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## **Analgesic and Hepatoprotective Activity of Methanolic Leaf Extract of *Ocimum gratissimum* (L.)**

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### **ABSTRACT**

The methanolic extract of *Ocimum gratissimum* (L.) leaves was screened for analgesic and hepatoprotective activity in albino rats, respectively. The use of the hot-plate method to study central analgesic activity of the leaves extract in albino rats indicated that the extract possesses the ability to significantly reduce pain threshold and also increase the response latency period to thermal stimuli in albino rats, similar to the reference drug acetylsalicylic acid. After treatment reaction time of albino rats was significantly increased to 10.92 sec with 40 mg kg<sup>-1</sup> of leaves extract, whereas acetylsalicylic acid also increased reaction time to 12.53 sec with 25 mg kg<sup>-1</sup>. A decline in the reaction time beyond 1.61 sec was observed by the reference drug and leaves extract. Albino rats whose livers were damaged with a hepatotoxin-Carbon tetrachloride (CCl<sub>4</sub>) 0.5 mL kg<sup>-1</sup> i.p. were used to test for hepatoprotective properties of the plant leaves extract. It reduced significantly (p<0.05) liver enzyme levels for animals treated with CCl<sub>4</sub> (0.5 mL kg<sup>-1</sup>) and the methanolic plant leaf extract (40 mg kg<sup>-1</sup>) concurrently compared to animals treated with CCl<sub>4</sub> only. Many histopathological changes in the liver such as marked dilation of the central vein, blood vessel congestion and inflammatory leucocytic infiltrations which were observed in the CCl<sub>4</sub> treated animals were not observed in the CCl<sub>4</sub> + plant extract treated animals. No apparent disruptions of the normal liver structure by histological and enzyme activities assessment were observed. The results show that the methanolic leaf extract is a potent analgesic and antihepatotoxic agent.

**Key words:** Pain perception, liver enzymes, histology, hepatoprotective, ethnomedicine, bioactivity

### **INTRODUCTION**

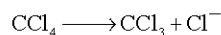
The direct use of plants for treatment of ailments is as old as man itself. In the third world countries, medicinal plants are of immense importance in the daily life of the people. Many plants are used as remedies and it is estimated that about 80% of the people in developing countries depend on traditional healing (WHO, 2001). Plants constitute an important source of active natural products that differ widely in terms of their structure and biological properties. Plants have played remarkable roles in the traditional medicine of various countries. The prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables or teas rich

in natural antioxidants (Virgili *et al.*, 2001; Singh *et al.*, 2004). Medicinal plant products have protective effects because of the presence of several components which have distinct mechanisms of action. Some of these components are enzymes and proteins and others are low molecular weight compounds such as vitamins, carotenoids and flavonoids (Zhang and Wang, 2002). Some of these components especially phytochemicals can significantly reduce the risk of cancer due to polyphenol antioxidant and anti-inflammatory effects, antihypertensive (Lee *et al.*, 2001), antihyperglycemic (Jeppesen *et al.*, 2002). Preclinical investigations also suggest that phytochemicals can prevent colorectal cancer and other cancers (Birt *et al.*, 2001; Higdon *et al.*, 2007). Many plants and plant products are recommended for the treatment of liver diseases and most times are claimed to offer significant relief (Arulkumaran *et al.*, 2009). *O. gratissimum* is one of those plants that have been recommended by traditional medicine practitioners in the South East of Nigeria (Adeyemi *et al.*, 2002).

*Ocimum gratissimum* (L.) is an erect multi-branched perennial shrub that grows up to a height of two meters with a tap root and many adventitious rootlets. The leaf is rich in essential oils especially thymol, others include flavonoids, saponins, steroids, camphor, estragol, linal, anethol, hydrocyanate and terpenes (Oforansi *et al.*, 2005). The plant has both culinary and medicinal uses. It is mainly used as a spice to flavor foods and meat. The components of *O. gratissimum* especially thymol has biological activity such as antiseptic, antitussive, antihelminthic, antispasmodic and antimicrobial. The fresh leaves are used as a laxative, while its infusion serves as a relief for headaches, fever, diarrhea, dysentery, pile and convulsion (Danziel, 1996). In Igbo area of South Eastern Nigeria the leaves extract are applied externally in treatment of conjunctivitis, rheumatic pain, dressing of wounds and lumbago (Iwu, 1993).

