

## PHARMACOLOGY

MITIGATION OF CARBON TETRACHLORIDE -INDUCED DAMAGE  
BY *PHYLLANTHUS AMARUS* IN LIVER OF MICE

RAJESH KRITHIKA and RAMTEJ J. VERMA\*

Department of Zoology, University School of Sciences, Gujarat University,  
Ahmedabad – 380 009, India

**Abstract:** Liver disease has become a global concern worldwide. In absence of reliable liver protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders as they are believed to be harmless based on their natural origin. The aim of the present study was to determine the hepatoprotective activity of *Phyllanthus amarus* plant extract against carbon tetrachloride (CCl<sub>4</sub>)-induced liver damage in female mice. Carbon tetrachloride administration caused a significant increase in liver and serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP), while total protein content significantly decreased as compared to vehicle control. The effect was dose-dependent. Oral administration of aqueous extract of *Phyllanthus amarus* along with carbon tetrachloride caused significant mitigation of CCl<sub>4</sub>-induced changes.

**Keywords:** carbon tetrachloride, liver, *Phyllanthus amarus* extract, serum

Medicinal plants are the local heritage with global importance. In recent times the pace of drug discovery from natural products has provided unprecedented opportunities due to mélange of natural product chemistry with modern biology demand for cost effective medication and need for biological agents for sustainable resources. The increased demand for botanical products is met by an expanding industry and accompanied by calls for assurance of quality, efficacy and safety (1). The preventive action of liver damage induced by CCl<sub>4</sub> has widely been used as a marker of hepatoprotective activity of drugs in general (2).

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 (3). *Phyllanthus amarus* Schum & Thonn (family Euphorbiaceae) is a medicinal herb used in connection with secondary hepatitis and other ailments, in ayurvedic medicine for over 2000 years (4). *P. amarus* is also a well known antiviral agent (5). However, attempts made earlier to demonstrate the hepatoprotective effect of *P. amarus* along with other plants of the same genus against CCl<sub>4</sub> had somehow unfortunately placed this plant at the bottom of the list (6, 7).

The present study was an effort to evaluate hepatoprotective property of aqueous extract of *P. amarus* against CCl<sub>4</sub>-induced liver toxicity.

## EXPERIMENTAL

## Materials and methods

Carbon tetrachloride was purchased from Merck, Mumbai, India. All other chemicals were procured from Himedia, Mumbai, India and were of analytical grade. Olive oil was obtained from Figaro, Madrid, Spain. *Phyllanthus amarus* Schum & Thonn was collected in the month of August and September 2007 from the herbal garden of Gujarat University, Ahmedabad, Gujarat, India. Herbarium specimen was prepared and authenticated by National Institute of Science Communication and Information Resources (Ref: NISCAIR/ RHMD/consult/-2007-08/938/122); New Delhi, India. The extract was prepared according to WHO protocol CG-04 (8). The aerial parts of the plant were washed thoroughly, cut into small pieces and dried under shade and powdered. Five grams of finely grounded plant powder was extracted in a Soxhlet apparatus using 100 mL of solvent containing 50% distilled water and 50% ethanol for 3 h. The extract

\* Corresponding author: phone no. 091-79-2630 2362; e-mail: ramtejverma2000@yahoo.com

was twice filtered with Whatman filter paper no. 1. It was thereafter concentrated in water bath and dried, yielding 8.5% of the product.

Inbred 6 – 8 week old Swiss strain female albino mice (*Mus musculus*) used in the study were obtained from Zydus Research Centre, Ahmedabad, India and weighed approximately 32 – 35 g at the initiation of the study. The animals were kept in well ventilated cages at  $25 \pm 2^\circ\text{C}$ . They were provided with normal food pellet (Amrut feeds, Mumbai, India) and water *ad libitum* and were maintained in 12 h light/dark cycle in central animal house facility of Zoology Department, Gujarat University, Ahmedabad, India. The research protocols were reviewed and approved by The Committee for the Purpose of Control and Supervision of Experiment on Animals (Reg – 167/1999/CPCSEA), New Delhi, India.

