

Evaluation of the Hepatoprotective Efficacy of *Moringa oleifera* Seed Oil on CCl₄-Induced Liver Damage in Wistar Albino Rats

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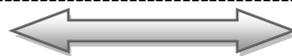
ABSTRACT

Studies have shown that certain parts of *Moringa oleifera* Lam plant, such as its leaves, roots, flowers, and seeds possess antihepatotoxic activity. Also in recent times, there has been a surge in local demand for *Moringa* seed oil, largely due to its reportedly high medicinal potential. The study was aimed at evaluating the hepatoprotective activity of *Moringa* seed oil against carbon tetrachloride (CCl₄)-induced hepatotoxicity in Wistar albino rats. Using the intragastric tube, *Moringa* seed oil (2mL/kg body weight) was administered orally on the CCl₄-intoxicated Wistar rats. Antihepatotoxic effects of *Moringa* seed oil was evaluated by assaying for the activities of liver marker enzymes alanine transaminase (ALT) (EC 2.6.1.2), aspartate transaminase (AST) (EC 2.6.1.1), and alkaline phosphatase (ALP) (EC 3.1.3.1) in serum. Histopathology of liver sections was also examined.

Substantially elevated serum activities of hepatic marker enzymes (ALT: 123.7±1.05 U/l and AST: 133.30±5.95 U/l) in CCl₄-intoxicated rats were significantly ($p < 0.05$) lowered towards control values (ALT: 64.33±11.90 and AST: 106.00±17.72) by the administration of *Moringa* seed oil. The histopathological appearances also clearly supported the biochemical findings. Results strongly indicated that *Moringa* seed oil possesses remarkable hepatoprotective potential underlining its ability to reverse CCl₄-induced liver injury in the experimental animals.

Keywords- CCl₄, Hepatoprotective, Liver injury, *Moringa oleifera* seed oil, Recovery

Date of Submission: 13 October 2013



Date of Acceptance: 30 November 2013

I. INTRODUCTION

The liver is a vital organ present in vertebrates and some other animals; it lies below the diaphragm in the abdominal-pelvic region of the abdomen, where it performs a wide range of metabolic activities necessary for homeostasis, nutrition and immune defence [1]. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions [2]. Most of the toxic compounds in the body are metabolized in liver. The entry of these toxicants into the body is principally via the gastrointestinal tract, and after absorption, they are transported through the hepatic vein to the liver. Carbon tetrachloride is one of the most potent hepatotoxins and is widely used in scientific research to evaluate hepatoprotective agents [3]. The metabolism of CCl₄ to the highly reactive and unstable CCl₃ radical by cytochrome P₄₅₀ in the liver leads to hepatocellular membrane damage [4]. Covalent binding of the metabolites of CCl₄, trichloromethyl (CCl₃) free radicals and subsequent derivatives to cell proteins is considered to be the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell necrosis [5].

The elevation of serum activities of hepatocellular enzymes ALT, AST and ALP is a reflection of their increased passage into the serum from damaged liver cells. Specific isoenzymes of AST are present in the liver cell mitochondria and cytoplasm whereas ALT is confined to the cytoplasm [6]. The transaminases are one group of enzymes that are sensitive indicators of liver cell injury [7]. Their serum levels are especially altered in hepatocellular disease particularly in acute diseases and they are often referred to as hepatocellular enzymes [6].

Many bioactive compounds and extracts from plants have thus been investigated for hepatoprotective and antioxidant effects against hepatotoxin-induced liver damage [8, 9]. Medicinally, herbal drugs have made a significant contribution for the treatment of hepatotoxicity [10, 11]. Prominent among those herbs is *Moringa oleifera* Lam (MO) which, owing to its high medicinal value has in recent times attracted much interest among Nigerians. *Moringa oleifera* Lam (MO) belongs to the genus *Moringaceae* (*Syns Moringa Pterigosperma Gaertn*). The plant is native to North India but it is now found throughout the tropics [12]. Instances of lifesaving nutritional rescue that are attributed to *Moringa* have been documented in the literature [13, 14]. The medical evidence for nutritional, therapeutic, and prophylactic properties of *Moringa oleifera* plant was described in a comprehensive literature review by Fahey [15]. Furthermore, most recent reviews [16, 17] have been focused on the medicinal uses, along with important phytochemical and pharmacological properties of different parts of the plant.

