Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 04, pp.29504-29509, April, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOCHEMICAL AND HISTOPATHOLOGICAL STUDY IN RATS INTOXICATED WITH CARBONTETRACHLORIDE AND TREATED *VERNONIA AMYGDALINA*

***Precious Barnes, Heckle Abban, Du-Bois Asante, Emmanuel Effah and Emmanuel Eli**

Department of Biomedical and Forensic, School of Allied Sciences, College of Health and Allied Sciences, University of Cape Coast

ARTICLE INFO

Article History:
Received 15th January, 2016
Received in revised form
29th February, 2016
Accepted 27th March, 2016
Published online 26th April, 2016

Key words:

Carbon Tetrachloride,
Alanine Transferase,
Vernonia amygdalina.

ABSTRACT

Introduction: *Vernonia amygdalina* belongs to the family Asterecae. *Vernonia amygdalina*contains active components or phytochemicals that can lead to liver regenerations in hepatotoxicity in hepatoxicity.
Aim: The aim of this study isto assess the chemotherapeutic and hepatoprotective effect of leaf extract of *Vernonia amygdalina*in rats.
Method: A total number of 15 albino rats were fed on standard diet and divided into three groups. Rats of the firstgroup were injected intraperitioneally with paraffin oil and tap water (control). Rats of the second and third groups were intraperitioneally injected with CCL_4, standard diet and tap water. The third group was treated with the leaf extract of *Vernonia amygdalina*. Blood and liver samples were collected for biochemical and histopathological analysis.
Results: Phytochemicals such as flavonoids, tannins were contained in the leaf extract of the plant. Levels of alanine ALT, GGT and AST were highest in the CCL_4 treated group with a mean and standard deviation of AST (88.25±14.22), ALT (89.25±8.99) and GGT (91.75±6.32) respectively as compared to the control and CCL_4 treated groups. Histopathologically, a greater amount of mononuclear cell infiltration, necrotic and few fibroblasts were observed in the liver of CCL_4 treated groups whiles liver regeneration was observed in the third group.
Conclusion: The results showed that the leaves of *Vernonia amygdalina* possessed hepatoprotective abilities.

Citation: Precious Barnes, Heckle Abban, Du-Bois Asante, Emmanuel Effah and Emmanuel Eli, R. 2016. "Biochemical and histopathological study in rats intoxicated with carbontetrachloride and treated *vernonia Amygdalina*", *International Journal of Current Research*, 8, (04), 29504-29509.

INTRODUCTION

Medicinal plants are rich source of novel drugs that forms the elements in traditional system of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and main compounds in synthetic drugs. *V. amygdalina*(bitter leaf) is used for treatment of jaundice, diarrhea, cancer; diabetes and tuberculosis (Muanya, 2013). Polyherbal preparations with bitter leaf as the active ingredients strengthen the immune system through many cytokines and chemokines regulations (Muanya, 2013). The herb not only lowers the body sugar level sufficiently, it also plays a role in the repair of pancreas. Bitter leaf is an abundant source of the poly unsaturated fatty acids, linoleic and linolenic acid and these poly unsaturated fatty acids have been found to be protective against cardiovascular disease (Tapsell, 2006).

****Corresponding author: Precious Barnes,***
Department of Biomedical and Forensic, School of Allied Sciences, College of Health and Allied Sciences, University of Cape Coast

It soothes inflamed joints and eradicates pains common with arthritis or rheumatism patients (Okoli *et al*., 2007). Many herbalists and naturopathic doctors recommend aqueous extracts of bitterleaf for their patients for emesis, loss of appetite induced ambrosia, dysentery and other gastrointestinal tract problems (Schippers, 2000). The main aim of the experiment is to investigate the therapeutic effects of *Vernonia amygdalina* on carbon tetrachloride induced hepatotoxicity in rats.

MATERIALS AND METHODS

The leaves of *V. amygdalina*were collected in the morning from Apewosika, University of Cape Coast (UCC). The plant materials were taxonomically identified and authenticated by the Department of Biomedical and Forensic Sciences, (UCC). A total of 15 albino rats were used for the research (60- 120g) and were obtained from the Laboratory house of Vertenary Medicine center, University of Ghana, Legon, Ghana. The animals acclimated for 2 weeks before starting the research.

