# Antihepatotoxic Effects Of *Ficus Vogelii* Ethanol Leaf Extract On The Liver Function Indices Of $Ccl_4$ – Induced Hepatotoxicity In Rats.

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**ABSTRACT:** This study was conducted to evaluate the anti-hepatotoxic effect of intraperitoneal administration of ethanol extract of *Ficus vogeli* (600mg/kg) in $CCl_4$-induced hepatotoxicity in male albino rats. Phytochemically, the leaf extract contains tannin, alkaloid, flavonoid, carbohydrates, protein, saponin, steroids, terpenoids, fats and oil. The administration of the *Ficus vogelii* extract was at one phase of the experiment according to the body weight of the test animals. The ethanol extracts of *Ficus vogelii* significantly reduced ($p<0.05$) the level of activity of the hepatic enzyme markers in the serum (Alanine amino transferase (ALT), Aspartate amino transferase (AST), alkaline phosphate (ALP) and total bilirubin) which occurred due to induced oxidative stress. Relative to the control group, treatment with $CCl_4$ significantly raised the levels of ALT, ASP, AST and total bilirubin in the serum. The animals that received *Ficus vogelii* showed not only reduced hepatocellular degeneration but also of hepatocellular regeneration when compared to the liver of those exposed to $CCl_4$ alone. Thus the histopathological studies also supported the anti-hepatpotoxic action of the ethanol extract of *Ficus vogelii*. The results of this study clearly indicate that *Ficus vogelii* ethanol extract has a potent anti-hepatotoxicity action against carbon tetrachloride induced liver damage in rats.

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**Keywords**: *Ficus vogeli*; Hepatotoxicity; Carbon tetrachloride; Liver Function Tests

## INTRODUCTION

Liver plays a vital role in regulating various physiological processes such as secretion, storage and metabolism. It detoxifies toxic substances and drugs absorbed in the body from the intestine which expose it to harmful substances that lead to the damage of the liver. Liver disease remains one of the major health problems and is quite challenging to the health care professionals including the pharmaceutical industry and drug regulatory agencies.

In addition, hepatic viral infections (hepatitis, A, B, C and D e.t.c) and the microbial infections of *Entamoeba histolytica* aids in hepatocellular damage. Globally, plant based drugs like *Silybium marinum, Picorrhiza kurna, Phyllanthus embalica* and *Phyllanthus amarus* are experimentally proven and successfully used in the clinical treatment of liver disorder (Thyagarajan *et al.,* 2002) These herbal drugs are rich in antioxidants which aid to cure liver diseases and they are widely prescribed in this modern age. It is therefore very important to provide scientific prove to justify the various medicinal uses of herbs.

The body cells and tissues identify almost all drugs or substances as non-self (i.e. xenobiotics) thereby subjecting them to various chemical processes (i.e. metabolism) to make them suitable for elimination. Almost all the tissue in the body has the ability to metabolize chemicals but the smooth endoplasmic reticulum in liver is the principal metabolic agent for both endogenous and exogenous substances. This exposes it to drug induced injury.

Many factors aid in the induction of hepatotoxicity. They include the following; age, nutritional status, gender, duration and dosage of drugs, ethnicity and race. Certain chemicals and drugs that induce liver damage are Carbon tetrachloride, Chloropromazine, Carbamazepine, Oral Contraceptive pills and tetracycline.

$CCl_4$ was formerly used as fire extinguisher and as a precursor for refrigerants but its applicability declined due to its severe adverse effect. It is among the most potent hepatotoxins. Under high temperature, it forms poisonous phosgene. It is synthesized from methane as shown in Fig. 1 below.

*Ficus* belong to the family of Moraceae. It is a genus of about 800 species and collectively known as Figs. It was indicated that *Ficus* is a relatively ancient species being about 60million years old. The fruits of most *Ficus* are edible while some are used as food resources for wild-life. Most culture uses *Ficus* as an object of worship while some use it for medicinal purposes.

The study aims at investigating into the effect of ethanol extract of *Ficus vogelii* on the liver of rats intoxicated with $CCl_4$.