#### Carbon tetrachloride-induced liver injury

The initial study was focused to determine the best possible dosage of  $\text{CCl}_4$  to induce liver damage in mice. Three doses of  $\text{CCl}_4$  were selected based on its oral  $\text{LD}_{50}$  value of 8260 mg per kg body weight (9). Fifty animals were randomly divided into five groups (ten per group) and caged separately. Group 1 (untreated control) were maintained without any treatment and were given free access to food and drinking water. Animals of group 2 received olive oil (0.2 mL/animal/day) for 30 days which is used as the vehicle to dissolve  $\text{CCl}_4$ . Animals of group 3, 4 and 5 were orally administered with  $\text{CCl}_4$  at doses of 275.3 (low dose), 413 (mid dose) and 826 (high dose) (dosage corresponding to  $1/30^{\text{th}}$ ,  $1/20^{\text{th}}$  and  $1/10^{\text{th}}$  of  $\text{LD}_{50}$  value of  $\text{CCl}_4$ , respectively) in 0.2 mL olive oil/kg body weight/day for 30 days using a feeding tube attached to a hypodermal syringe.

#### $\text{LD}_{50}$ determination of *Phyllanthus amarus*

Acute oral toxicity (AOT) studies of *P. amarus* was performed using main dose test of up and down procedure as per Organisation for Economic Co-operation and Development (OECD) guideline 425 (10) using female mice. The animal was fasted for 3 h prior to the experiment and orally administered single dose of the extract and observed for mortality for up to 48 h. Based on this, the dose of the next animal was determined. All dosed animals were also observed for 14 days to study the long term toxicity. The  $\text{LD}_{50}$  of the extract was calculated using “AOT 425” software provided by the Environment Protection Agency, Washington, D.C, USA (11).

#### Phytochemical screening of *Phyllanthus amarus*

Quantitative determinations of tannins, saponins, flavonoids and alkaloids were carried out according to the methods described by Sofowara (12), Trease and Evans (13) and Harborne (14). The phytochemicals were later estimated quantitatively. Tannins were estimated by the method of Van-Burden and Robinson (15). Saponins were quantified by Obadoni and Ochuko method (16). Flavonoids were quantitatively assayed according to Boham and Kocipai-Abyazan (17) and alkaloids were determined using Harborne (14) method. Analysis was done in triplicates and values were expressed as the mean  $\pm$  S.E.M.

#### *Phyllanthus amarus* treatment

Seventy animals were divided into seven groups (n = 10) and caged separately. Group 1 animals (untreated control) were maintained and were given free access to food and drinking water. Animals of group 2 received olive oil (0.2 mL/animal/day) which is used as the vehicle to dissolve  $\text{CCl}_4$ . Group 3 animals were given *Phyllanthus amarus* extract (300 mg/kg body weight/day) in 0.2 mL of distilled water, which served as plant control group. Animals of group 4 received HD of  $\text{CCl}_4$  as mentioned above. Group 5, 6 and 7 animals were orally treated with HD of  $\text{CCl}_4$  along with doses of 100, 200 and 300 mg/kg body weight/day of *Phyllanthus amarus* extract dissolved in 0.2 mL distilled water for 30 days.

The treatment was continued for 30 days in all groups. The blood was collected by cardiac puncture under mild ether anesthesia. The serum was separated by centrifugation at  $1000 \times g$  for 10 min. The animals were then sacrificed by cervical dislocation. The liver was dissected out quickly, washed in ice-cold saline and blotted free of blood. A 10% homogenate of the liver tissue was prepared in 0.1 M Tris-HCl buffer (pH 7.4) and utilized for further biochemical analysis.

#### Assessment of liver function

The ALT and AST activity were assayed according to the method of Reitman and Frankel (18). ALP activity was determined as described by Bessey et al. (19). ACP activity was analyzed by the method described in Sigma Technical Bulletin (20). The total protein content was determined by the method of Lowry et al. (21) using bovine serum albumin as a standard. Among the parameters studied, ALT, AST and ALP are considered as reliable markers for liver injury (22).