Most parts of the plant have also been used in folk medicine in Africa and South Asia [15]. The World Health Organization [18] estimated that 80% of the populations of some Asian and African countries depend on traditional medicine for primary health care. Numerous studies now point to the elevation of a variety of detoxification and antioxidant enzymes and biomarkers as a result of treatment with *Moringa* or with phytochemicals isolated from *Moringa* [19, 20, 21, 22].

Hepatoprotective activity of the *M. oleifera* leaves, flowers, roots and seeds have been well documented in the literature [12, 23, 24, 25]. Aside the common use of this plant both as food and as herbal medicine in Nigeria, there is also a growing increase in consumption of *Moringa* seed oil among local consumers. In view of this, the study was therefore designed to evaluate the hepatoprotective efficacy of the oil in carbon tetrachloride (CCL₄)-intoxicated Wistar rats.

II. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty four Wistar albino rats (all male) weighing 150 to 180g were purchased from the Department of Animal science, University of Ibadan, Ibadan, Nigeria. The experimental animals were thereafter kept in cages at the animal house of the Department of Biochemistry, University of Benin, Benin City, Nigeria. The process of acclimatization lasted for two weeks as they were fed with standard diet (Grower's mash) and water. Treatment lasted for ten days as the animals were distributed into four groups (1, 2, 3 and 4) of six animals each and **Group 1** served as control. The different doses of *Moringa oleifera* seed oil administered to the other groups are:

- **Group 2** (CCL₄-treated) animals received 1ml CCL₄/kg body weight once (on the 10th day);
- **Group 3** animals (treated with CCL₄ + *Moringa* seed oil) received 2ml of *Moringa* oil/Kg body weight for 10 days and intoxicated with CCL₄ (1ml/Kg) once (on the 10th day);
- **Group 4** (*Moringa* seed oil-treated) animals received 2ml of *Moringa* oil/Kg body weight for 10 days.

2.2 Extract (*Moringa oleifera* Seed Oil)

The *Moringa oleifera* oil was obtained from Millennium Quality Oil factory in Gombe, Gombe state, Nigeria.

2.3 Collection of Blood and Liver Tissue Samples

At twenty four hours after administering all the appropriate doses, blood samples were collected through cardiac puncture and liver tissues were removed. Serum samples were obtained by centrifugation of the clotted blood at 3500 rpm for 15 minutes using a bench top centrifuge, and preserved at -20°C for analysis within 48hours.

The liver tissue samples removed were subjected to the following histological procedure:

- Fixation of tissue specimen in 10% formaline for 24 hours.
- Dehydration in increasing grades of alcohol (70%, 90%, 100%).
- Cleared in xylene in three changes for 1 hour each.
- Infiltrated in three changes of paraffin at 60°C oven for 1 hour each.
- Embedded in paraffin wax
- Paraffin tissue block attached to wooden block, trimmed and nickel at one edge.
- Sectioned at 5 microns (5μ) using rotary microtome.
- Deparaffinization and hydration of tissue sections followed by staining with haematoxylin and eosin (H&E) staining method.
- Examination under the light microscope

2.4 Assay for Alanine Transaminase (ALT)

The alanine transaminase (ALT) activity in the plasma and tissue homogenate was estimated by the method of Reitman and Frankel [26]. The assay is based on the following reaction of the enzyme:
Glutamate + pyruvate \rightleftharpoons α -ketoglutarate + alanine

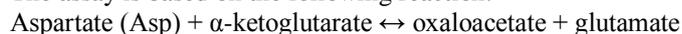
By reaction with 2, 4, dinitrophenylhydrazine, the keto acid produced (in the equation above) will then form the corresponding colored hydrazone.

The assay for the activity of the enzyme was carried out by adding 0.2ml of serum sample to 1ml of ALT buffered substrate. The mixture was incubated at 37°C for 30mins. After incubation, 1ml of color reagent (2, 4, dinitrophenylhydrazine) was added to the mixture. The solution was allowed to stand at room temperature for 20 mins. Sodium hydroxide (10 ml of 0.4 M NaOH) was then added, mixed and allowed to stand for at least five minutes. A blank containing 0.2ml of distilled water was similarly treated. Absorbance of the sample was read against the blank at 505nm.

