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# Hepatoprotective Effect of *Phyllanthus reticulatus* Leaves against Carbon Tetrachloride-Induced Hepatic Damage in Rats

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## ABSTRACT

***Methanolic extract of Phyllanthus reticulatus (leaves), was evaluated for its hepatoprotective activity against carbon tetrachloride ($CCl_4$)-induced hepatic damage in Wistar rats; by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin. Histological studies were also carried out to support our hypothesis. Administration of the extract (200 and 400 mg/kg) orally markedly prevented $CCl_4$-induced elevation of serum GPT, GOT, ALP and total bilirubin levels. A comparative histopathological study of liver in treated groups exhibited similarity to normal tissue architecture, compared to $CCl_4$-treated group.***

***Keywords: Phyllanthus reticulatus and Hepatoprotective.***

## INTRODUCTION

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment to its functions may lead to many implications on one's health. Management of liver disease is still a challenge to the modern medicine. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis (Sherlock S., Dooley J. 2002). *Phyllanthus reteculatus* is a broad spectrum medicinal plant that has received world- wide recognition (Srividiya and Perival, 1995). *P. reteculatus* is generally employed to reduce pain, expel intestinal gas, to stimulate and promote digestion, as anti-helminthes to expel intestinal worms and act as a mild Laxative. *P. reteculatus* also has antiseptic, diuretic, antiviral, anti-diabetic, hypotensive and antipyretic properties, and is also used in the treatment of jaundice, diarrhoea, dysentery, wound, ulcers and urogenital diseases (Calixto *et al.,* 1998). The plants of the genus *Phyllanthus* are widely distributed in most tropical and subtropical countries and have long been used in traditional medicine to treat chronic liver disease (Liu *et al*., 2003).

Plants contain numerous constituents; some tend to possess some level of toxicity. Cases of this toxicity in plants have been reported (Santos et al., 1995; Shaw et al., 1997; Kaplowitz, 1997). *P. reteculatus* has been classified among plants with a low potential for toxicity, with an LD50 averaging 2000 mg/kg/day (Krithika and Verma, 2009). The phytochemical analysis of the *P. reteculatus* extract confirmed the presence of tannins, saponins, flavonoids and alkaloids. The plant extract have been found to contain high levels of saponins, tannins, flavonoids and alkaloids (Fernand, 1998; Naaz, 2007; Krithika and Verma, 2009).

Therefore, in view of these reasons, we have selected it to study the Hepatoprotective activity of its leaves.

## MATERIAL AND METHODS

### *Plant Material and Extraction*

Fresh leaves of the plant *P. reteculatus* were collected in the morning from Khartoum area in the year 2015, dried and powdered. Methanolic extract was prepared by maceration of leaves powder (1000g) with methanol (3L) for 48 hours with intermittent stirring. After extraction, the solvent was filtered and concentrated under reduced pressure. The extract (yield: 27%) obtained was stored at -20°C until being used.

### Animals

Experiments were performed in healthy male Wistar Albino rats (11-12 weeks old). Rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan, under illumination at night and early morning with feed and drinking water provided *ad libitum*. The rats were divided randomly to four groups (n=5). Animals were fasted for 16 h prior to the administration of $CCl_4$. The study protocol was approved by the Institutional Ethical Committee.

### Chemicals

Assay kits for the estimation of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were purchased from Randox, UK. 5, 5' dithio bis-(2-nitrobenzoic acid) was purchased from Sigma Chemical Co., USA. All other chemicals were of analytical grade.

### Acute toxicity study

Wistar Albino rats of either sexes weighing between 100 and 150 gms were used in present investigation. The animals were fasted overnight prior to the experimental procedure. The

methanolic extract was administered orally only once in doses of 1000 and 2000 mg/kg to two groups of rats (n=5), and percentage mortality was reported after 7 days (Kamel, M.S. *et al.,* 1991).