Table 1. Effect of carbon tetrachloride on biochemical parameters in the liver of mice

Parameters	Experimental groups				
	Untreated control 1	Vehicle control 2	275.3 mg / kg body weight/ day (LD) 3	413 mg / kg body weight / day (MD) 4	826 mg / kg body weight / day (HD) 5
Alanine transaminase (mU/mg protein/30 min)	1.52 ± 0.03	1.52 ± 0.04	2.05 ± 0.04 <sup>abde</sup>	2.51 ± 0.05 <sup>abce</sup>	3.07 ± 0.09 <sup>abcd</sup>
Aspartate transaminase (mU/mg protein/60 min)	1.82 ± 0.03	1.79 ± 0.05	2.60 ± 0.04 <sup>abde</sup>	3.29 ± 0.06 <sup>abce</sup>	4.35 ± 0.06 <sup>abcd</sup>
Alkaline phosphatase (µmoles <i>p</i> -nitrophenol released/30 min/mg protein)	0.28 ± 0.004	0.28 ± 0.004	0.32 ± 0.003 <sup>abde</sup>	0.35 ± 0.001 <sup>abce</sup>	0.40 ± 0.006 <sup>abcd</sup>
Acid phosphatase (µmoles <i>p</i> -nitrophenol released/30 min/mg protein)	1.27 ± 0.01	1.24 ± 0.01	1.55 ± 0.01 <sup>abde</sup>	1.85 ± 0.02 <sup>abce</sup>	2.14 ± 0.06 <sup>abcd</sup>
Total protein (mg/100 mg)	22.96 ± 0.25	22.92 ± 0.37	21.04 ± 0.18 <sup>abde</sup>	17.99 ± 0.17 <sup>abce</sup>	15.90 ± 0.15 <sup>abcd</sup>

<sup>a</sup> as compared to group 1,  $p \leq 0.05$ , <sup>b</sup> as compared to group 2,  $p \leq 0.05$ , <sup>c</sup> as compared to group 3,  $p \leq 0.05$ , <sup>d</sup> as compared to group 4 :  $p \leq 0.05$ , <sup>e</sup> as compared to group 5,  $p \leq 0.05$ . Values are the arithmetic mean  $\pm$  S.E.M.;  $n = 10$

Table 2. Effect of carbon tetrachloride on biochemical parameters in the serum of mice

Parameters	Experimental groups				
	Untreated control 1	Vehicle control 2	275.3 mg / kg body weight/ day (LD) 3	413 mg / kg body weight / day (MD) 4	826 mg / kg body weight / day (HD) 5
Alanine transaminase (mU/mg protein/30 min)	12.68 ± 0.28	12.13 ± 0.19	20.33 ± 0.32 <sup>abde</sup>	32.40 ± 0.55 <sup>abce</sup>	44.77 ± 0.35 <sup>abcd</sup>
Aspartate transaminase (mU/mg protein/60 min)	39.93 ± 0.53	± 0.53 ± 0.55	55.39 ± 0.64 <sup>abde</sup>	72.02 ± 0.76 <sup>abce</sup>	92.36 ± 0.35 <sup>abcd</sup>
Alkaline phosphatase (µmoles <i>p</i> -nitrophenol released/30 min/mg protein)	0.42 ± 0.01	0.42 ± 0.05	0.53 ± 0.03 <sup>abde</sup>	0.62 ± 0.10 <sup>abce</sup>	0.73 ± 0.03 <sup>abcd</sup>
Acid phosphatase (µmoles <i>p</i> -nitrophenol released/30 min/mg protein)	0.21 ± 0.03	0.20 ± 0.03	0.24 ± 0.01 <sup>abde</sup>	0.29 ± 0.03 <sup>abce</sup>	0.34 ± 0.06 <sup>abcd</sup>
Total protein (mg/100 mg)	4.72 ± 0.05	4.71 ± 0.07	3.92 ± 0.18 <sup>abde</sup>	3.18 ± 0.07 <sup>abce</sup>	2.28 ± 0.05 <sup>abcd</sup>

<sup>a</sup> as compared to group 1,  $p \leq 0.05$ , <sup>b</sup> as compared to group 2,  $p \leq 0.05$ , <sup>c</sup> as compared to group 3,  $p \leq 0.05$ , <sup>d</sup> as compared to group 4,  $p \leq 0.05$ , <sup>e</sup> as compared to group 5,  $p \leq 0.05$ . Values are the arithmetic mean  $\pm$  S.E.M.;  $n = 10$

### Statistical analysis

The results are expressed as the mean  $\pm$  S.E.M. The data were statistically analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey test. The level of significance was accepted with  $p = 0.05$ .