2.5 Assay for Aspartate Transaminase (AST) Activity

The Aspartate transaminase activity in the plasma and tissue homogenate was estimated by the method of Reitman and Frankel [26].

The assay is based on the following reaction:



Procedure for the assay of AST activity was carried out by adding 0.2ml of serum sample to 1ml of AST buffered substrate. The mixture was incubated at 37°C for 60mins. After incubation, 1ml of color reagent (2, 4, dinitrophenylhydrazine) was added to the mixture. The solution was allowed to stand at room temperature for 20 mins. Sodium hydroxide (10 ml of 0.4 M NaOH) was then added, mixed and allowed to stand for at least five minutes. A blank containing 0.2ml of distilled water was similarly treated. Absorbance of the sample was read against the blank at 505nm.

2.6 Assay for Alkaline Phosphatase (ALP) Activity

The activity of this enzyme was determined in the plasma and tissue homogenates by the method of Annino and Giese [27]. When P-nitrophenylphosphate is incubated with alkaline phosphatase, it is hydrolyzed to P-nitrophenol (with a change in color from almost colorless to yellow in alkaline solution). Addition of sodium hydroxide stops the enzyme reaction and brings out the color of the P- nitrophenol.



The assay for the enzyme activity was carried out by adding 0.1ml of sample to 1ml of alkaline phosphate buffered substrate. The solution was mixed and incubated at 37°C for exactly 30mins, after which 10ml of 0.02M NaOH was added. A blank that contained 0.1ml of water was also similarly treated. Absorbance was read at 410nm setting the blank at zero.

2.7 Statistical Analysis

The experimental data were statistically analyzed using SPSS version 17 for windows and a one-way ANOVA (analysis of variance) method was employed to compare the mean differences observed among the various groups. The results are presented as mean \pm SD (standard deviation) with the level of significance set at $p < 0.05$.

III. RESULTS

The levels activity of Aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline phosphate (ALP) were taken as indices for hepatotoxicity induced by CCl_4 , and the hepatoprotective efficacy of *Moringa oleifera* seed oil. Serum levels of the enzyme activities were therefore analyzed in the different groups and the results are presented in Table 1.

Significantly higher ALT and AST activities ($p < 0.05$) were observed in both groups II (ALT- 123.7 ± 1.05 ; AST- 133.30 ± 5.95) and III (ALT- 64.33 ± 11.90 ; AST- 106.00 ± 17.72) compared to group 1 (ALT- 34.33 ± 4.94 ; AST- 70.00 ± 5.36). However, significantly lower ($p < 0.05$) AST activity was observed in group 4 (50.50 ± 3.84) in comparison with the control (70.00 ± 5.36). Significantly elevated ALP activity ($p < 0.05$) was also observed in group 3 (16.68 ± 4.71) compared with the control (5.53 ± 1.31).

The results of histopathological examinations of the liver sections as depicted in figures 1-4 also indicate the hepatoprotective efficacy of the *Moringa* seed oil.

Table1. Effect of *Moringa oleifera* Seed Oil on the Serum Activities of ALT, AST, and ALP in CCl_4 -Induced Hepatotoxicity in Rats.

GROUP	ALT (U/l)	AST (U/l)	ALP (U/l)
1 (CONTROL)	34.33 ± 4.94^a	70.00 ± 5.36^a	5.53 ± 1.31^a
2 (CCl_4)	123.7 ± 1.05^b	133.30 ± 5.95^b	15.05 ± 2.49^b
3 (CCl_4 +OIL)	64.33 ± 11.90^c	106.00 ± 17.72^{ab}	16.68 ± 4.71^{ba}
4 (OIL ONLY)	27.33 ± 4.10^a	50.50 ± 3.84^{ac}	7.47 ± 0.76^a

n=6 for each group; b and c values are significantly different from 'a' (control) values at $p < 0.05$

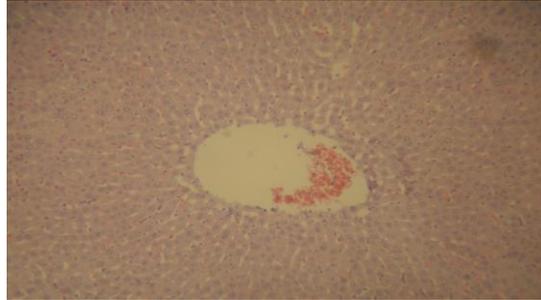


Fig 1. Control Hepatic cells

Liver section of the control, with normal architecture: the central veins, portal tracts, hepatocytes and sinusoids appear normal. The lobular unit is also well identified.

