractor at a 1:3 extract-to-n-hexane ratio, as described by Efosa *et al.* (2016). The powder residue was dried using a water bath while the oily – residual filtrate was subjected to a rotary evaporator, collecting the used hexane and concentrating the oil-extract. Crude extraction of plant material was done using microwave-assisted extraction (MAE). The defatted powdered form of the seeds was dissolved in 70% aq. ethanol and heated at 80°C for one minute in a microwave. The extract was filtered using a *Whatman* filter paper (No. 1) and the filtrate was concentrated under reduced pressure using a rotary evaporator (Efosa *et al.*, 2016).

2.2 Solvent Fractionations

The crude extract (filtrate) was partitioned using different solvents of increasing polarity index-petroleum ether, chloroform, ethyl acetate and methanol with the aid of a Soxhlet extractor at a temperature of 40°C for 2-3 hours. Solvent extracts obtained were concentrated under reduced pressure by a rotary evaporator and subsequently labeled.

2.3 Determination of Total Flavonoid Content (TFC)

The total flavonoid content of each solvent fraction was determined by Aluminium chloride colorimetric assay with slight modifications as reported by Laghari *et al.* (2011). Quercetin was used as a standard to construct the calibration curve. The flavonoid content was calculated as mean \pm SD (n=3) and expressed as mg/g of quercetin equivalent of dry *Irvingia gabonensis* seed extract.

The flavonoid content of each solvent fraction was estimated using the linear regression equation obtained from the standard curve of quercetin.

$$y = 0.578x + 0.0954, r^2 = 0.995$$

Where y = Absorbance of each solvent fraction.

$$\text{Flavonoid Content} = \frac{V \times C}{M}$$

Where C = concentration of quercetin from the calibration curve in mg/ cm³, V = volume of extract in cm³ and M = weight of extract in grams.

2.4 Isolation of Total Crude Flavonoid Extract

The ethyl acetate fraction was dissolved in water and extraction was done with chloroform in a separating funnel and 10% NaCl solution was added drop wise to the aqueous layer in order to precipitate out the tannins. The supernatant was

then partitioned with ethyl acetate and the solvent was evaporated to yield the crude flavonoid fraction (Laghari *et al.*, 2011).

3.1 Reducing Power Assay (RP)

The reducing power of the ethyl acetate extract of *Irvingia gabonensis* was determined by the method of Oyaizu (1986). The assay method is based on the principle of increase in the absorbance of the reaction mixtures. The higher the absorbance value, the higher the antioxidant activity.

3.2 Induction of Liver Damage in Mice

Tetrachloromethane (CCl₄)-induced liver damage was achieved by injecting 3 ml/kg of CCl₄ (3 ml/kg body weight dissolved in groundnut oil) intraperitoneally after fourteen days of animal's acclimatization and feeding with pellet and water *ad libitum* as described by Adewale *et al.* (2014).

Experimental Animals

Twenty-seven (27) male albino mice of about five to six weeks old, weighing between 25 – 34g were purchased from Department of Pharmaceutical Science, Kaduna State University. The mice were randomly distributed into nice groups of three mice each (Group I, II, III, IV, V, VI, VII, VIII, and IX).

The mice were acclimatized for fourteen days and maintained under standard conditions of temperature (23°C \pm 2°C), control humidity ad 12-hours light/dark cycle. The mice were fed standard feed and water was given to them *ad libitum*. The animals handling protocols were adopted based on the guidelines of the National Institution of Health for laboratory animal cares (NIH, 1998). Animals in group I served as the normal control group with no extract and CCl₄

given to them, while animals in group II served as the negative control group. Animals in this group were administered CCl_4 but no extract was given. Group III animals served as positive control group. Animals in this group were administered with CCl_4 , followed by daily administration of Resveratrol $0.6\text{mg}/\text{cm}^3/\text{kg}$ body weight dissolved in distilled water. Interestingly, animals in group IV-VI were administered CCl_4 with subsequent daily administration of 25, 50 and $100\text{mg}/\text{kg}$ of the flavonoid rich extract for two weeks while animals in group VII to IX were given the same dose of the extract for four weeks. Twenty-four (24) hours after last feeding at two weeks and four weeks, blood samples were obtained from each experimental mouse via the

3.0 RESULT AND DISCUSSION

heart vessels into non-heparinized bottles for organ function analyses.

Determination of Biochemical Indices

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities were carried out according to the procedure described by Reitman and Frankel (1957), While Alkaline Phosphatase was determined according to the method adopted by Rec (1957)

Statistical Analysis

Individual group difference was analysed by one-way analysis of variance (ANOVA) using SPSS 2014 at 5% level of significance ($P \leq 0.05$).