### RESULTS AND DISCUSSION

Herbal medicine is gaining popularity. Scarcity of scientific data on their safety and efficacy has

raised concerns regarding toxicity. The acute toxicity test performed has indicated that *Phyllanthus amarus* extract is non toxic up to a dosage of 2000 mg/kg body weight. The toxicity study conducted suggests that the extract is safe as evidenced by its high LD<sub>50</sub> value.

The phytochemical analysis of the *Phyllanthus amarus* extract confirmed the presence of tannins, saponins, flavonoids and alkaloids. The plant extract contained high levels of saponins (2.89 %  $\pm$  0.17) and tannins (5.19 %  $\pm$  0.32). The alkaloid (2.39 %  $\pm$

Table 3. Effect of *P. amarus* on carbon tetrachloride induced biochemical changes in the liver of mice

Parameters	Experimental groups						
	Untreated control 1	Vehicle control 2	<i>P. amarus</i> Control 3	HD CCl <sub>4</sub> 4	<i>P. amarus</i> 100 mg + HD CCl <sub>4</sub> 5	<i>P. amarus</i> 200 mg + HD CCl <sub>4</sub> 6	<i>P. amarus</i> 300 mg + HD CCl <sub>4</sub> 7
Alanine trasaminase (mU/mg protein/30 min)	1.54 ± 0.04	1.54 ± 0.07	1.55 ± 0.04	3.10 ± 0.02 <sup>abcefg</sup>	2.51 ± 0.30 <sup>abcdfg</sup>	2.05 ± 0.06 <sup>abcdeg</sup>	1.56 ± 0.21 <sup>def</sup>
Aspartate trasaminase (mU/mg protein/60 min)	1.94 ± 0.25	1.94 ± 0.31	1.91 ± 0.08	4.29 ± 0.27 <sup>abcefg</sup>	3.5 ± 0.48 <sup>abcdfg</sup>	2.64 ± 0.19 <sup>abcdeg</sup>	1.96 ± 0.15 <sup>def</sup>
Alkaline phosphatase (μ moles <i>p</i> -nitrophenol released/30 min/mg protein)	0.283 ± 0.01	0.282 ± 0.01	0.287 ± 0.05	0.413 ± 0.01 <sup>abcefg</sup>	0.355 ± 0.01 <sup>abcdfg</sup>	0.320 ± 0.02 <sup>abcdeg</sup>	0.286 ± 0.01 <sup>def</sup>
Acid phosphatase (μmoles <i>p</i> -nitrophenol released/30 min/mg protein)	1.26 ± 0.01	1.25 ± 0.01	1.25 ± 0.02	2.14 ± 0.03 <sup>abcefg</sup>	1.84 ± 0.02 <sup>abcdfg</sup>	1.53 ± 0.04 <sup>abcdeg</sup>	1.25 ± 0.04 <sup>def</sup>
Total protein (mg/100 mg)	22.24 ± 0.31	22.97 ± 0.31	23.05 ± 0.09	15.72 ± 0.27 <sup>abcefg</sup>	20.59 ± 0.06 <sup>abcdfg</sup>	21.74 ± 0.08 <sup>abcdeg</sup>	22.93 ± 0.07 <sup>def</sup>

<sup>a</sup> as compared to group 1,  $p \leq 0.05$ , <sup>b</sup> as compared to group 2,  $p \leq 0.05$ , <sup>c</sup> as compared to group 3,  $p \leq 0.05$ , <sup>d</sup> as compared to group 4,  $p \leq 0.05$ , <sup>e</sup> as compared to group 5,  $p \leq 0.05$ , <sup>f</sup> as compared to group 6,  $p \leq 0.05$ , <sup>g</sup> as compared to group 7,  $p \leq 0.05$ . Values are the arithmetic mean ± S.E.M.; n = 10

Table 4. Effect of *P. amarus* on carbon tetrachloride-induced biochemical changes in serum of mice