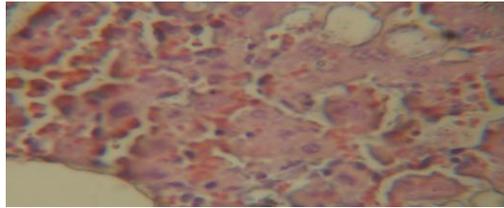


Fig 2. CCl₄-damaged hepatic cells

Extensive areas of patchy and confluent hepatic necrosis, and lobular inflammation; sinusoidal spaces are flooded with inflammatory cells.

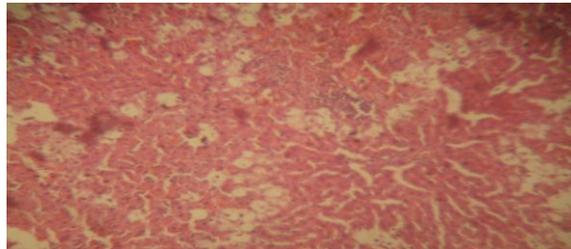


Fig 3. CCl₄+Moringa Seed Oil-Treated Hepatocytes

Hepatotoxicity of CCl₄ appears to be prevented as no necrosis is seen. The central vein and portal triads also appear normal.

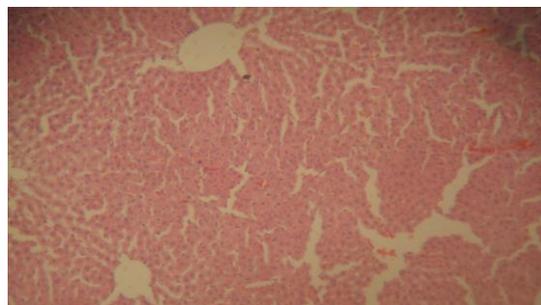


Fig 4. Moringa Seed Oil-Treated Hepatic Cells

Normal hepatocellular architecture. There are no significant pathological changes compared to the control.

IV. DISCUSSION AND CONCLUSION

Carbon tetrachloride CCl₄ is widely used for experimental induction of liver damage [28]. The study was undertaken to demonstrate the protective ability of *Moringa oleifera* seed oil on CCl₄-induced hepatic damage in Wistar rats. From the study, markedly raised serum hepatocellular enzyme (ALT and AST) activities in rats treated with only CCl₄ (group 2) compared with the control, strongly suggest the high level of hepatotoxicity inherent in acute exposure to carbon tetrachloride, and is consistent with earlier reports by Hamza [29].

This evidence of CCl₄-induced hepatotoxicity was further demonstrated by the significantly elevated activities of the enzymes (ALT & AST) in both group 2 and 3 animals as against the controls. Administration of *M. oleifera* seed oil therefore appeared to exert a protective effect against the CCl₄-induced hepatic damage as indicated by the marked decrease in ALT and AST activities in the group 3 animals treated with both Moringa oil and CCl₄, compared with the group II animals treated with only CCl₄ (**Table 1**). This finding is also consistent with those previously reported from similar studies [30, 31, 32, 33].

The histological features of liver sections from the various groups (**fig.1-4**) appear to be occasioned by the biochemical changes that occurred in the liver cells. The abnormal histology revealed by figure II clearly demonstrated the extent of hepatic damage induced by CCl₄ intoxication. This evidence supports the findings recently reported by Ezejindu et al. [1] indicating extensive fatty change (steatosis) and presence of necrosis as parts of the histological features of CCl₄-damaged hepatocytes. Evidences from histological examination of group 3 animals in this study were also consistent with further reports by Ezejindu et al. [1] which indicated very little fatty change in liver sections, with an appearance suggesting the recovery of most areas of the hepatic cells of Wistar rats treated with both Moringa extract and carbon tetrachloride.