The experimental animals were housed at a conducive temperature (room temperature) and 60- 65% of relative humidity and kept in a 12 hours dark/ 12 hours light cycle. All animals experiment, procedures and techniques used in the study were conducted in compliance with National Institute of Health Guilelines for Care and Use of Laboratory animals and other known International acceptable guidelines.

Preparation of Extract

Fresh leaves of *V. amygdalina* were obtained from the *V. amygdalina* plant. The leaves were air dried for 1 weeks after which the leaves were oven dried at 55^{o}C for about 4 hours. The leaves were blended using Sanyo SM (G300) blender. Solvent extraction was the next step. During this process, 100g of the powdered blended leaves were dissolved in 450ml of 80% methanol after which 150ml of distilled water was added. The volume of the mixture obtained was 600ml. The mixture was allowed to stand for 3 days and shaken vigorously each day. The mixture was stirred at regular intervals (3-5 minutes) for 1 hour and was then filtered using Whatman filter paper to separate the crude sample and the solution. The sample was concentrated using a rotary evaporator at 60^{o}C to recover the solvent. Further heating was done on a hot plate at 80 ^{o}C where a paste- like substance was obtained. The extract was covered with an aluminium foil and was stored in a refrigerator until it was required for use.

Phytochemical Analysis

Solvent Extraction

The extract to be administered was prepared by dissolving 0.3515g of the crude extract in a volume of 56ml of distilled water. The concentrations of the extract (0.134ml, 0.981ml, 0.654ml, 0.431ml, 1ml and 0.556ml) to be administered were taken into consideration by calculating the volume and the corresponding weight of the animal. The extract was given to the animals for 14 days.

Blood and tissue Collection

At the end of the experiment, the overnight fasted animals (the control and experimental animals) were sacrificed under light ether anesthesia. Blood samples were collected by cardiac puncture before incision of the abdomen; 5 ml of blood samples were collected in anti-coagulant tubes, serum was collected and frozen at -30°C until the time of analysis. The blood collected was transferred on to a centrifuge tube after 30min using Wisperfuge model 1384 centrifuge. Diagnostic kits for serum alanine aminotransferase (ALT) and aspartate amino transferase (AST), alkaline phosphatase (ALP), carbon tetrachloride and other chemicals and solvents were of highest grade commercially available. Liver and kidney tissues were also obtained and cut in small pieces and immersed in neutral buffered formalin 10% for histopathology.

Induction of Hepatotoxicity

Liver toxicity was induced with the intraperitoneal injection of CCl_4 (1 ml/kg b.wt.), 1:1 diluted with paraffin oil, for two successive days of the experiment (Karikus *et al*., 2011).

Biochemical Parameters

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT) were estimated in serum using commercially available kits and a standard BS-120 Mindray Chemistry Analyzer.

Histological Analysis

Part of liver samples was submitted in 10%phosphatebuffered formalin. The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydratedand stained with hematoxylin and eosin (H & E) using standard histological technique described earlier (Bancroft and Gamble, 2002). The extent of tissue damage induced by CCl_4 was then evaluated by assessing the morphological changes in the liver sections by employing light microscopy.

Experimental group design and protocol

The 15 albino rats used were divided into three groups with each group comprising of 5 rats and were fed with the same diet throughout the experimental period.

RESULTS

Table 1. The various qualitative phytochemical screening in *Vernonia amygdalina*

Phytochemicals	Preliminary Phytochemical Analysis
Anthraquinones	-
Terpenoids	+
Saponins	+
Tannins	+
Alkaloids	+
Glycosides	+
Steroid	+

+ = indicates presence of phytochemicals and
- = indicates absence of phytochemicals

Table 2. The various treatments and the corresponding groups

Groups	Treatments
I.	Basal diet and tap water.
11.	Basal diet, tap water, and carbon tetrachloride.
III.	Basal diet, tap water, carbon tetrachloride and treatment with *Vernonia amygdalina*.