**Hepatoprotective activity**

Hepatic injury was induced in rats by subcutaneous administration of a single dose of 1.0 ml/kg $CCl_4$ mixed with equal volume of olive oil on the $7^{th}$ day (Salwa, A., *et al.*, 1988). Animals were divided in to 5 groups of 5 animals each: Group 1: Control group, treated with 2% w/v gum acacia in water at the dose of 2.0 ml (orally) for 7 days, then injected with olive oil treatment (1.0 ml/kg, s.c.) on day 7. Group II: Treated with vehicle (2.0 ml, orally) for 7 days followed by $CCl_4$ on day 7. Group III: Treated with the standard drug silymarin (100 mg, orally) for 7 days followed by $CCl_4$ on day 7. Group IV and V: treated with methanolic extract of *P. reteculatus* suspended in 5% gum acacia in water at doses of 200 and 400 mg/kg (orally) for 7 days followed by $CCl_4$ on day 7, respectively.

**Estimation of Biochemical Parameters**

The rats were sacrificed 24 hours after the administration of the last dose under anesthesia using halothane. The blood was collected and the serum was separated by centrifugation. The serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate trasnsaminase (SGPT), serum alkaline phosphatase (ALP) and total bilirubin were estimated by the spectrophotometer (Hatapakki B.C., *et al.,* 2006).

**Statistical analysis**

All values are expressed as means $\pm$ S.D. the data were subjected to Student's t-test; and one-way ANOVA followed by Newman-Keuls multiple comparison test; and $P<0.05$ were considered significant (Thakur V.D., Mengi S.A. 2005).

**Histopathological Studies**

Liver was rapidly excised immediately after the sacrifice, and was washed with normal saline (0.09%), fixed in formalin (10%) and embedded in paraffin wax. Sections of 4-5µ thickness were made and stained with haematoxylin-eosin. Histological observations were made under light microscope (Sethuraman M.G., *et al*., 2003).

## RESULTS AND DISCUSSION

**Results**

**Acute toxicity study**

Oral administration of the methanolic extract of *P. reteculatus* at doses of 1000 and 2000 mg/kg did not produce any overt changes in behavior or symptoms of toxicity. The extract was found to be safe up to a dose 2000 mg/kg in rats. Thus two doses (200 and 400 mg/kg) which were found to be safe, were employed for further pharmacological studies.

**Biochemical estimations**

The results for the effect of *P. reteculatus* on serum enzymes SGOT, SGPT, ALP and Total serum bilirubin are shown in table **1**. The induced hepatic damage by $CCl_4$ in rats caused significant rise in marker enzymes SGOT, SGPT, ALP and Total serum bilirubin. Oral administration of *P. reteculatus* extract 400 mg/kg was observed to significantly lower the levels of marker enzymes SGOT, SGPT and Total bilirubin ($P < 0.001$). It also lowered ALP ($P < 0.05$). While, *P. reteculatus* extract 200 mg/kg was observed to lower significantly the levels of SGOT ($P < 0.01$), SGPT ($P < 0.05$) and Total bilirubin ($P < 0.01$) but did not lower ALP. The effect of Silymarin seemed dose dependent and offered relatively greater protection. The toxic effect of $CCl_4$ was significantly controlled in the animals treated with methanolic

extract of *P. reteculatus* by way of restoration of the levels of liver function biochemistry similar to that of standard drug silymarin.

**Histopathology**

Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, cenusoidal spaces and central vein (Figure 1a). Group II animals exhibited disarrangement of normal hepatic cells with intense centrilobular necroses, vacuolization of cytoplasm and fatty degeneration (Figure 1b). The liver sections of the rats treated with methanolic extract of *P. reteculatus* and silymarin followed by $CCl_4$ intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure 1c, 1d, 1e).