We can thus infer from this study that *M. oleifera* seed oil possesses a very high therapeutic potential which underlines its ability to bring about recovery from CCl₄-induced hepatic damage. It also appears *M. oleifera* seed oil is capable of reversing or inhibiting lipid peroxidation in liver cells; further studies are therefore needed.

V. ACKNOWLEDGEMENTS

We are grateful to Dr. Jerry Orhue, Dr. Agoreyo (Mrs.), Mr. Sylvanus Inni, Mr. Agu, and Mr. Ogunbiyi Oluwabenga for the technical assistance they offered while the study was being carried out.

REFERENCES

- [1] Ezejindu DN, Chinweife KC, Ihentuge CJ. **The Effects of Moringa Extract on Liver Enzymes of Carbon Tetrachloride Induced Hepatotoxicity In Adult Wister Rats.** *The International Journal of Engineering and Science (IJES)*, Volume 2 (Issue 7), 2013, Pages 54-59.
- [2] Maton A, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, Wright JD, *Human Biology and Health* (Englewood Cliffs, New Jersey, USA: Prentice Hall, 1993). ISBN 0-13-981176-1. OCLC32308337.
- [3] Doherty, R.E. "A History of the Production and Use of Carbon Tetrachloride, Tetrachloroethylene, Trichloroethylene and 1, 1, 1-Trichloroethane in the United States: Part 1—Historical Background; Carbon Tetrachloride and Tetrachloroethylene". *Environmental Forensics*, 1 (1), 2000, 69–81.
- [4] Ohta Y, Sasaki E, Nishida K, Kongo M, Hayashi T, Nagata M & Ishiguro L. Inhibitory effect of Oren-gedoku-to (Huanglian-Jie-Du-Tang) extract on hepatic triglyceride accumulation with the progression of carbon tetrachloride-induced acute liver injury in rats. *Journal Ethnopharmacology*, 61(1), (May 1998), 75-80.
- [5] Recknagel RO, Glende EA Jr, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. *Pharmacology & Therapeutics*, 43, 1989, 139–54.
- [6] Wilkinson HJ, *Diagnostic enzymology* (Edward Arnold Publishers Ltd., London.775., 1976).
- [7] Mayers CW and Jones RS, *Textbook of liver and biliary surgery* (J.B. Lippin-Cott Company Philadelphia 47-8, 61, 209, 1990).
- [8] Yousef MI, Omar SA, El-Guendi MI, Abdelmegid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food and Chemical Toxicology*, 48, 2010, 3246–3261.
- [9] Choi, JH, Choi CY, Lee KJ, Hwang YP, Chung YC, Jeong HG. Hepatoprotective effects of an anthocyanin fraction from purple-fleshed sweet potato against acetaminophen induced liver damage in mice. *Journal of Medicinal Food*, 12, 2009, 320–326.
- [10] Li R, Guo W, Fu Z, Ding G, Zou Y and Wang Z. Hepatoprotective Action of Radix Paoniae Rubra Aqueous Extract against CCl₄-Induced Hepatic Damage. *Molecules*, 16(10), 2011, 8684-8694.
- [11] Yen FL, Wu TH, Lin LT and Lin CC. Hepatoprotective and Antioxidant Effects of Cuscuta Chinensis against Acetaminophen-Induced Hepatotoxicity in Rats. *Journal of Ethnopharmacology*, 111, 2007, 123-128.
- [12] Fakurazi S, Nanthini U and Hairuszah I. Hepatoprotective and antioxidant action of *Moringa oleifera* Lam. against acetaminophen induced hepatotoxicity in rats. *International Journal of Pharmacology*, 4(4), 2008, 270-275.
- [13] Fuglie LJ, The Miracle Tree, in *Moringa oleifera: Natural Nutrition for the Tropics*. (Church World Service, Dakar, 1999) 68 pp. Revised in 2001 and published as *The Miracle Tree: The Multiple Attributes of Moringa*, 172 pp. http://www.echotech.org/bookstore/advanced_search_result.php?keywords=Miracle+Tree.
- [14] Fuglie LJ. New Uses of Moringa Studied in Nicaragua. *ECHO Development Notes* #68, June, 2000. <http://www.echotech.org/network/modules.php?name=News&file=article&sid=194>.
- [15] Fahey JW. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic and prophylactic properties. *Trees for Life Journal*, 1, 2005, 5.
- [16] Pandey A, Pandey RD, Tripathi P, Gupta PP, Haider J, Bhatt S, and Singh AV. *Moringa oleifera* Lam. (Sahijan) - A Plant with a Plethora of Diverse Therapeutic Benefits: An Updated Retrospection. *Medicinal & Aromatic Plants*, 1, 2012, 1.