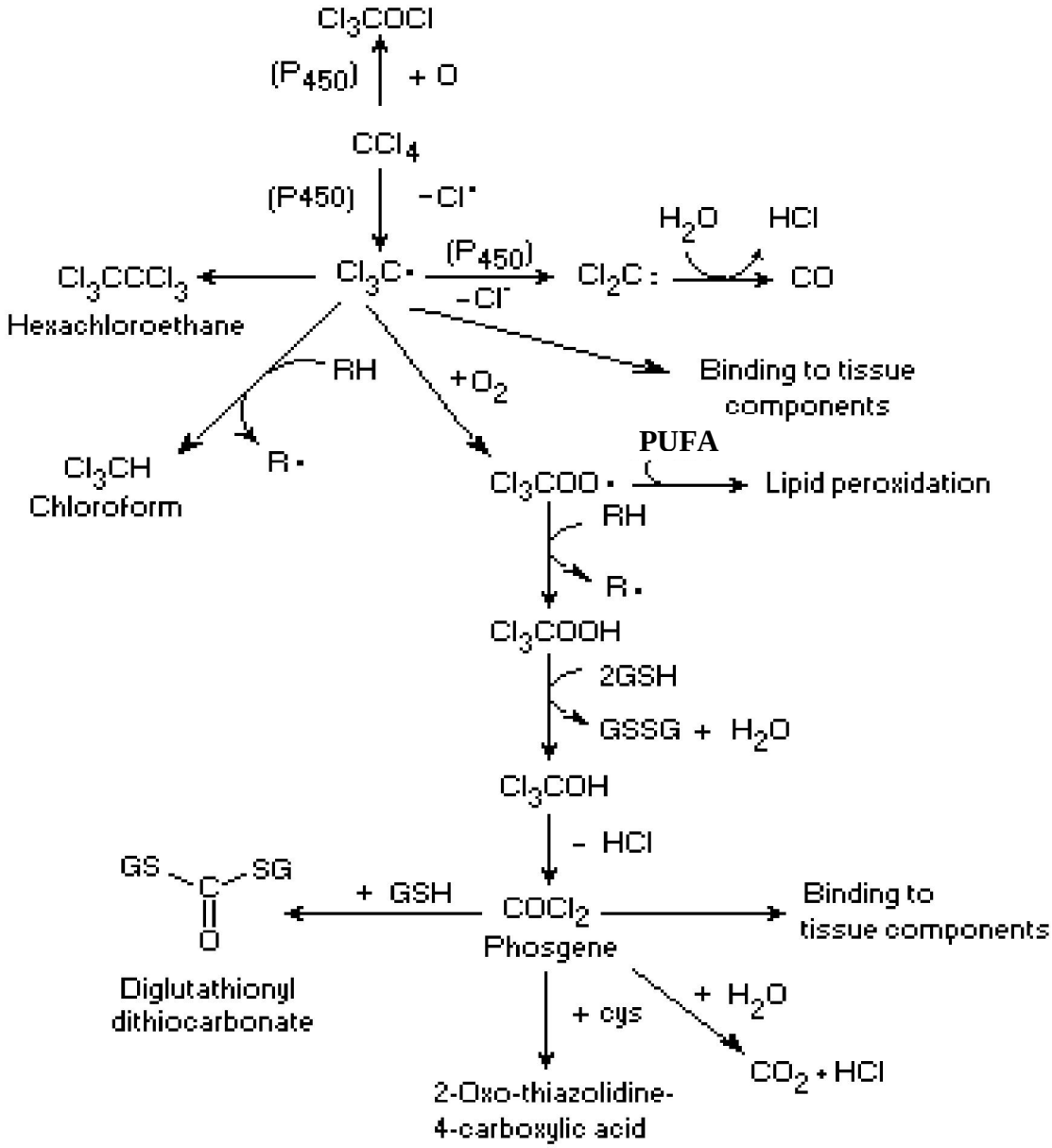


Fig. 1: Biotransformation of carbon tetrachloride
(From Harris and Anders, 1981; Anders and Jakobson, 1985; McGregor and Lang, 1996)

## MATERIALS AND METHODS

**Animals.** Twenty (20) albino rats and nine mice were obtained from the Animal House of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

**Plant Materials.** The leaves of *Ficus vogelii* were used for this study. They were collected in the forest within Nsukka environ by a botanist at Botany Department, University of Nigeria, Nuskka on the 5th of May, 2008**.**

The freshly collected leaves of *Ficus vogelii* were chopped into pieces and dried for two weeks at room temperature. The dried leaves were weighed and50g was defatted using petroleum ether (60 – 80°c) in a chamber.