## DISCUSSION AND CONCLUSION

Hepatotoxicity of liver is due to the consequence of $CCl_4$ activation by cytochrome P-450 to trichloromethyl free radical ($CCl_3$) and which in turn disrupts the structure and function of lipid and protein macromolecules in the membrane of the cell organelles (Obidah W., *et al*., 2009) Carbon tetrachloride is the one of the most commonly used hepatotoxins in the experimental study of liver diseases (Johnson D.E., Kroening C., 1998). It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration (Shi G.F., Q. Li, 2005). The increased level of SGOT, SGPT, ALP and total bilirubin is sensitive indicators of liver injury (Vadivu R.1., *et al*., 2008). The increase in the levels of serum bilirubin reflected the depth of jaundice. The increase in transaminases and alkaline phosphatase was the clear indication for the loss of functional integrity of the cell membrane (Watt J.M., Breyer-Brandwijk M.G. 2002). The leaves of *P. reteculatus* were reported possessing flavonoids. It was suggested that the plants containing flavonoids possess hepatoprotective activity (Reitman S., Frankel S. 1957). In previous studies, The extracts of leaf, stem, stem bark and root of *P. reteculatus* were screened for hepatoprotective activity in Wistar albino rats. The stem bark extracts of the plant showed significant ($P < 0.05$) hepatoprotective effects as revealed by a decrease in the activity of serum transaminase and alkaline phosphatase enzymes as compared to control rats.

In the present study, also it was seen that administration of $CCl_4$ elevates the levels of serum marker enzymes SGOT, SGPT, ALP and total bilirubin. The Sylimarin-treated groups exhibited lower levels of marker enzymes as compared to $CCl_4$-treated groups. The stabilization of marker enzyme levels by *P. reteculatus* extract is a clear indication of the improvement of the functional status of the liver cells (Valeer J.D. 2003). These findings were further confirmed with histopathological studies. The histopathological examination clearly reveals that the hepatic cells and central vein were similar to normal tissue in the group treated by *P. reteculatus* extract (400 mg/kg) in contrast to the group which received $CCl_4$.

Thus, *P. reteculatus* can be considered an effective hepatoprotective drug as it restores liver damage caused by $CCl_4$. Hence, this extract can be used in poly herbal formulations to provide a synergistic effect with other hepatoprotective drugs and thereby preventing the process of initiation and progress of hepatocellular diseases (Mujumdar, A.M. *et al*., 1998).

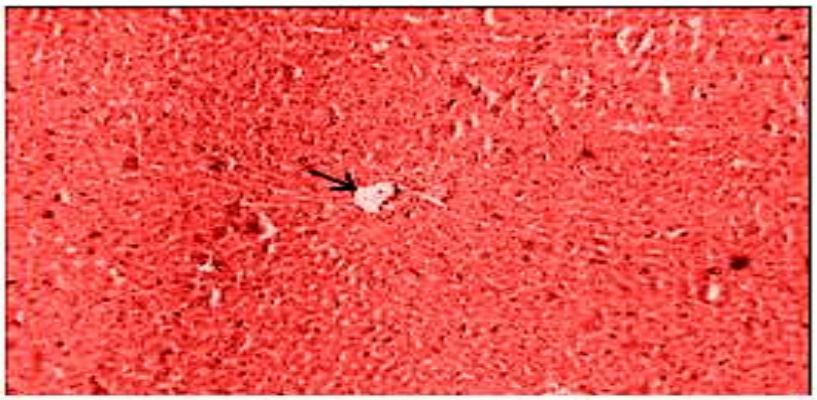
**Table 1. Effect of using *P. reteculatus* extract as treatment on different biochemical parameters in the serum of rats.**

| Parameters | Control | $CCl_4$ | Silymarin (100 mg/kg) | B.aegyptiaca (200 mg/kg) | B.aegyptiaca (400 mg/kg) |
|---|---|---|---|---|---|
| **SGOT (U/ml)** | 40.64±4.31 | 110.24±8.15* | 52.26±6.54** | 93.43±6.18*** | 65.71 ±7.29** |
| **SGPT (U/ml)** | 33.17± 6.09 | 126.75±6.23* | 43.18± 4.62 | 116.45±5.47** | 82.56 ±5.58** |
| **ALP (KA/unit)** | 14.52± 2.65 | 25.21± 2.91* | 17.22±2.58** | 23.78± 2.24 | 19.17± 2.16** |
| **T. Bilirubin (mg/dl)** | 0.72± 0.05 | 1.40± 0.06* | 0.61± 0.05** | 1.27± 0.07*** | 0.87± 0.06** |