Parameters	Experimental groups						
	Untreated control 1	Vehicle control 2	<i>P. amarus</i> Control 3	HD CCl <sub>4</sub> 4	<i>P. amarus</i> 100 mg + HD CCl <sub>4</sub> 5	<i>P. amarus</i> 200 mg + HD CCl <sub>4</sub> 6	<i>P. amarus</i> 300 mg + HD CCl <sub>4</sub> 7
Alanine trasaminase (mU/mg protein/30 min)	12.16 ± 0.14	12.23 ± 0.14	12.17 ± 0.08	44.46 ± 0.45 <sup>abcefg</sup>	16.31 ± 0.38 <sup>abcdfg</sup>	14.29 ± 0.36 <sup>abcdeg</sup>	12.23 ± 0.08 <sup>def</sup>
Aspartate trasaminase (mU/mg protein/60 min)	39.9 ± 0.34	39.9 ± 0.78	39.64 ± 0.45	92.58 ± 0.23 <sup>abcefg</sup>	52.46 ± 0.78 <sup>abcdfg</sup>	46.42 ± 0.23 <sup>abcdeg</sup>	39.73 ± 0.11 <sup>def</sup>
Alkaline phosphatase (μ moles <i>p</i> -nitrophenol released/30 min/mg protein)	0.421 ± 0.06	0.424 ± 0.09	0.422 ± 0.02	0.706 ± 0.07 <sup>abcefg</sup>	0.491 ± 0.01 <sup>abcdfg</sup>	0.463 ± 0.06 <sup>abcdeg</sup>	0.421 ± 0.01 <sup>def</sup>
Acid phosphatase (μmoles <i>p</i> -nitrophenol released/30 min/mg protein)	0.209 ± 0.05	0.206 ± 0.03	0.209 ± 0.03	0.349 ± 0.08 <sup>abcefg</sup>	0.255 ± 0.04 <sup>abcdfg</sup>	0.234 ± 0.07 <sup>abcdeg</sup>	0.203 ± 0.04 <sup>def</sup>
Total protein (mg/100 mg)	4.72 ± 0.56	4.73 ± 0.78	4.73 ± 0.34	2.23 ± 0.10 <sup>abcefg</sup>	3.15 ± 0.55 <sup>abcdfg</sup>	3.82 ± 0.60 <sup>abcdeg</sup>	4.73 ± 0.13 <sup>def</sup>

<sup>a</sup> as compared to group 1,  $p \leq 0.05$ , <sup>b</sup> as compared to group 2,  $p \leq 0.05$ , <sup>c</sup> as compared to group 3,  $p \leq 0.05$ , <sup>d</sup> as compared to group 4,  $p \leq 0.05$ , <sup>e</sup> as compared to group 5,  $p \leq 0.05$ , <sup>f</sup> as compared to group 6,  $p \leq 0.05$ , <sup>g</sup> as compared to group 7,  $p \leq 0.05$ . Values are the arithmetic mean ± S.E.M.; n = 10

0.02) and flavonoid (1.53 % ± 0.04) contents were also determined.

The results shown in Table 1 and 2 revealed no significant alterations between untreated and vehicle control groups (Group 1, 2). Oral administration of CCl<sub>4</sub> caused a significant ( $p \leq 0.05$ ), dose-dependent increase in liver and serum ALT, AST, ALP and ACP enzyme activities. On the other hand, total pro-

tein content was decreased in all these three groups; the most significant decrease was observed in the HD group.

The results shown in Table 3 and 4 indicate that oral administration of aqueous extract of *Phyllanthus amarus* (300 mg/kg body weight) alone did not cause any significant effect in all parameters as compared to vehicle control. However, oral administration of

the *P. amarus* along with CCl<sub>4</sub> for 30 days significantly ( $p \leq 0.05$ ) mitigated the toxic effects of CCl<sub>4</sub> in liver and serum parameters in a dose-dependent manner. Various investigators (23-25) have reported significant rise in these enzyme activities by CCl<sub>4</sub>. The serum levels of ALT, AST and ALP have been reported to be sensitive indicators of liver injury (26). These are of major importance in assessing and monitoring liver cytolysis, thus they increased presence in the serum may give information on organ dysfunction (27). Gao et al. (28) have reported that ALT activity is an important indice to measure a degree of cell-membrane damage, while AST is an indicator of mitochondrial damage since it contains 80% of this enzyme. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum, located predominantly in the microvilli of the bile canaliculi. Thus, an increase in ALP activity reflects the pathological alteration in biliary flow (29). A damage to lysosomal integrity was evidenced by a sharp dose-dependent increase in the activities of ACP in the liver and serum. The significant increase seen in the acid phosphatase activity after toxin administration may be due to increased synthesis of lysosomal enzymes as a response to increased cellular degeneration and other pathological liver injury (30). This also suggested high tissue catabolism and cellular autophagy which are possible sequences leading to tissue damage (31).