Analgesics that act on pain perception within the central nervous system are used mainly to relieve pain that originate in the viscera or arise from severe injuries, burns or neoplasm (Adeyemi *et al.*, 2002).

Carbon tetrachloride (CCL<sub>4</sub>) is a hepatotoxin which can have adverse effect on the liver. The affected group of cells may be very small in number or may be of grave and catastrophic proportion as in acute yellow atrophy of the liver where 90% of the parenchyma cells are destroyed. It is known to induce oxidative stress and causes liver injury by formation of free radicals (Manna *et al.*, 2006). The mechanism of Carbon tetrachloride induced hepatic injury is mediated by a reactive metabolite trichloromethyl free radical which is formed by the hemolytic cleavage of carbon tetrachloride:



Trichloromethyl peroxy free radical is more reactive than carbon tetrachloride. The toxicity produced by carbon tetrachloride is thought to be due to the reaction of free radicals with lipid and protein. These free radicals cause the peroxidation of the polyenoic lipids of the endoplasmic reticulum and the generation of secondary free radicals derived from these lipids starts a chain reaction (Valko *et al.*, 2007).

The effectiveness of *Ocimum gratissimum* (L.) as a therapy and in offering any protection against the hepatotoxin depends largely on its ability to either inhibit the activation of carbon tetrachloride to its reactive metabolite carbon trichloride (CCl<sub>3</sub>) or its central uptake by the liver free radical generating system (Lee *et al.*, 2004).

The present study is therefore designed to evaluate the analgesic and hepatoprotective effects of the methanolic leaves extract of *O. gratissimum* (L.) in albino rats against claims by ethno-medicine practitioners that the plant leaf has both analgesic and hepatoprotective properties.

## **MATERIALS AND METHODS**

**Plant materials:** Fresh leaves of *O. gratissimum* (L.) were collected from plants growing in the campus of Abia State University, Uturu, Nigeria as ornamental plants on the 24th of February 2011. The plant was authenticated at the Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria. Voucher specimen of the plant was deposited at the departments' herbarium.

**Preparation of methanol extract:** Samples of the fresh leaves were first washed with distilled water to remove dirt and contaminants. They were oven dried at a temperature of 27°C for three days. The dried sample was then pulverized into fine powder. About 600 g of the milled sample was extracted in methanol 100 mL using the Soxhlet apparatus. The volume of the extract collected was 60.5 mL. Standardization of the extract gave 9.16 mg mL<sup>-1</sup>.

**Experimental procedure:** Approval for animal studies was obtained from the College of Health Sciences, Animal Ethics Committee, Abia State University, Uturu. Inbred male albino rats weighing between 150 to 200 g, aged six weeks were used for the investigation. The animals were housed in standard cages and maintained on standard rat pellets and water *ad libitum*. Twenty four hours before the experiment, food was with-held but the animals had free access to water. The animals were randomly divided into five groups of five animals each. Groups one to three received 100, 200, 300 mg kg<sup>-1</sup> of the extract, respectively. Group four received 25 mL kg<sup>-1</sup> of acetylsalicylic acid, while group five a placebo of normal saline (0.5 mL) which is the vehicle for the extract as control by oral administration. Two end points were used in the investigation; Pre-treatment Reaction Time (PTRT) and After Treatment Reaction Time (ATRTR).

**Analgesic studies:** Analgesic effect was investigated by inducing pains in the animals through thermal means as recommended by International Association for the study of pain (Annegowda *et al.*, 2010) and the guidelines on ethical standards for the investigation of pain in animals (Bhandare *et al.*, 2010).