Table 3. The mean and standard deviations on the serum alanine aminotransferase, aspartate aminotransferase and gamma glutamyl transferase activities of carbon tetrachloride treated rats in different groups

Groups	AST (u/l)	ALT (u/l)	GGT (u/l)
Control	21.25±1.71	21.75±2.21	19.75±2.74
Carbon tetrachloride only	89.25±8.99	88.25±14.22	91.75±6.32
Carbon tetrachloride and extract	30.50±6.14*	30.25±8.09*	30±6.32*

(n=5), Values were recorded as mean ± standard error of mean.p<0.05 was considered statistically significant. (*)=statistical difference between control and extract treated groups.

Qualitative phytochemical test and analysis

The extract was tested for the presence of bioactive compounds by using standard methods described elsewhere (Trease, 1993; Parekh, 2008; Asante *et al*., 2015). Results Mean and standard deviation of the mean of the liver enzymes in the various groups

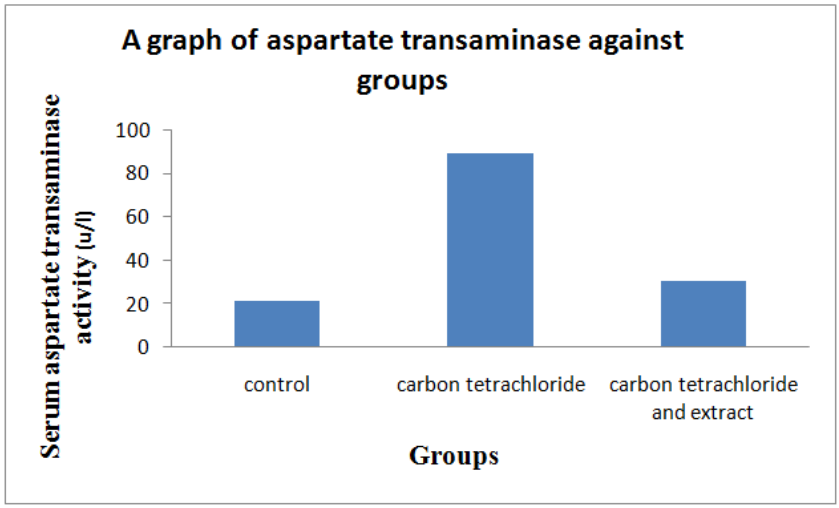

Fig. 1. The serum aspartate aminotransferase (AST) activity in different animals groups. The serum AST activity is the highest in CCL_4 group in comparison with control and $CCl_4$and extract group. The serum AST activity shows significant ($p<0.05$) decline in CCl_4 induced hepatoxicity in rats

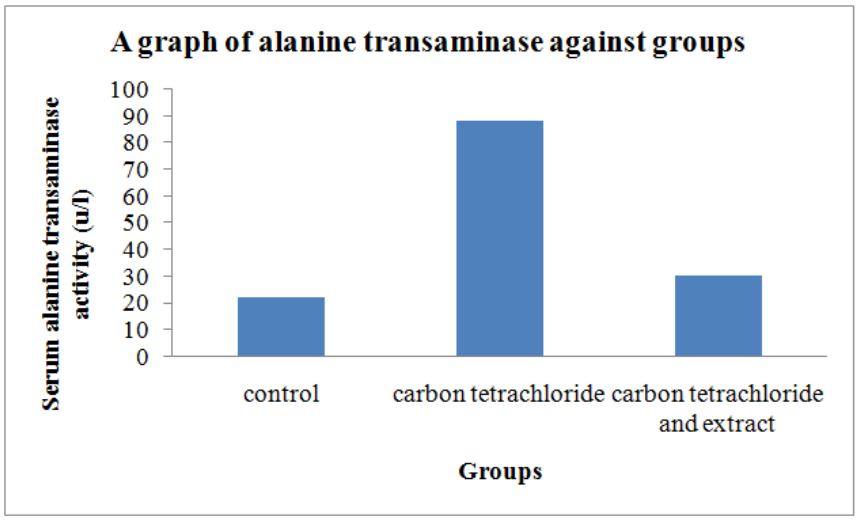

Fig. 2. The serum alanine aminotransferase (ALT) activity in different animals groups. The serum ALT activity is the highest in CCl_4 treated group in comparison with control and carbon tetrachloride group. The serum ALT activity shows declining administered carbon tetrachloride and extract groups and a t-test value ($p<0.0001$)