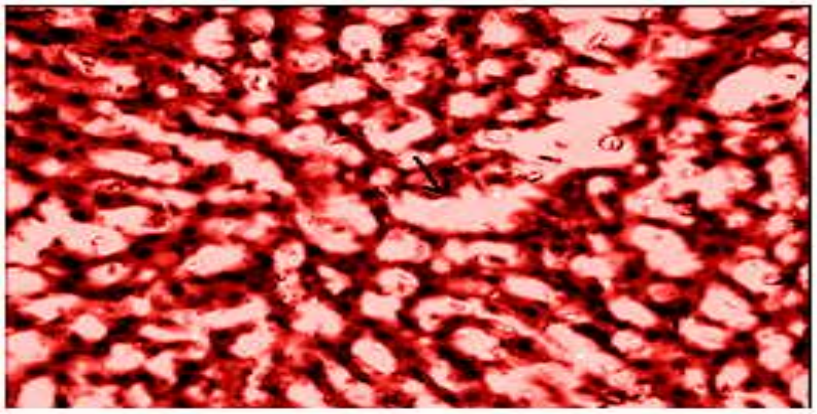
Values expressed as mean ± S.D. of five animals in each group.

*P < 0.001 as compared with the group I.

**P < 0.05, ***P < 0.01 as compared with the group II.

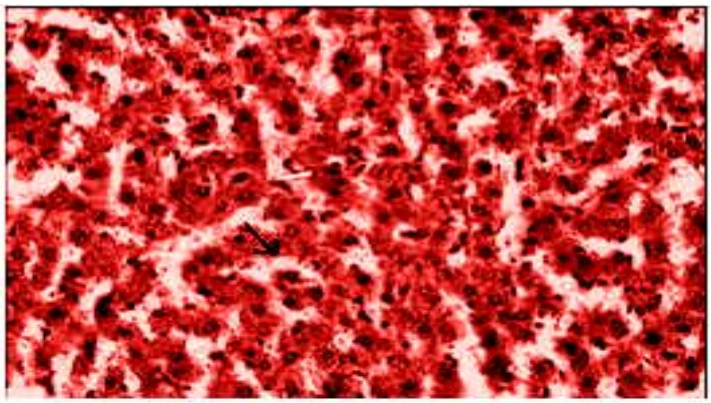


(a)

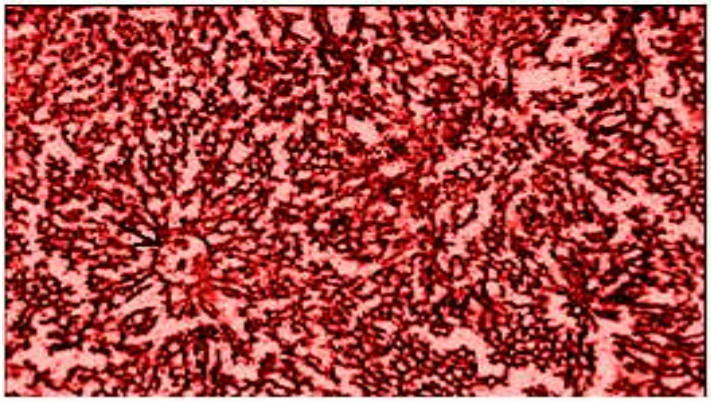


(b)

**Figure 1. a. Section through the liver tissue of control rats showing normal histology.**
**Figure 1. b. Section of the liver tissue of rats treated with $CCl_4$ showing necrosis and fatty vacuoles.**