- [17] Anwar F, Latif S, Ashraf M and Gilani AH. *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. *PHYTOTHERAPY RESEARCH* 21, 2007, 17–25.
- [18] World Health Organization (WHO). Traditional Medicine, December 2008. www.who.int/mediacentre/factsheets/fs134/en/
- [19] Fahey JW, AT Dinkova-Kostova, and P Talalay, The “Prochaska” microtiter plate bioassay for inducers of NQO1, Chapter 14 in *Methods in Enzymology*, Vol. 382, Part B, 2004, pp. 243-258 (Eds.) (H. Sies & L. Packer, Elsevier Science, San Diego, CA.)
- [20] Kumar NA, and Pari L. Antioxidant action of *Moringa oleifera* Lam. (drumstick) against antitubercular drugs induced lipid peroxidation in rats. *Journal of Medicinal Food*, 6(3), 2003, 255-259.
- [21] Rao KNV, Gopalakrishnan V, Loganathan V, and Shanmuganathan S. Antiinflammatory activity of *Moringa oleifera* Lam. *Ancient Science of Life* 18(3-4), 1999, 195-198.
- [22] Faizi S, BS Siddiqui, R Saleem, S Siddiqui, K Aftab, and AH Gilani. Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. *Journal of Natural Products* 57, 1994, 1256-1261.
- [23] Alaaeldin A and Hamza. *Curcuma longa*, *Glycyrrhiza glabra* and *Moringa oleifera* ameliorate diclofenac – induced hepatotoxicity in rats. *American Journal of Pharmacology and Toxicology*, 2(2), 2007, 80-88.
- [24] Ruckmani K, Kavimani P, Anandan R and Jaykar B. Effect of *Moringa oleifera* Lam on paracetamol – induced hepatotoxicity. *Indian Journal of Pharmaceutical Sciences*, 60, 1998, 33-35.
- [25] Gilani AH, Janbaz KH and Shah BH. Quercetin exhibits hepatoprotective activity in rats. *Biochemical Society Transactions*, 25, 1997, 85.
- [26] Reitman S & Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28, 1957, 56-63.
- [27] Annino JS, Giese RW. *Clinical chemistry, principles and procedures*, 4th edition, (Little Brown and Company, Boston. pp. 76-82, 1976).
- [28] Parola, M, Leonarduzzi, G, Biasi, F, Albono, M, Biocca, G, Polic, Dianzani, MU. Vitamin E dietary Supplementation. Protects against CCl₄ induced chronic liver damage and cirrhosis. *Hepatology*, 16, 1992, 1014-1021.
- [29] Hamza AA. Ameliorative effects of *Moringa oleifera* Lam. seed extract on liver fibrosis in rats. *Food and Chemical Toxicology*, 48, 2010, 345-355.
- [30] Ujah OF, Ujah IR, Johnson JT, Ekam VS and Udenze ECC. Hepatoprotective property of ethanolic leaf extract of *Moringa oleifera* on carbon tetrachloride (CCl₄) induced hepatotoxicity. *Journal of Natural Product and Plant Resources* 3 (2), 2013, 15-22.
- [31] Rajesh MG and Latha MS. Protective activity of *Glycyrrhiza glabra* Linn. on carbon tetrachloride-induced peroxidative damage. *Indian Journal of pharmacology* 36, 2004, 284-287.
- [32] Yan-Jun L, Jie-ping Y, Zhao- hong S, Wang L. Ginkgo biloba extract reverses CCl₄-induced liver fibrosis in rats. *World Journal of gastroenterology*, 10, 2004, 1037-1042.
- [33] Ulicna O, Greksak M, Vancova O, Zlatos L, Galbavy S, Bozek P, Nakano M. Hepatoprotective effect of rooibos tea (*Aspalathus linearis*) on CCl₄-induced liver damage in rats. *Physiological Research*, 52, 2003, 461-466.