**Hepatoprotective studies:** Albino rats (males) weighing between 145 and 160 g and aged six weeks were used to test for hepatoprotective effect. The animals were divided randomly into three groups consisting of five animals each. Group 1 served as control which received a placebo of normal saline (0.5 mL) which is the vehicle for the extract for 7 days. Group 2 received CCl<sub>4</sub> 0.5 mL kg<sup>-1</sup> i.p. for 7 days while group 3 received CCl<sub>4</sub> 0.5 mL kg<sup>-1</sup>, i.p. and the plant extract (40 mg kg<sup>-1</sup> orally) concurrently for 7 days. After 7 days of treatment, the rats were kept overnight fasting and killed by cervical dislocation. Blood samples were collected by direct cardiac puncture under ether anesthesia and the serum was used for the assay of the marker enzymes. Serum Aspartate Aminotransferase (AST), Serum Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were determined using Randox diagnostic kit. Determination was based on the principle described by Reitman and Frankel (1957).

**Histopathological studies:** Histological studies on the liver were done according to procedures described by Dacie and Lewis (1991) and Disbrey and Rack (1970).

**Statistical analysis:** Values were represented as Mean±SD. Data obtained were subjected to one way Analysis of Variance. (ANOVA) and group means were compared using Duncan's multiple range tests. p-values ( $p < 0.05$ ) were considered significant.

## RESULTS AND DISCUSSION

**Histopathological examination:** Microscopic examination of the control group liver, Fig. 1a showed a normal morphology with the central vein in the centre of the lobule. The liver cells are radially arranged to form sheets. The venous sinusoids converge upon the central vein. The liver cells possess distinct cell outline.

Histopathological examination of  $\text{CCl}_4$ -treated rat liver, Fig. 1b revealed centrilobular fatty degeneration and swelling, hepatic damage, damaged sinusoidal architecture with broad patches of hepatic cellular necrosis and fibrosis.

In liver histology of animals treated with  $\text{CCl}_4$ +plant extract, Fig. 2a and b show normalization of hepatic cells, central vein and portal triad. Liver histology improved as compared to  $\text{CCl}_4$  treated animals with few necrotic patches and very little fatty change. The lamina of the hepatic cells is well-defined. Concurrent administration of  $\text{CCl}_4$  and *O. gratissimum* (L.) leaf extract preserved the histological structure of liver though there was mild congestion and regeneration of liver tissue.

Table 1 shows the result on the analgesic activities of methanolic leaf extract of *O. gratissimum* (L.). With the hot plate method, the rats were affected by heat while the extract reduced the

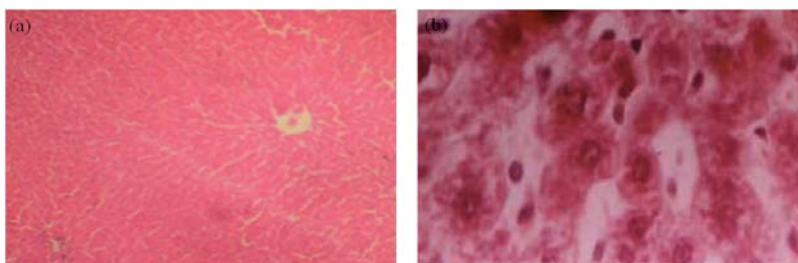


Fig. 1 (a-b): (a) Normal control liver and (b) Liver of animals treated with  $\text{CCl}_4$ . Photo-microphotographs of a cross-section of liver of rats. MAGx40

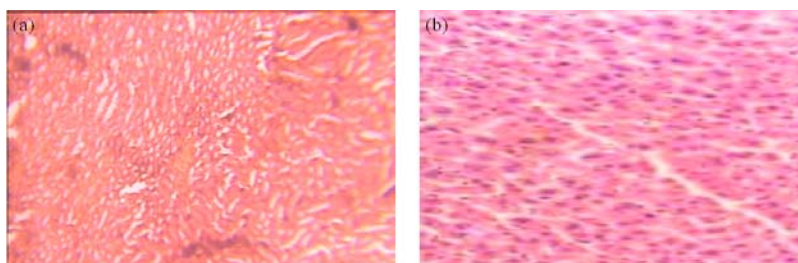


Fig. 2 (a-b): Photo-microphotographs of a cross-section of liver of rats administered  $\text{CCl}_4$ +plant extract. MAGx40