It is well known that CCl<sub>4</sub> is activated in the liver, producing products which are metabolized by the mixed function oxidase. The toxicity of the reactive compounds and their metabolites may result from covalent interaction with the critical target molecules such as DNA, lipids, proteins, or carbohydrates, or from alteration of target molecules *via* secondary bond formation (lipid peroxidation, generation of reactive oxygen species, alteration of reduced or oxidized glutathione) (32).

Oral administration of CCl<sub>4</sub> along with *P. amarus* aqueous extract caused significant amelioration in CCl<sub>4</sub> induced toxicity in the liver of mice. The reduction in the levels of ALT and AST by the extract is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl<sub>4</sub> (33). Promythin et al. (34) have also reported that *P. amarus* extract decreases ALT and AST levels in rats administered with ethanol to induce hepatotoxicity. The treatment with *Phyllanthus amarus* extract for 30 days along with the high dose of the toxin significantly decreased ALP (35) and ACP activity in the liver and serum. This indicates the ability of *P. amarus* extract to stabilize biliary dysfunction and suggests a protective

mechanism against rupture of lysosomes thereby restoring the cell integrity.

Smuckler et al. (36) have shown that the administration of CCl<sub>4</sub> to rats results in a widespread dislocation of ribonucleoprotein particles from the membranes of the rough endoplasmic reticulum. This results in a depressed capacity of liver microsomes to incorporate aminoacids causing a generalized inhibition of protein synthesis. Oral administration of the *P. amarus* extract enhanced the protein synthesizing function of the liver and increased the total protein content.

Several active compounds have been identified in *P. amarus* extract. Lignins like phyllanthin and hypophyllanthin, flavonoids like quercetin and astragaln, ellagitannins like amarinic acid and hydrolyzable tannins like phyllanthisiin D (37) isolated from this plant were reported to possess antioxidative properties. The overall recovery process and stabilization of the disease status by *P. amarus* extract might be due to its antioxidative property (38).

It is concluded that the oral administration of *Phyllanthus amarus* extract along with CCl<sub>4</sub> significantly ameliorated the CCl<sub>4</sub>-induced changes in the liver tissue.

#### Acknowledgment

The Authors thankfully acknowledge Rameshwardasji Birla Smarak Kosh, Mumbai, India for their financial assistance.

#### REFERENCES

1. Sucher N.J., Carles M.C.: *Planta Med.* 74, 603 (2008).
2. Yu C., Wang F., Jin C., Wu X., Chan W.K., McKeehan W.L.: *Am. J. Pathol.* 161, 2003 (2002).
3. Liu J., Lin H., McIntosh H.: *J. Viral Hepat.* 8, 358 (2001).
4. Naaz F., Javed S., Abdin M.Z.: *J. Ethnopharmacol.* 113, 503 (2007).
5. Thyagarajan S.P., Jayaram S., Valliammai T., Madanagopalan N., Pal V.G., Jayaraman K.: *Lancet.* 336, 949 (1990).
6. Sane R.T., Kuber V.V., Mary S.C., Menon S.: *Curr. Sci.* 20, 1243 (1995).
7. Lee C.Y., Peng W.H., Cheng H.Y., Chen F.N., Lai M.T., Chiu T.H.: *Am. J. Chin. Med.* 34, 471 (2006).
8. WHO Protocol CG-06. APJF/IP, 1001A, Geneva; World Health Organisation 1983.