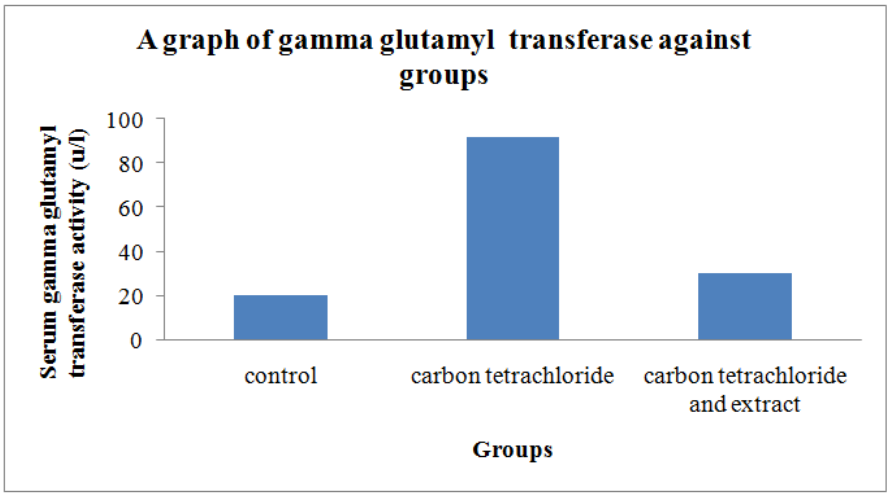

Fig. 3. The serum gamma glutamyl transpeptidase activity in different animals groups. The serum gamma glutamyl transpeptidase activity is the highest in carbon tetrachloride treated group in comparison with control and carbon tetrachloride intoxicated group. The serum gamma glutamyltransferase activity shows declining in administered carbon tetrachloride and extract with a t-test value of $p<0.0007$