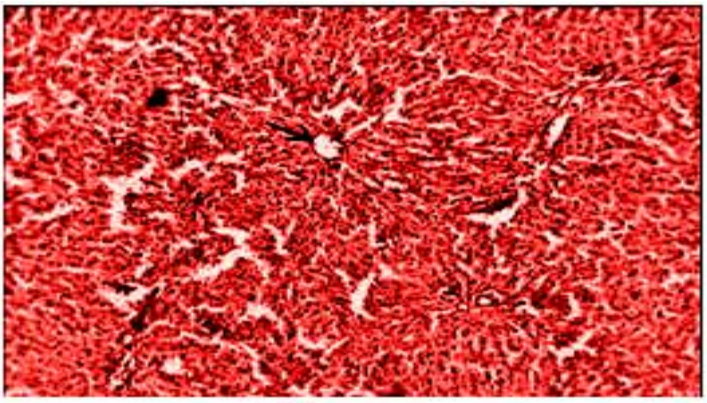


(c)



(d)

**Figure 1.c. Section of the liver tissue of silymarin- treated rat showing normal hepatocytes.**
**Figure 1.d. Section of the liver tissue of methanol extract (200mg/kg) treated rat showing normal arrangements of hepatocytes around the central vein.**



(e)

**Figure 1.e. Section of the liver tissue of methanol extract (400mg/kg) treated rat showing normal arrangements of hepatocytes around the central vein, absence of necrosis.**

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## REFERENCES

**Breyer, J.M. and Brandwijk, M.G. (1982).** The medicinal and poisonus plants of Southern and Eastern Africa. 2 nd ed. London: Livingstone. p. 1064-5.

**Calixto, J.B., Santos, A.R.S, Cechinel-Filho, V. and Yunes, R. A. (1998).** A Review of the plants of the Genus *Phyllanthus*: their Chemistry, Pharmacology and Therapeutic Potential. Med. Res. Rev. 18: 225-258

**Fernand, V.E. (1998).** Initial characterization of crude extracts from *Phyllanthus amarus schum* and *thonn* and *Quassia amara L*. using normal phase thin layer chromatography [Thesis]. Louisiana State University; pp6-13.

**Hall, J.B. (1992).** Ecology of a key African multipurpose tree species *Balanites aegyptiaca* Del. (Balanitaceae): The state of knowledge. Forest Ecol Manag. 50:1-30.

**Hatapakki, B.C., Hukkeri, V.I., Gopalkrishna, B., Patil, D.N. and Kandale, J.B. (2006).** Hepatoprotective Activity Fruit Pulp of Balanites roxburghii Indian Drugs-Bombay. 43 (7): 583-585.

**Johnson, D.E. and Kroening, C. (1998).** Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes, Pharmacol Toxicol. 83: 231-917.

**Kamel, M.S., Ohtani, K., Kurokawa, T., Assaf, M.H., El-Shanawany, M.A., Ali, A.A., *et al*. (1991).** Studies on *Balanites aegyptiaca* fruits, an antidiabetic Egyptian folk medicine. Chem Pharm Bull (Tokyo). 39:1229-33.

**Kaplowitz, N. (1997).** Hepatotoxicity of Herbal Remedies. Insight into the intricacies of plant- animal warfare and Cell Death. Gastroenterology. 113: 1408 – 1412.

**Khan, F.M. (2009).** Ethno-veterinary medicinal usage of flora of greater cholistan desert (Pakistan). Pak Vet J. 29:75-80.

**Kirtikar, B.D. and Basu, B.D. (1933).** Indian Medicinal Plants. Deheradun: International Book Distributors; Vol. 3. p. 1823-4.

**Koko, W.S., Ahmed Mesaik, Yousaf, S., Galal, M. and Igbal Choudhary, M. (2008).** In vitro immunomodulating properties of selected Sudanese medicinal plants. Journal of Ethnopharmacology. 118 (1): 26-34.

**Krithika, R. and Verma, R. J. (2009).** Mitigation of carbon tetrachloride-induced damage by *Phyllanthus amarus* in liver of mice. Acta Pol Pharm. 2009 Jul-Aug; 66(4):439-44.