**Preparation of Plant Extract.** The defatted leaves were macerated for two days using 1,200ml of 70% ethanol as the solvent. The filtrate obtained was concentrated using a rotary evaporator to remove the

ethanol content and kept in an oven at 40°c to remove the water content of the extract to obtain a semi-solid extract and dark brown vicious residue. The extract was subjected to phytochemical analysis for the presence of alkaloids, saponins, reducing sugars, oils, resins, fat, emulsion, and steroids using the method described by Harborne (1973).

**Concentration of Extract.** The extract was concentrated using the rotary evaporate to remove the ethanolic content of the extract and also the laboratory bench oven at 40°c to remove any trace ethanol and water content of the extract. A watch glass was weighed and 2ml of the extract was poured into it and weighed again. The extract with the watch glass was reweighed until a constant weight is achieved.

**Determination of Yield of Plant.** A quantity of the air dried chopped *Ficus vogelii* leave was packed into the soxhlet extractor. The weight of the evaporated extract was calculated using the formula:

$$\frac{Weight(g)\ of\ Evaporated\ Extract}{Weight(g)\ of\ Packed\ Ficus\ vogelii} \times 100$$

**Preparation of Working Materials**

a) **Normal saline:** 0.9g of NaCl was dissolved in 100ml of distilled water.
b) **Five percent ethanol in normal saline** was prepared and used as the vehicle in dissolving the extract .The 5% ethanol was gotten by measuring 5ml of 96% ethanol into a 250ml beaker and 95ml of distilled water was added to give a 100ml solution.
c) **Stock Solution:** A quantity, 0.4g of extract of *Ficus vogelii* was dissolved in 5ml of normal saline to give 400mg in 5ml of solution (80mg/ml). 1ml of the stock solution was added to another test tube and 4ml of normal saline was added to make it 5ml solution containing 80mg/5ml which is 16mg/ml.
d) **$CCl_4$ Model for Evaluation of anti-hepatotoxic activity:** The $CCl_4$ model described by Rojikind (Rojikind 1973) was used for scheduling the dose regimen. 1.5ml/kg i.p.of $CCl_4$ diluted in olive oil (1:3) was employed for inducing liver toxicity.

**Experimental Design**

Twelve albino rats were used for this study with three rats each in groups of four.

**Group One**: The rat weighing (173.8g 162.0g & 171.4g) was used for this group. This group was administered intraperitoneally at the dose of 1.5ml/kg b. w. of the carbon tetrachloride before the extract at the dose of 600mg/kg b. w.

**Group Two:** The rats weighing (159.7g, 155.6g & 159.0g) were used for this group. This group was administered intraperitoneally at the dose of 1.5ml/kg b.w. with the toxicant (carbon tetrachloride).

**Group Three**: The rat weighing (153.0g, 155.6g & 157.1g) was used for this group. This group were administered intraperitoneally at the dose of 5ml/kg b.w. of the vehicle (olive oil) together with water and fed.

**Group Four:** The rats weighing (145.6g 147.7g & 147.0g) was used for this group. This group were given the normal fed and water li bidum.