Histopathological Examinations

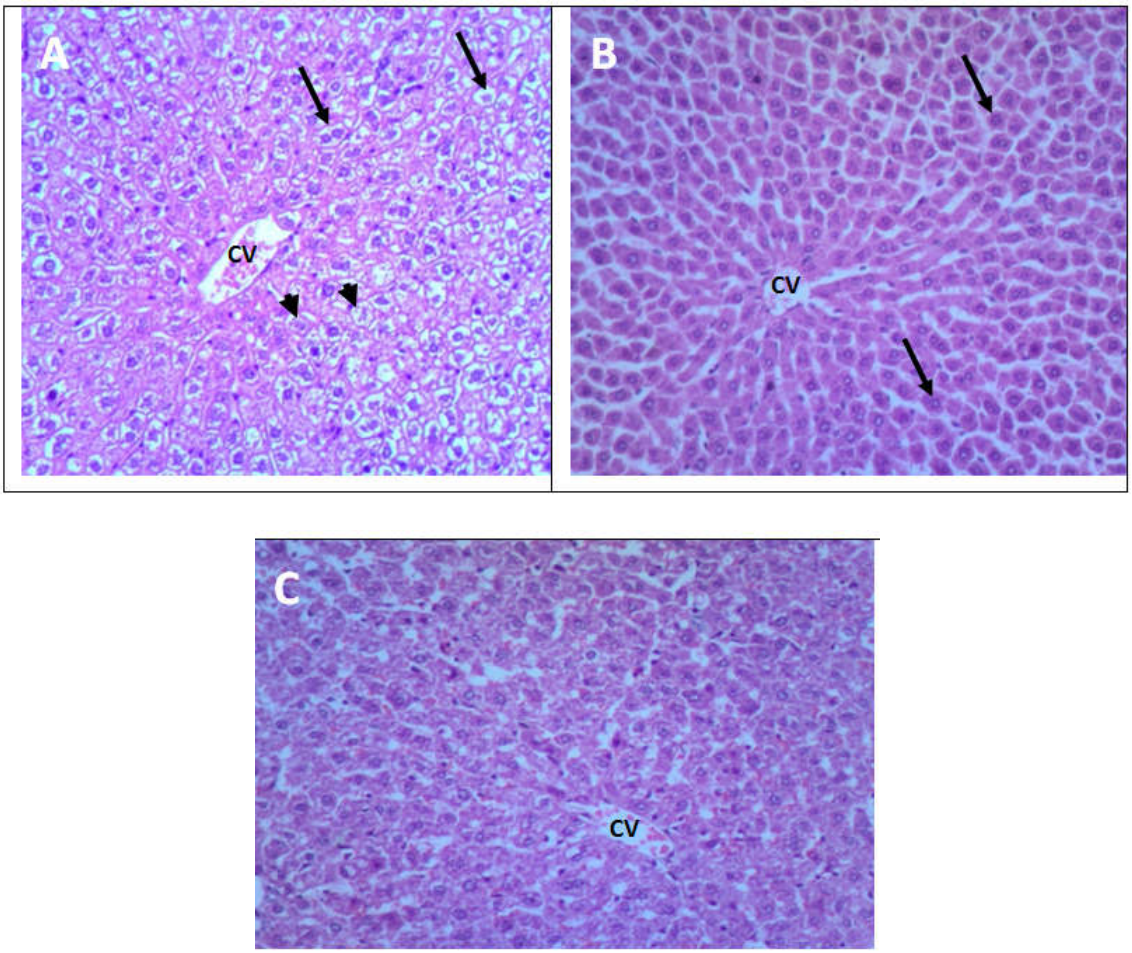

Figure 1. Photomicrograph of liver sections of experimental rats. (A) Section shows fatty changes (black arrows) in hepatocytes, seen here as vacuoles around nuclei in rats given only CCl_4. These changes are pronounce and conspicuous in cells that are further away from the central vein (CV), and with degeneration of nuclei in other cells (Black arrow head). (B) Normal histology of hepatocytes (Black arrows) surrounding the CV in normal control rats. (C) Slightly eodematous with few activated Kupffer cells and leucocyte infiltration, seen in rats given both CCl_4 plus the extract

DISCUSSION

This present study investigated the chemotherapeutic properties of *V. amygdalina* leaf extract in the treatment of carbon tetrachloride induced liver diseases with histopathogical and biochemical characteristics of the liver. In the present study,CCl_4 had an adverse effects on the liver. Histopathologically, with reference to figure 1, the hepatocytes appeared irregularly arranged with disorganization of hepatic architecture, kupffer cell activation, mild hemoderosis, severe necrosis and induced fibrosis and this was in support of Junnila *et al.*, (2000) and Karakus *et al.*, (2011). The hepatocytes appeared large with numerous microscopic and macroscopiccytoplasmic vacuoles, a condition known as fatty change as describe previously (Mohan, 2010; Karakus *et al.*, 2011). The central vein appeared dilated and congested with blood. Also, there were focal necrotic changes along with infiltration in the CCl_4induced rats and this in agreement with Canbay *et al.*, 2007. These results indicate that CCl_4 at the administered dose in the study produced severe oxidative damage evidenced as necrotic changes in the liver.

This was in agreement with a study by Zimmerman *et al.*, (1965) who reported the toxicity of carbon tetrachloride on the liver. Kupffer cells and hepatocytes were full of brown granular deposits of hemosiderin which was due to the accumulation of excess iron in the liver and this was in agreement with the works done by Jurczuk *et al.,* (2004). Kupffer cells produced liver fibrosis that led to the formation of scar tissue in response to liver damage and revealed a small amount of the extract regenerated the liver and this correlated to Arhoghro *et al.*, (2009) who stated that as low as 15% of extract could even completely revert liver change to normal in the treated animals. It should be emphasized that replacement of the lost hepatic mass was mediated through proliferation of mature adult hepatocytes and the other hepatic cell types. It is not mediated by proliferation of a selective subpopulation of stem cells (as in skin and small intestine). Normal liver weight was reestablished within 5–7 days. At the end of regeneration, the size of the liver lobules was remarkably larger and the thickness of the hepatocyte plates is almost twice the size of the normal one cell thickness and this was in support of Michalopoulos and Doherty, (2000). Previous studies also