perception of pain (group 1) mean PTRT  $3.4 \pm 0.15$  sec while mean ATRT was  $10.92 \pm 0.2$  sec compared to (group 5-positive control) PTRT  $3.6 \pm 0.41$  sec and ATRT  $7.15 \pm 0.376$  sec. This shows it took a significantly ( $p < 0.05$ ) longer time for the animals to feel the heat when the *O. gratissimum* (L.) leaf extract was administered. Table 1 also shows the effect of acetylsalicylic acid on pain perception in the animals (group 4 animals). PTRT  $3.5 \pm 0.32$  sec and ATRT  $12.53 \pm 1.27$  sec. This result shows that the drug (acetylsalicylic acid) had analgesic effect on the animals compared to the plant extract (group 1-3).

A comparison of mean ATRT for the administered extract (Table 1)  $10.92 \pm 0.21$ ,  $11.20 \pm 0.11$  and  $11.56 \pm 0.15$  sec and mean ATRT for the administered drug (group 4)  $12.53 \pm 1.27$  sec shows that the acetylsalicylic acid had a non-significantly ( $p < 0.05$ ) greater analgesic effect compared to leaf extract of *O. gratissimum* (L.) which is a decline of about 1.61 sec. Our findings seem to be in agreement with those of Chandrashekar *et al.* (2004) who reported that an analgesic is considered positive when the animal fails to respond to painful stimulus for a period corresponding to the PTRT plus 4 sec. Adeyemi *et al.* (2002) and Choi and Hwang (2004) also reported similar values for analgesic activity of *Persea Americana* and *Foeniculum vulgare*, respectively. In this study the results of  $10.92 \pm 0.21$  sec as ATRT and  $3.4 \pm 0.15$  sec as PTRT (Table 1) seem to agree with this theory.

Table 2 shows that carbon tetrachloride ( $\text{CCl}_4$ ) caused liver damage as is evident in the significantly ( $p < 0.05$ ) high concentration of hepatic enzymes in the  $\text{CCl}_4$  treated rats (group 2 animals),  $48.6 \pm 3.84$ ,  $54.4 \pm 2.62$  and  $55.4 \pm 0.27$   $\text{U L}^{-1}$  for serum aspartate aminotransferase; serum alanine aminotransferase and serum alkaline phosphatase, respectively, as compared to the control (group 1),  $25.8 \pm 1.26$ ,  $27.0 \pm 1.14$  and  $26.2 \pm 1.32$   $\text{U L}^{-1}$  for AST, ALT and ALP, respectively. Measurement of enzyme activities in tissues and body fluids play significant roles in disease investigation, diagnosis and detection of tissue cellular damage (Chang *et al.*, 2004). It is quite possible that the increase in activity of these enzymes may be due to the hepatotoxic effect of carbon tetrachloride on the liver (Weber *et al.*, 2003). Adesokan and Akanji (2003) and Jensen and Freeese (2009) also reported the significant increase of serum aspartate aminotransferase and serum alanine aminotransferase levels by hepatotoxins.

For the group 3 animals treated with  $0.5 \text{ mL kg}^{-1}$  of  $\text{CCl}_4$  and  $100 \text{ mg kg}^{-1}$  of the extract of *O. gratissimum* (L.) concurrently, the enzyme concentrations were significantly ( $p < 0.05$ ) reduced  $28.4 \pm 2.35$ ,  $31.2 \pm 2.86$  and  $30.2 \pm 0.01$   $\text{U L}^{-1}$  for AST, ALT and ALP, respectively (Table 2). The

Table 1: The hot plate method

Drug and dose (n = 5)	PTRT (sec)	ATRT (sec)
Group 1 $100 \text{ mg kg}^{-1}$ of extract	$3.4 \pm 0.15$	$10.92 \pm 0.21$
Group 2 $200 \text{ mg kg}^{-1}$ of extract	$3.5 \pm 0.31$	$11.20 \pm 0.11$
Group 3 $200 \text{ mg kg}^{-1}$ of extract	$3.2 \pm 0.25$	$11.56 \pm 0.15$
Group 4 $25 \text{ mL kg}^{-1}$ of drug	$3.5 \pm 0.32$	$12.53 \pm 1.27$
Group 5 $0.5 \text{ mL}$ saline	$3.6 \pm 0.41$	$7.15 \pm 0.36$