**Lui, R-LH. and Huang, Y.L. (2003).** Genus *Phyllanthus* for chronic hepatitis B virus infection: A systemic review. Viral Hepatitis (8): 358-366.

**Mohamed, A.H., Eltahir, K.E.H, Ali, M.B., Galal, M., Ayeed, I.A., Adam, S.I. and Hamid, O.A. (1999).** Some pharmacological and toxicological studies on Balanites aegyptiaca bark. *Phytotherapy Research*. 13 (5), 439-441.

**Mujumdar, A.M., Upadhye, A.S. and Pradhan, A.M. (1998).** Effect of *Azaderachta indica* leaf extract on $CCl_4$ induced hepatic damage in albino rats, Indian Journal of Pharmaceutical Sciences. 60: 363-367.

**Naaz, F., Javed, S. and Abdin, M.Z. (2007).** Hepatoprotective effect of ethanolic extract of *Phyllanthus amarus* Schum. et Thonn. on aflatoxin B1-induced liver damage in mice. J Ethnopharmacol; 113: 503-509.

**Ndoye, M., *et al.* (2004).** Reproductive biology in *Balanites aegyptiaca* (L.) Del., a semi-arid forest tree. Afr J Biotechnol. 3:40-6.

**Neuwinger, H.D. (2004).** Plants used for poison fishing in tropical Africa. Toxican. 44 (4): 417-430.

**Obidah, W., Nadro, M.S., Tiyafo, G.O. and Wurochekke, A.U. (2009).** Toxicity of crude *Balanites aegyptiaca* seeds oil in rats. J Am Sci. 5:6-13.

**Pandey, C.N. (2005).** Medicinal plants of Gujarat. Gujarat, India: Gujarat Ecological Education and Research Foundation. p. 387.

**Reitman, S. and Frankel S. (1957).** A colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases, American journal of Clin. Path. 28: 56-63.

**Salwa, A., Maksoud and Hadidi M.N.E. (1988).** The flavonoids of Balanites aegyptiaca (Balanitaceae) from Egypt. Plant systematic and evolution. 160 (3-4), 153-158.

**Santos, A.R.S, Ailho, V.C, Yunes, R.A. and Calixto, J.B. (1995).** Analysis of the mechanism underlying the Anti-nociceptive Effect of the Extracts of plants from the Genus Phyllanthus. General Pharmacol. 26: 1499- 1506

**Sethuraman, M.G., Lalitha, K.G. and Rajkapoor, B. (2003).** Hepatoprotective activity of Sarcostemma brevistigma against carbon tetrachloride induced hepatic damage in rats. Current Science. 84; 1186-1187.

**Shaw, D., Leon, C., Koleu, S. and Murray, V. (1997).** Traditional Remedies and Food Supplements. A Five-year Toxicology Study (1991, 1995). Drug Safety. 17: 342- 356.

**Sherlock, S. and Dooley, J. (2002).** Disease of liver and biliary system. Blackwel scientific publications. 11;322-356.

**Shi, G.F. and Q. Li (2005).** Effects of oxymetrine on experimental hepatic fibrosis and its mechanism in-vivo. World J. Gastroenterol. 11: 268-271.

**Staerk, D., Chapagain, B.P., Lindin, T., Wiesman, Z. and Jaroszewski, J.W. (2007).** Structural analysis of complex saponins of *Balanites aegyptiaca* by 800 MHz 1H NMR spectroscopy. Magn Reson Chem. 44:923-8.

**Thakur, V.D. and Mengi, S.A. (2005).** Neuropharmacological profile of Eclipta alba (Linn.) Hassk, Journal of Ethnopharmacology. 102: 23-31.

**Vadivu, R.1., Krithika, A., *et. al*. (2008).** International Journal of Health Research. 1(3): 163-168.

**Valeer, J.D. (2003).** Liver tissue examination, Journal of Hepatology. 39: 543-549.

**Watt, J.M. and Breyer-Brandwijk M.G. (2002).** The Medicinal and Poisonous Plants of South and East Africa. Edinburgh and London: E. and S. Livingstone. p. 164-165.

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