suggested that there were slow lobular reorganization taking place for several weeks, and eventually liver histology became indistinguishable from the original by Wagenaar *et al*., 1993). A key endpoint of liver regeneration was the restoration of the total number and mass of hepatocytes, the main functional cells of the liver responsible for delivering most of the hepatic functions important for body homeostasis. On the other hand, administration of aqueous extracts of *Vernonia amygdalina* to the carbon tetrachloride treated animals did not completely regain the hepatocytes to normal. Hepatocytes were enlarged and had light and foamy cytoplasm filled with numerous vacuole-like spaces, and mild degenerative changes as observed in figure 6. Thus, the results suggested that *Vernonia amygdalina* extract acted as a potent hepatoprotective agent against carbon tetrachloride induced hepatotoxicity in rats as suggested by Arhoghro *et al*., (2009).

The result also indicated that the use of the leaf extract of *V. amygdalina* for the management of all kinds of diseasesin traditional medicine is justified and was in agreement with (Gill, 1992). With reference to table 2, phytochemical screening of V. *amygdalina* have shown the presence of some phytochemicals such as flavonoids, terpenoids, saponins, alkaloids, tannins, glycosides, steroids in the plant and this was in conformity with Ayoola *et al*., 2008.The liver regenerative ability, management of liver diseases such necrosis and the suppression of liver enzymes by the extract of *V. amygdalina* may be due to one or a combination of these phytochemicals present in the plant. Research at the Linus Pauling Institute and the European Food Safety Authority shows that flavonoids are poorly absorbed in the human body (less than 5%), with most of what is absorbed being quickly metabolized and excreted.Inflammation has been implicated as a possible origin of numerous local and systemic diseases, such as cancer. Preliminary studies indicated that flavonoids may affect anti-inflammatory (Izzi *et al.,* 2012). These chemical compounds in the extract may be acting singly or in synergy with one another to exert anti-inflammatory, liver regeneration and diseases management activities. This study has therefore established the rationale for traditional use of *Vernonia amygdalina* as a remedy for liver diseases and this was in agreement with Muanya, (2013).

Biochemically, treatment of the rats with carbon tetrachloride caused a significant increase of serum aspartate transaminase, alanine transaminase and gamma- glutamyl transferase with reference to tables 3, 4 and 5 and this was in support of Recknagel *et al*. (1989); Brent and Thnaian et al., (2013) who stated that the increased serum levels of hepatic enzymes have been attributed to the liver injury, because these enzymes are place in cytoplasmic area of the cell and are released into circulation in case of cellular damage. Oral administration of the aqueous extract from *V. amygdalina* leaves accelerated the reversion of liver damage through reduction of liver marker enzymes, which are aspartate aminotransferase (AST) aspartate transaminase, alanine transaminase (ALT).

Conclusion

The research carried out shows that leaf extract of *V. amygdalina* has a chemotherapeutic and hepatoprotective properties against carbon tetrachloride induced hepatotoxicity. These results suggest that the extract of *V. amygdalina* has potential clinical applications for treating liver disorders.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We acknowledge Mr Daniel Konja who assisted as a phlebotomist and Isaac Annan who assisted with the statistical analysis.Authors are also grateful to the NoRHS (Network of Researchers in Health Sciences) for their immense support and contribution to the success of this work.

REFERENCES

Arhoghro, E.M., Ekpo, K.E., Anosike, E.O., Ibeh, G.O. 2009. Effect of aqueous extract of bitter leaf (*Vernonia amygdalina Del*) on carbon tetrachloride (CCl4) induced liver damage in albino wistar rats. *Eur. J. Sci. Res*., 26: 122-130.