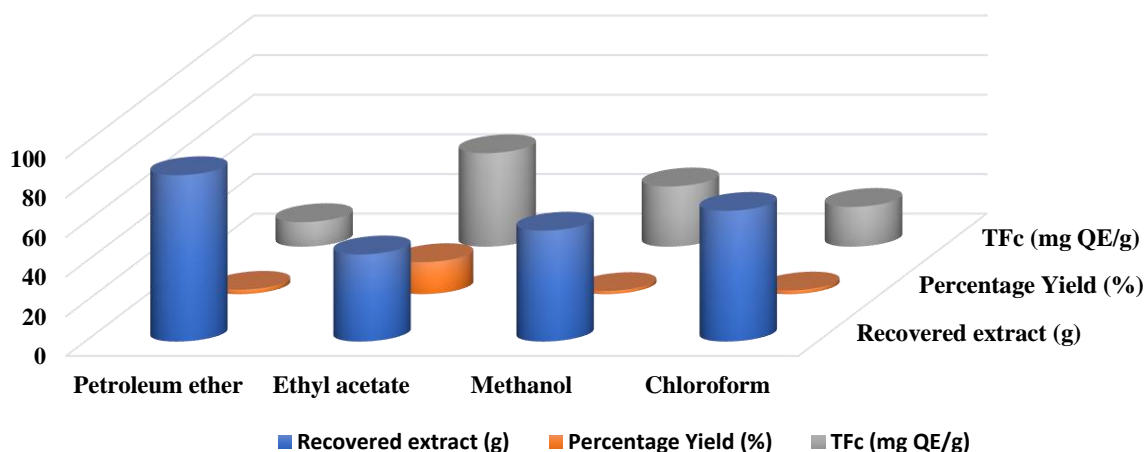


Figure 2: The TFCs of the various crude extracts expressed in terms of Quercetin (QE)

The TFCs of the various crude extracts are expressed in terms of Quercetin (QE) and are presented in above. Figure 2 shows the total flavonoid extracts extrapolated from four different extract of increasing polarity. Among

the different solvents used, highest amount of flavonoid ($47.20\text{mg QE}/\text{g}$) was recorded in Ethyl acetate extract with Petroleum ether having the lowest flavonoid content.

Table 1: Reducing Power (RP) of the Flavonoid rich extract (ethyl acetate fraction) of *Irvingia Gabonensis* (IG) Compared with Ascorbic Acid Standard.

Concentration ($\mu\text{g}/\text{ml}$)	IG Extract Absorbance values	Vitamin C Absorbance values
20	0.33 ± 0.02	0.25 ± 0.01
40	0.34 ± 0.03	0.28 ± 0.01
60	0.41 ± 0.02	0.30 ± 0.01
80	0.47 ± 0.04	0.33 ± 0.01
100	0.48 ± 0.01	0.36 ± 0.01

Values are measure of triplicate estimation Means in the same column at $P \leq 0.05$ significant difference

Reducing power of a phytochemical compound is based on the level of antioxidant activity of that compound (Oktay *et al.*, 2003). In the present study, the reducing power of *Irvingia gabonensis*

ethyl acetate seed extract increased in a concentration-dependent manner and was found to be slightly higher than that of the ascorbic acid standard used (Table 1). This data reflects the

significant antioxidant activity of the flavonoid rich fraction of the seed and may justify the use of *Irvingia gabonensis* seed extract for traditional or cultural medicinal use.

The fruit of the plant was reported to be rich in vitamin C and is consumed as a desert fruit throughout Western and Central Africa (Leakey and Newton, 1994).

Several studies have revealed that antioxidant activity may be from compounds, such as

flavonoid, isoflavones, flavones, anthocyanins, catechins, and other phenolics (Kahkonen *et al.*, 1999, Alothman and Bhat, 2009 and Isabelle *et al.*, 2010). The high anti-inflammatory and scavenging effect of flavonoid has been reported to be due to the oxidizing species, hydroxyl groups complexes present in the flavonoid structure which led to stabilization of free radicals and reduction of oxidative damage (Manach *et al.*, 2004).

Table 2: Serum Liver Function Indices of CCl₄-induced Liver Damage in Mice after Two- and Four-Weeks Oral Administration of Flavonoid Rich Fraction of *Irvingia gabonensis*.