**Determination of Serum Glutamate Pyruvate Transaminase (SGPT) or Alanine Amino Transaminase (ALT).**

The colorimetric method for *in-vitro* determination of SGPT/ALT in serum was done using a Quimica Clinica Applicada test kit (Reitman, et al; 1957). Here, 0.5ml of Reagent A was put in a test tube and incubated for 5minutes at 37°C. 0.1ml of serum sample was added to the incubated sample and incubated again for 30minutes at 37°C. The standards were prepared as follows:

- Tube 1—0.1ml deionized water + 0.5ml Reagent A
- Tube II—0.1ml deionized water + 0.45ml Reagent A + 0.05ml of standard.
- Tube III—0.1ml deionized water + 0.40ml Reagent A + 0.10ml of standard
- Tube IV—0.1ml deionized water + 0.35ml Reagent A+ 0.15ml of standard
- Tube V--0.1ml deionized water + 0.30ml Reagent A + 0.20ml of standard

Next, 0.5ml of colour developer (Reagent B) was added to sample tubes and the standards, allowed to stand for 20minutes at room temperature.0.5ml of NaOH working solution (diluted Reagent C) was added and allowed to stand for 15minutes at room temperature. The transmittance of the samples and the standard were read at 505nm against deionized water blank. The mixture is stable for up to an hour. The SGPT/ALT activity/values of the samples were gotten by interpolating the transmittance obtained for the samples in the calibration curve made from the standards. The results were express in SI units (International units per liters (IU/L)).

**Determination of Serum Glutamic Oxaloacetic Transaminase (SGOT) or Aspartate Amino Transferase (AST).**

The colorimetric method for *in-vitro* determination of SGOT/AST in serum was done using a Quimica Clinica Applicada test kit (Reitman, et al;

1957). Here, 0.5ml of Reagent A was put in a test tube and incubated for 5minutes at $37^0$C. 0.1ml of serum sample was added to the incubated sample and incubated again for 60minutes at $37^0$C. The standards were prepared as follows:

- Tube 1—0.1ml deionized water + 0.5ml Reagent A
- Tube II—0.1ml deionized water + 0.45ml Reagent A + 0.05ml of standard.
- Tube III—0.1ml deionized water + 0.40ml Reagent A + 0.10ml of standard
- Tube IV—0.1ml deionized water + 0.35ml Reagent A+ 0.15ml of standard
- Tube V--0.1ml deionized water + 0.30ml Reagent A + 0.20ml of standard

Next, 0.5ml of colour developer (Reagent B) was added to sample tubes and the standards, allowed to stand for 20minutes at room temperature.0.5ml of NaOH working solution (diluted Reagent C) was added and allowed to stand for 15minutes at room temperature. The transmittance of the samples and the standard were read at 505nm against deionized water blank. The mixture is stable for up to an hour. The SGOT/AST activity/values of the samples were gotten by interpolating the transmittance obtained for the samples in the calibration curve made from the standards. The results were express in SI units (International units per liters (IU/L)).

**Determination of Serum Alkaline Phosphatase**

The phenolphthalein monophosphate method for the *in vitro* determination of alkaline phosphatase in serum using Quimica Clinca Applicada test kit. Here, the colour developer was prepared by adding one vial of colour developer salt to 250ml of de-ionized water. 1.0ml of deionised water was added to a clean test tube and a drop of chromogenic substrate was added also, mixed and incubated at $37^0$C for 5minutes. 0.1ml of serum sample was added to the test tube, mixed and incubated at$37^0$C for 20minutes. 5ml of colour developer was added. The absorbance was read against a water blank at wavelength 550nm.For the standard, 1ml of water was added to a test tube and a drop of the chromogenic substrate, mixed and incubated at $37^0$C for 20minutes before the addition of 5ml of colour developer and the absorbance was read at 550nm. The result was express in SI unit (IU/L)

**Determination of Serum Bilirubin**

The *in vitro* determination of total bilirubin in serum was done using Quimica Clinica Applicada bilirubin test kit (Doumas *et al.*, 1973). In this case, 0.2ml of sulphuric acid solution (Reagent A) was poured into a test tube and a drop of sodium nitrite (Reagent D) and 1ml of caffeine solution (Reagent B) were added with 0.05ml of serum sample. The contents were mixed and allowed to stand for 10minutes at room temperature. Lastly, 1ml of tartarate solution (Reagent C) was added, mixed and allowed to stand for 5minutes at room temperature. The absorbance was read at 578nm against the blank content. The result was expressed in SI unit (mg/dl).