9. Sigma-Aldrich material safety data sheet for carbon tetrachloride.
10. OECD Guideline No. 425, Organisation for Economic Co-operation and Development, Paris, Cedex 16, France (2001).
11. Gujrati V., Patel N., Rao V.N., Nandakumar K., Gouda T.S., Md. Shalam., Shanta Kumar S.M.: *Ind. J. Pharmacol.* 39, 43 (2007).
12. Sofowara A.: *Medicinal Plants and Traditional Medicine in Africa*. p. 289, Spectrum Books Ltd., Ibadan, Nigeria 1993.
13. Trease G.E., Evans W.C.: *Pharmacognosy*. 13<sup>th</sup> edn., pp. 176-180, Bailliere Tindall, London 1989.
14. Harborne J. B.: *Phytochemical methods*. pp. 49 – 188 Chapman and Hall Ltd., London 1973.
15. Van-Burden T.P., Robinson W.C.: *J. Agric. Food Chem.* 1, 77 (1981).
16. Obdoni B. O., Ochuko P. O.: *Pure Appl. Sci.* 8b, 203 (2001).
17. Boham B. A., Kocipai-Abyazan R.: *Pacific Sci.* 48, 458 (1974).
18. Reitman S., Frankel S.: *Am. J. Clin. Pathol.* 28, 56 (1957).
19. Bessey O.A., Lowry O.H., Brock M.J.: *J. Biol. Chem.* 164, 321 (1946).
20. Sigma Technical Bulletin No. 104, Sigma Chemical Co., MO. USA.
21. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J.: *J. Biol. Chem.* 193, 265 (1951).
22. Hilaly J., Israili Z.H., Lyoussi B.: *J. Ethnopharmacol.* 91, 43 (2004).
23. Sadeghi H., Ghaitasi E., Mazrooghi N., Sabzali S.: *J. Shahrekord Univ. Med. Sci.* 9, 38 (2007).
24. Samudram P., Rajeshwari H., Vasuki R., Geetha A., Sathiy M.P.: *African J. Biochem. Res.* 2, 61 (2008).
25. Hayashi S., Itoh A., Isoda K., Kondoh M., Kawase M., Yagi K.: *Eur. J. Pharmacol.* 580, 380 (2008).
26. Molander D.W., Wroblewski F., La Due J.S.: *Clin. Res. Proc.* 3, 20 (1955).
27. Wells R.M., McIntyre R.H., Morgan A.K., Davie P.S.: *Comp. Biochem. Physiol.* 64A, 565 (1986).
28. Gao J., Tang X., Dou H., Fan Y., Zhao X., Xu Q.: *J. Pharm. Pharmacol.* 56, 1449 (2004).
29. Ploa G.L., Hewitt W.R.: *Detection and evaluation of chemically induced liver injury*, in: *Principles and Methods of Toxicology*, 2nd ed., Wallace Hayes, A. (Ed.), pp. 399–628, Raven Press, New York 1989.
30. Verma R.J., Vasu A., Saiyed A.A.: *J. Environ. Sci.* 16, 447 (2004).
31. Abraham P., Welfred G.: *Ind. J. Pharmacol.* 32, 250 (2000).
32. Weber L.W.D., Boll M., Stampfl A.: *Crit. Rev. Toxicol.* 33, 105 (2003).
33. Jain A, Soni M, Deb L, Jain A, Rout S.P., Gupta V.B., Krishna K.L.: *J. Ethnopharmacol.* 115, 61 (2008).
34. Pramyothin P., Ngamtin C., Pongshompoo S., Chaichantipyuth C.: *J. Ethnopharmacol.* 114, 169 (2007).
35. Jeena K.J., Joy K.L., Kuttan R.: *Cancer Lett.* 136, 11 (1999).
36. Smuckler E.A., Iseri O.A., Benditt E.P.: *J. Exp. Med.* 116, 55 (1962).
37. Rajeshkumar N.V., Kuttan R.: *J. Ethnopharmacol.* 73, 215 (2000).
38. Harish R., Shivanandappa T.: *Food Chem.* 95, 180 (2006).

*Received: 06. 12. 2008*

Erratum to “Effect of low-dose tacrolimus coadministered with raloxifene on the skeletal system in male rats”,  
Acta Poloniae Pharmaceutica – Drug Research, 66(2), 207-212, 2009.

Table	Column	Line	Is	Should be
1, 2, 3	4	1	III – Raloxifene (5 mg/kg <i>po</i> )	III – Raloxifene HCl (5 mg/kg <i>po</i> ) *
2	4	5	213.42 ± 2.65	213.42 ± 2.65 <sup>cc</sup>
2	5	5	<sup>cc</sup> 214.80 ± 4.60 <sup>cc, t</sup>	214.80 ± 4.60 <sup>cc, t</sup>
2	6	5	p < b 0.01	p < 0.01
2	5	11	90.73 ± 1.81 <sup>ccc</sup>	90.73 ± 1.81 <sup>ccc, ttt</sup>
2	6	11	<sup>ttt</sup> p < 0.001	p < 0.001
3	1	6	At breaking lod	At breaking load
3	6	7	N	NS