\*Values are Mean $\pm$ SD of triplicate determinations. PTRT: Pretreatment reaction time, ATRT: After treatment reaction time

Table 2: Enzyme activities ( $\text{U L}^{-1}$ )

Enzyme	Group 1 (Control)	Group 2	Group 3
AST	$25.8 \pm 1.26$	$48.6 \pm 3.84$	$28.4 \pm 2.35$
ALT	$27.0 \pm 1.14$	$54.4 \pm 2.62$	$31.2 \pm 2.86$
ALP	$26.2 \pm 1.32$	$55.4 \pm 0.27$	$30.2 \pm 0.01$

\*Values are Mean $\pm$ SD of triplicate determinations (n = 5). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

reduction in enzyme concentration for the animals co-administered CCL<sub>4</sub> and the leaf extract (group 3) is significant (p<0.05) when compared to enzyme concentrations of animals administered CCL<sub>4</sub> only (group 2 animals): 48.6±3.84, 54.4±2.62 and 55.4±0.27 U L<sup>-1</sup> for AST, ALT and ALP, respectively. The significant (p<0.05) reduction in enzyme concentrations suggests that methanolic leaves extract of *O. gratissimum* (L.) has hepatoprotective effect and clearly showed that this is a preventive action on damaged liver by *O. gratissimum* leaf extract.

The co-administration of the leaf extract and carbon tetrachloride revealed remarkable inhibition of CCL<sub>4</sub> hepatotoxicity. The significantly (p<0.05) low concentrations of the liver enzymes in group 3 animals compared to those of group 2 showed the ability of the plant extract to inhibit the NADPH<sub>2</sub>-ADP-Fe<sup>2+</sup> system which is responsible for the activation of CCL<sub>4</sub> as reported by Chandrashekar and Prasanna (2010). Among the various methods involved in the hepatotoxic effect of Carbon tetrachloride, one is oxidative damage through free radical generations (Ali *et al.*, 2009; Dhanasekaran and Ganapathy, 2011) and antioxidant property is claimed to be one of the mechanisms of hepato-protective effect of indigenous drugs (Chandrashekar and Prasanna, 2010). Some investigators have also made similar reports on medicinal plants, such as leaves of *Melia azedarach* and seeds of *Piper longum* (Samudram *et al.*, 2008), *Chamomile recutita* (Gupta *et al.*, 2006) and *Terminalia arjuna* (Venkatesh *et al.*, 2007) and *Coptidis* rhizome (Ye *et al.*, 2009).

Reports on effect of long term consumption of *O. gratissimum* in male rats shows the animals had increased weight, reduction in serum protein, cholesterol, lipid peroxidation and haemoglobin. Superoxide dismutase was significantly increased but changes in glutathione-s-transferase, ALT, AST and ALP were not significant (Iweala and Obidoa, 2010). Effraim *et al.* (2003) reported that at high concentrations the *O. gratissimum* leaf extract could be toxic. The essential oil in *O. gratissimum* has been associated with its toxicity (Orafidiya *et al.*, 2004).

## CONCLUSION

The results from this study showed the *O. gratissimum* plant leaf extract increased pain perception time comparable to the drug. It also decreased hepatic enzymes when administered concurrently with carbon tetrachloride in animals as histopathological observations showed remarkable reverting changes in liver sections of the *O. gratissimum* treated group than in the untreated group. Enzyme analyses were also in good agreement with biochemical changes. In conclusion the present study demonstrates that *O. gratissimum* methanolic leaf extract has analgesic properties and hepatoprotective effect against CCL<sub>4</sub><sup>-</sup> induced hepatotoxicity which is preventive. The *O. gratissimum* leaves may be used to achieve these objectives as practiced by ethno-medicine practitioners. *O. gratissimum* should have potential for developing drug for liver disorders.