**Statistical Analysis**

The values were expressed as mean ± SEM (Standard Error Measure). The statistical analysis was carried out by one way analysis of variance (ANOVA). The Pearson value ($p<0.05$) were considered significant. Biochemical parameter results are presented in the Tables and Figures respectively. The descriptive statistics for total bilirubin, liver enzymes and lipid profile were analyzed using SPSS.

## RESULTS

**Effect on the Serum Glutamate pyruvate Transferase (SGPT) or Alanine aminotransaminase (ALT)**

Significant difference ($P<0.05$) exists in comparing group II with groups I, III, IV, and V. Non-significant difference ($P>0.05$) exists in comparing groups I with groups III, IV, and V. This graph showed a significant increase in group II when compared with other groups.

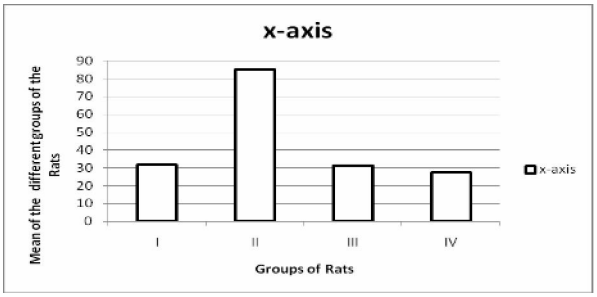


Fig. 1: The effect of *Ficus vogelii* on Alanine aminotransferase (ALT) or SGPT.

**Effect on the Serum Glutamate Oxalo-acetate Transferase (SGOT) or Asparate aminotrancferase (AST):**

Fig. 2 shows that significant difference ($P<0.05$) exists in comparing group II with groups I, III, IV, and V. Non-significant difference ($P>0.05$) exists in comparing groups I with groups III, IV, and V. The graph (Fig. 2) shows a significant increase ($P<0.05$) in group II when compared with other groups.

**Effect on the Serum Alkaline Phosphatase (ALP):**

Significant difference ($P<0.05$) exists as shown in Fig. 3 in comparing group II with groups I, III, IV, and V. Non-significant difference ($P>0.05$)

exists in comparing group I with groups III, IV, and V. Fig. 3 below shows a significant increase (P<0.05) in group II when compared with other groups.

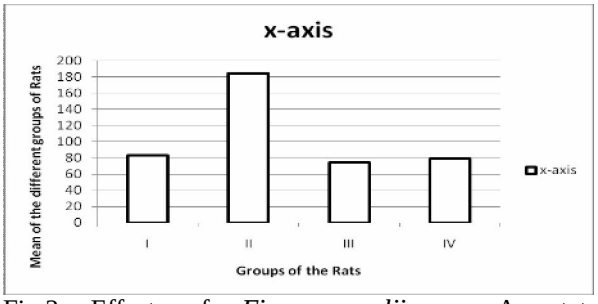


Fig.2 Effect of *Ficus vogelii* on Aspartate aminotransferase (AST) or SGOT

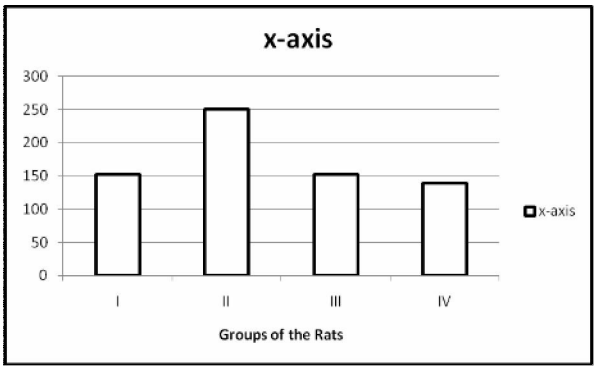


Fig. 3 Effect of *Ficus vogelii* on Alkaline Phosphatase (ALP)

**Effect on the serum Total Bilirubin:**

In Fig. 4, significant difference (P<0.05) exists in comparing group II (the toxicant) with group I (the toxicant + 600mg/kg b. w. of Ext.), group III (5ml/kg b. w. of olive oil),group IV (600mg/kg b. w. of Ext. + the toxicant) and group V (control) and vice versa. Non-significant difference (P>0.05) exists in comparing group I with group III, group IV, and group V; group III with group I, group IV and group V; group IV with group I, group III, and group V and the group V with group I, group III and group IV.