Asante, D., O.E. Ameyaw, E. Effah-Yeboah, A.P. Gyamenah, and J. Djabanor, 2015. "Hepatoprotective effect of ethanolic leaf extracts of *Abrus precatorius* in *Plasmodium berghei* infected imprinting control region (ICR) mice; A histopathological perspective," *International Journal of Biological and Pharmaceutical Research,* vol.6, no. 10, pp. 814-821,

Bancroft, J.D., Gamble, M. 2002. Theory and Practice of Histological Techniques. Churchill Livingstone, London, New York and Philadelphia 54 (20): 2191–3;

Canbay, A., Feldstein, A., Baskin-Bey, E., *et al.* 2007. The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse*. J Pharm Exp Ther 3:1191-1196*

Doherty RE (2000). "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): 69–81.*

Gill, L.S. 1992. Ethno medicinal use of plants in Nigeria. University of Benin Press, Benin-City.

Izzi, V., Masuelli, L., Tresoldi, I., Sacchetti, P., Modesti, A., Galvano, F *et al.* 2012. "The effects of dietary flavonoids on the regulation of redox inflammatory networks". *Frontiers in bioscience (Landmark edition)* 17 (7): 2396–2418

Junnila, M., Rahko, T., Sukura, A., *et al*. 2000. Reduction of carbon tetrachloride-induced hepatotoxic effects by oral administration of betaine in male Han-wistar rats: a morphometric histological study. *Vet Pathol* 37:231-238

Jurczuk, M, Brzoska, M.M., Moniuszko-Jakoniuk, J., Galazyn-Sidorczuk M, and Kulikowska-Karpinska E. 2004. Antioxidant enzymes activity and lipid peroxidation in liver and kidney of rats exposed to cadmium and ethanol. *Food Chem Toxicol* 429-438

Karakus, E., Karadeniz, A., Simsek, N., *et al*. 2011. Protective effect of Panax ginseng against serum biochemical changes

and apoptosis in liver of rats treated with carbon tetrachloride (CCl4). *J Hazard Mater* 195:208-213

Munaya, C. 2013. Bitter leaf-based extracts cures hepatitis co-inferation and others. *The Guardian Newspaper*

Okoli, R.I., Aigbe, O., Ohafu-Obode, J.O. and Mensah, J.K. 2007. Medicinal herbs used for managing some common ailments among Esan people Edo State, Nigeria. *Pakistan Journal of Nutrition* 6(5):470-490.

Parekh, J., Chanda, S. 2008. Phytochemicals screening of some plants from western region of India. *Plant Arch*., 8: 657-662

Recknagel RO, Glende JEA, Dolack JA, et al. 1989. Mechanisms of carbon tetrachloride toxicity. *Pharmacol Ther 43:139-154*

Schiffers, R.R. 2000. African indigenous vegetables: an overview of the cultivated species. University Greenwich Press, England.

Tapsell, 2006. Health benefits to herbs and spices: the past, the present and future. *Medicinal Journal of Austria* 1:170-190.

Thnaian Althnaian, Ibrahim Albokhadaim, and Sabry M El-2013. Bahr Biochemical and histopathological study in rats intoxicated with carbontetrachloride and treated with camel milk,Springerplus. 2: 57.

Trease, G.E., Evans, W.C. 1989. Pharmacognosy, 11th edition., Bailliere Tindall, London, pp. 45-50.

Wagenaar, G.T., Chamuleau, R.A., Pool, C.W., de Haan, J.G., Maas, M.A., Korfage, H.A., Lamers, W.H. 1993. Distribution and activity of glutamine synthase and carbamoylphosphate synthase upon enlargement of the liver lobule by repeated partial hepatectomies. 397–407 *J Hepatol*

Zimmerman, H.J., Kodera, Y., West, M. 1965. Effects of carbon tetrachloride poisoning on the plasma levels of cytoplasmic and mitochondrial enzymes in animals with nutritional fatty metamorphosis. *J Lab Clin Med* 66:324-333

APPENDIX

Abbreviation

ALT- Alanine Aminotransferase
AST- Aspartate Aminotransferase
CCL_4- Carbon tetrachloride
GGT- Gamma- glutamyl transpeptidase

****