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## REFERENCES

- Adesokan, A.A. and M.A. Akanji, 2003. Effect of administration of aqueous extract of *Enantia chlorantha* on the activities of some enzymes in the small intestine of rats. Nigerian J. Biochem. Molec. Biol., 18: 103-105.
- Adeyemi, O.O., S.O. Okpo and O.O. Ogunti, 2002. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). Fitoterapia, 73: 375-380.

- Ali, S.A., T.H. Al-Amin, A.H. Mohamed and A.A. Gameel, 2009. Hepatoprotective activity of aqueous and methanolic extracts of *Capparis decidua* stems against carbon tetrachloride induced liver damage in rats. *J. Pharmacol. Toxicol.*, 4: 167-172.
- Annegowda, H.V., M.N. Mordi, S. Ramanathan and S.M. Mansor, 2010. Analgesic and antioxidant properties of ethanolic extract of *Terminalia catappa* L. Leaves. *Int. J. Pharmacol.*, 6: 910-915.
- Arulkumaran, K.S.G., A. Rajasekaran, R. Ramasamy, M. Jegadeesan, S. Kavimani and A. Somasundaram, 2009. Cassia roxburghii seeds protect liver against toxic effects of ethanol and carbontetrachloride in rats. *Int. J. Pharm. Tech. Res.*, 1: 237-246.
- Bhandare, A.M., A.D. Kshirsagar, N.S. Vyawahare, A.A. Hadambar and V.S. Thorve, 2010. Potential analgesic, anti-inflammatory and antioxidant activities of hydroalcoholic extract of *Areca catechu* L. nut. *Food Chem. Toxicol.*, 48: 3412-3417.
- Birt, D.F., S. Hendrich and W. Wang, 2001. Dietary agents in cancer prevention: Flavonoids and isoflavonoids. *Pharmacol. Ther.*, 90: 157-177.
- Chandrashekar, K.S., D. Satyanarayana and A.B. Joshi, 2004. Analgesic activity of *Leucas lavandulaefolia* rees. *Indian Drugs*, 42: 78-80.
- Chandrashekar, K.S. and K.S. Prasanna, 2010. Hepatoprotective activity of *Leucas lavandulaefolia* against carbon tetrachloride-induced hepatic damage in rats. *Int. J. Pharma Sci. Res.*, 2: 101-103.
- Chang, J.S., L.C. Chiang, F.F. Hsu and C.C. Lin, 2004. Chemoprevention against hepatocellular Carcinoma of *Comus officinalis*-*in vitro*. *Am. J. Chin. Med.*, 32: 717-725.
- Choi, E.M. and J.K. Hwang, 2004. Anti-inflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. *Fitoterapia*, 75: 557-565.
- Dacie, J.V. and S.M. Lewis, 1991. *Practical Hematology*. 7th Edn., Edinburgh, Churchill Livingstone, England, pp: 37-85.
- Danziel, A.C., 1996. *The Useful Plants of West Africa*. Crown Agents, London, pp: 162.
- Dhanasekaran, J.J. and M. Ganapathy, 2011. Hepatoprotective effect of *Cassia auriculata* L. leaf extract on carbon tetrachloride intoxicated liver damage in wister albino rats. *Asian J. Biochem.*, 6: 104-112.
- Disbrey, B.D. and J.H. Rack, 1970. *Histological Laboratory Methods*. Livingstone, Edinburgh, ISBN: 0443006946, pp: 56-128.
- Effraim, K.D., T.W. Jacks and O.A. Sodipo, 2003. Histopathological Studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of rabbit. *Afr. J. Biomed. Res.*, 6: 21-25.
- Gupta, A.K., H. Chitme, S.K. Dass and N. Misra, 2006. Antioxidant activity of *Chamomile recutita* capitula methanolic extracts against CCl<sub>4</sub>-induced liver injury in rats. *J. Pharmacol. Toxicol.*, 1: 101-107.
- Higdon, J.V., B. Delage, D.E. Williams and R.H. Dashwood, 2007. Cruciferous vegetables and human Cancer risk: Epidemiologic evidence and mechanistic basis. *Pharmacol. Res.*, 55: 224-236.
- Iweala, E.E.J. and O. Obidoa, 2010. Studies on some biochemical and histological changes associated with long term consumption of leaves of *Ocimum gratissimum* L. in male rats. *Am. J. Food Technol.*, 5: 376-384.
- Iwu, M.M., 1993. *Handbook of African Medicinal Plants*. 1st Edn., CRC Press, Boca Raton, FL., ISBN-10: 084934266X, pp: 464.
- Jensen, J.E. and D. Freese, 2009. *Liver Function Tests*. Colorado Center for Digestive disorders 205s, Suit A Longmont Co., pp: 51-163.