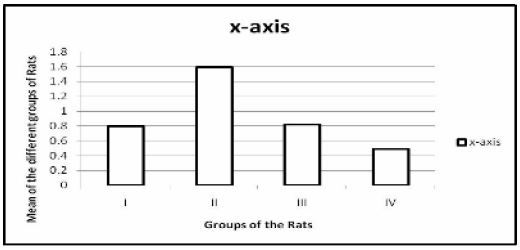


Fig. 4 The Effect of *Ficus vogelii* on the Total bilirubin

Table 1: Effect of *Ficus vogelii* on the Serum levels of AST,ALT, ALP and bilirubin of rats induced with$CCl_4$

| GROUPS | TREATMENTS | AST(U/L) | ALP(U/L) | ALT(U/L) | Bilirubin |
|---|---|---|---|---|---|
| **I** | $CCl_4$(1.5ml/kgi.p.)+600mg/kg i.p of Ext. | 82.8500±13.4 | 152.5867±14.2 | 31.800±12.4 | 0.7933±1.1 |
| **II** | $CCl_4$ (1.5ml/kg i.p.) | 184.3600±17.2 | 250.3533±27.8 | 85.3500±10.5 | 1.6433±0.1 |
| **III** | 5ml/kg(1:3) of olive oil | 74.2467±6.8 | 152.9400±17.7 | 31.22633±6.2 | 0.8200±3.8 |
| **IV** | Control | 78.9267±16.9 | 139.4100±59.3 | 27.4400±21.5 | 0.5767±0.2 |

## DISCUSSION

By nature, the liver possesses regenerative capacity and this should be considered in the experimental design by including a toxic control. The elevation in the serum marker analysis namely AST, ALT and ALP is one of the causes of hepatotoxicity of $CCl_4$ (Kaplowitz *et al.*, 1986). The liver function test studies demonstrate that $CCl_4$ (compared to normal) induces degeneration in hepatocytes. Following the spectrophotometric reading, increase in the liver enzyme induced by $CCl_4$ was remarkably reduced by the administration of the extract obtained from *Ficus vogelii* which is in good agreement with the histopathological result. This may be attributed to the recovery of the liver damage induced by $CCl_4$.

The hepatotoxicity effect of $CCl_4$ is largely due to its active metabolite "Trichloromethyl radical (Srivastava *et al.*, 1990), which binds to the macromolecule and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (Sengottuvelu *et al.*, 2007). The tichloromethyl radical also causes functional and morphological changes in the cell membrane. The hepatic cells are involved in a variety of metabolic activities. They consist of higher concentration of AST and ALT in the cytoplasm and AST in particular exist in mitochondria (Kumar *et al.*, 2007). Due to the damage caused to hepatic cells, the leakage of the membrane will lead to increase in levels of hepatospecific enzyme in serum. The elevated serum

enzyme levels like AST and ALT are indicative of cellular leakage and functional integrity of cell membrane in liver. The antihepatotoxicity of a drug can be evaluated by its capacity to cure the injuries or to restore the normal hepatic physiology by regeneration mechanisms, which have been induced by a hepatotoxin. This study has, therefore, shown that ethanol extract of *F. vogelii* contain an antioxidant effect that leads to its hepatocurative effect.

The results of this study indicate that the ethanol extract of *F. vogelii* could protect liver against $CCl_4$ induced hepatotoxicity. The component of the plant that is responsible for this effect was not investigated though the phytochemical analysis indicate the presence of alkaloids, flavonoids, carbohydrates, protein, saponins, tannins, Fats and oil, steroids and terpenoids. Most of this anti-oxidant is known to have anti-inflammatory property while most of them aid in the regeneration of a damaged liver (Kumar *et al*., 2007). In order to elucidate the mechanism by which *Ficus vogelii* extract component exhibit the anti-hepatotoxicity effect which was demonstrated in this study, further studies with the isolated components will follow to indicate the component of the leaf that has the antihepatotoxic activity.

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