Groups	AST (U/dl)	ALT (U/dl)	ALP (U/dl)
Group I	82.63±6.56 ^c	32.13±5.29 ^d	52.87±7.64 ^d
Group II	183.13±3.85 ^a	63.53±5.80 ^a	168.77±4.29 ^a
Group III	73.87±9.87 ^c	34.37±4.02 ^{cd}	54.07±6.47 ^d
After two weeks administration			
Group IV	120.04±5.03 ^b	50.03±5.06 ^b	94.26±7.01 ^b
Group V	103.93±8.30 ^{bc}	46.37±4.64 ^c	86.78±7.58 ^{ab}
Group VI	95.87±8.65 ^c	42.83±6.56 ^{bc}	81.67±5.13 ^c
After four weeks administration			
Group VII	97.74±7.61 ^c	42.67±6.69 ^{bc}	84.33±7.02 ^{ab}
Group VIII	90.08±8.47 ^c	38.13±2.71 ^c	73.67±3.06 ^{cd}
Group IX	83.67±4.16 ^c	35.23±2.36 ^{cd}	56.03±5.51 ^d

Values are measure of triplicate estimation Means in the same column followed by different letters are significant at $P \leq 0.05$, IG = *Irvingia gabonensis*

The above result shows a significant difference ($P \leq 0.05$) between the experimental groups in the liver parameters analyzed (Table 2). Significant increase ($P \leq 0.05$) in the serum level for ALT, AST, ALP from groups IV to VI and a significant decrease after two weeks of extract administration was observed. While groups VII to IX had reduced in the level of biomarkers after four weeks of extract administration. The normalization in the mice liver enzymes (AST, ALT and ALP) in this study reveals a curative medicinal effect of the flavonoid rich fraction of *Irvingia gabonensis* seed extract. CCl₄ elicited the elevation in the levels of liver marker enzymes (AST, ALT, and ALP) resulting in significant hepatic damage. The elevated levels of these biochemical parameters are direct reflection of alterations in the hepatic structural integrity of the mice livers. The elevation of liver marker enzymes in this study correlates with the findings of Prakash *et al.* (2008) who observed significant hepatic damage in rats treated with a single dose of CCl₄. The damage indicates a

cellular leakage and loss of functional integrity of the liver. However, the normalization in the levels of marker enzymes ALT and AST in mice administered with flavonoid rich fraction of *Irvingia gabonensis* seed extract as reported in this study is also in agreement with the accepted view of Adewale *et al.* (2014). The view stated that serum levels of transaminases return to normal with healing hepatic parenchyma and the regeneration of hepatocytes. Interestingly, Okolo *et al.* (1995) earlier speculated on the use of the plant bark in the treatment as analgesic in traditional medicine. The results in this study suggest that treatment of mice for two weeks and four weeks with *Irvingia gabonensis* flavonoid rich seed extract at varying dosages to CCl₄ administration significantly reversed the increase in serum liver enzymes as compared with the negative control. It would be deduced, therefore, that the antioxidant effects of *Irvingia gabonensis* flavonoid rich seed extract could perhaps be the reason behind the hepato-curative effect recorded in this study. Consequently, the findings in this

study indicated that treatment of mice with *Irvingia gabonensis* flavonoid rich seed extract for duration of time after administration of CCl₄ can cause a marked decrease in the levels of hepato-specific serum enzymes. It is believed that the flavonoid rich fraction enhances the activity of intracellular antioxidant enzymes to reduce trichloromethyl oxidative capacity, with subsequent down-regulation of CCl₄ alkylation, and blocking peroxidation leading to structural destabilization. Anti-inflammatory activity of the extract reduced tissue inflammation and increase the activation of anti-inflammatory molecules while down-regulating pro-inflammatory molecules as seen in figure 2 above. It thus implies that *Irvingia gabonensis* flavonoid rich seed extract may be a potential cure against CCl₄-induced liver damage.

4.0 CONCLUSION

The utilization of flavonoid rich fraction of *Irvingia gabonensis* seed extract in this study has generated a promising outcome in the treatment of liver damage. The result obtained in this study could be due to the high antioxidant activity of the flavonoid rich fraction leading to oxidative inhibitory potential of extract. Therefore, the flavonoid rich fraction of *Irvingia gabonensis* seed may be potential source of alternative medicine in the treatment of liver diseases.

5.0 RECOMMENDATION

This study could be very helpful in the exploitation of the medicinal properties of flavonoids present in the plant part in the treatment of liver diseases. Consumption of the plant part might also be recommended, especially for people with preexisting liver problem.

ETHICAL APPROVAL

All authors declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of Bayero University Kano with the ethical approval number BUK/CHS/REC/112.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used 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 the personal efforts of the authors.

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