- Jeppesen, P.B., S. Gregersen, K.K. Alstrup and K. Hermansen, 2002. Stevioside induces antihyperglycaemic insulinotropic and glucagonostatic effects *in vivo* Studies in the diabetic goto Kakizaki (GK) rats. *Phytomedicine*, 9: 9-14.
- Lee, C.N., K.L. Wong, J.C. Liu, Y.J. Chen, J.T. Chen and P. Chan, 2001. Inhibitory effect of stevioside on calcium influx to produce antihypertension. *Plant. Med.*, 67: 796-799.
- Lee, K.J., E. Woo, C.Y. Choi, D.W. Shin, D.G. Lee and H.J. You, 2004. Protective effect of acetoside on carbon tetrachloride induced hepatotoxicity. *Life Sci.*, 74: 1051-1064.
- Manna, P., M. Sinha and P.C. Sil, 2006. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC Complementary Alternative Med.*, 6: 33-33.
- Oforokansi, K.C., C.O. Esimonu and C.R. Anele, 2005. Evaluation of the *in-vitro* combined effects of the leaf of the *Bryophyllum pinnatum* and *Ocimum gratissimum*. *Plant Prod. Res. J.*, 9: 23-27.
- Orafidiya, L.O, E.O. Agbani, E.O. Iwalewa, K.A. Adelusola and O.O. Oyedapo, 2004. Studies on the acute and sub-chronic toxicity of essential oil of *Ocimum gratissimum* L. leaf. *Phytomedicine*, 11: 71-76.
- Reitman, S. and S. Frankel, 1957. Determination of liver enzymes activity. *Am. J. Clin. Pathol.*, 28: 56-56.
- Samudram, P., H. Rajeshwari, R. Vasuki, A. Geetha and P.S. Moorthi, 2008. Hepatoprotective activity of bi-herbal ethanolic extract on Ccl4-induced hepatic damage in rats. *Asian J. Biochem.*, 32: 308-314.
- Singh, U.P., D.P. Singh, S. Maurya, R. Maheshwari, M. Singh, R.S. Dubey and R.B. Singh, 2004. Investigation on the phenolics of some spices having pharmacotherapeutic properties. *J. Herb Pharmacother.*, 4: 27-42.
- Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.*, 39: 44-84.
- Venkatesh, S., G.K. Mohan, E. Pallavi, B.R. Kumar and M. Ramesh, 2007. Hepatoprotective activity of *Ficus carica* Linn. leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. *DARU*, 15: 162-166.
- Virgili, F., C. Scaccini, L. Packer and G. Rimbach, 2001. Cardiovascular Disease and Nutritional Phenolics. In: *Antioxidants in Food*, Pokorny, J., N. Yanishlieva and G. Rimbach (Eds.). Woodhead Publishing Ltd., Cambridge, ISBN: 1-85573-463-X pp: 87-99.
- WHO, 2001. Traditional medicine. Technical report series pp 3.
- Weber, L.W., M. Boll and A. Stampfl, 2003. Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.*, 33: 105-136.
- Ye, X., Y. Feng, Y. Tong, K.M. Ng and S. Tsao *et al.*, 2009. Hepatoprotective effects of *Coptidis rhizome* aqueous extract on carbon tetrachloride-induced Acute liver hepatotoxicity in rats. *J. Ethnopharmacol.*, 124: 130-136.
- Zhang, H.Y. and L.F. Wang, 2002. Theoretical elucidation on structure-antioxidant activity relationships for indolinonic hydroxylamines. *Bioorganic Med. Chem. Lett.*, 12: 225-227.