

Effect of *Phyllanthus niruri* Linn. treatment on liver, kidney and testes in CCl₄ induced hepatotoxic rats

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Phyllanthus niruri extract is extensively used in treating liver ailments. Effects of aqueous extract of *P. niruri* on liver, kidney and testes of CCl₄ induced hepatotoxic rats were studied. High levels of malondialdehyde (MDA) were observed in the CCl₄ test group with significant reduction of MDA levels in all groups on *P. niruri* extract administration. Highest levels of glutathione (GSH) were found in *P. niruri* group. Activities of alanine transaminase, aspartate transaminase and alkaline phosphatase enzymes were significantly reduced in the curative group (*P. niruri* treatment after CCl₄ injection). Histopathology of liver showed lesser degree of inflammation in all *P. niruri* treated groups while the renal and seminiferous tubules showed eosinophilic protein casts with signs of tubular damage and degeneration. Testes also showed decreased amount of mature spermatozoa. The results suggest that *P. niruri* has anti-oxidant and hepato-protective activity with associated deleterious effects on kidney and testes.

Keywords: CCl₄, Hepatotoxicity, Oxidative stress, *Phyllanthus niruri*

Hepatitis of varied etiology is commonly encountered in clinical practice. Drugs available for the treatment are mainly herbal combinations. Ancient systems of medicine like Ayurveda, Unani etc. have used plants of the genus *Phyllanthus* in treating various liver disorders¹. These plants are also known to have diverse biological activities like hypoglycemic, hypotensive, diuretic, antioxidative and anti-inflammatory². Traditional uses of *Phyllanthus* have been confirmed by a number of biochemical, pharmacological and clinical studies³⁻⁷. These activities have been attributed to the flavonoids, terpenes, benzenoids, lignans, lipids, vitamin C, steroids etc, that have been purified from the plant⁸. The species *Phyllanthus niruri* (Linn.) also known as *P. amarus* (Hindi-*Bhumi amlaki*, English-*Stone Breaker*, *Shatter stone*, Kannada-*Nela nelli*) is being used for the treatment of jaundice extensively in folklore medicine. Experiments in animal models and humans have shown the plant extract to possess inhibitory activity against Hepatitis B virus and related Hepadna viruses^{3,5}.

Most of the studies have focused on the hepatoprotective effects of *P. niruri* and there is a

lacuna in the understanding of its side effects on other organs. Liver is not the only target organ of CCl₄ toxicity but includes other organs like kidneys, heart, lung, testes, brain etc⁹⁻¹¹. The present study has been designed to investigate the effects of *P. niruri* on CCl₄ induced hepatic, renal and testicular toxicity.

Materials and Methods

Animals—Male albino rats of Wistar strain weighing 150-200 g used for the study were maintained in hygienic environment and fed with commercially available pellets of rat chow and water *ad libitum*.

Study design—The rats were divided into following 5 groups of 6 animals each.

Group I (Control group): was injected with distilled water, ip for 7 days.

Group II (*P. niruri* group): whole plant extract was given @ 100 mg/ kg body wt, po for 7 days

Group III (CCl₄ test group): 0.5 ml CCl₄/kg body wt, ip for 7 days

Group IV (Prophylactic group): 0.5ml CCl₄/kg body wt, ip for 7 days with simultaneous administration of *P. niruri* extract @ 100 mg/ kg body wt, po for 7 days

Group V (Curative group): 0.5 ml CCl₄/kg body wt, was given ip for 7 days followed by administration

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of *P. niruri* extract @ 100 mg/kg body wt, po for next 7 days.

CCl₄ was obtained from Qualigens Fine Chemicals, a division of Glaxo India Ltd.

Chemicals used for all other estimations were of reagent grade and purchased locally.

Preparation of plant extract—Locally available young *P. niruri* plants with roots were collected from a single area. The plant was identified and authenticated by reputed botanists of St. Aloysius College, and the Department of Botany of Mangalore University. The plants were washed with water, dried and weighed. A decoction was prepared by boiling in water (1:4, w/v). Boiling was continued till the water level reduced to half. Pure extract was obtained by filtering through fine muslin. Concentration of the extract was estimated by taking dry weight of 1ml of the extract. The weight was found to be 30 mg/ml. The extract was refrigerated at 4°C until use. Fresh extract was prepared every week and concentration was standardized.

On day 8 of study period the animals were sacrificed with light ether anesthesia. Blood was collected by cardiac puncture. Serum was used for assay of hepatic enzymes. Liver, kidney and testes were dissected out and processed for histopathological studies. A small portion of the liver (10% w/v) was used to prepare a homogenate with ice cold saline.

Assessment of oxidative stress—Suitably diluted liver homogenate was used for the estimation of malondialdehyde (MDA)¹² and reduced glutathione (GSH)¹³.

Serum enzymes—Estimation of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total bilirubin (TB) levels in serum was carried out on Hitachi 917 Auto analyzer using commercially available kits at, KMC Hospital Ambedkar circle.

Histopathology—Liver, kidney and testes of all the animals were fixed in 10% formalin and processed by the usual method for paraffin embedding. Sections of 5 µm thickness were taken, stained with H&E and were evaluated for pathological changes using binocular light microscope.

Statistical analysis—The data were analysed using Student's *t* test and ANOVA.

Results

The weight changes seen in body and body organs are given in Table 1. The test group (Group III) and the prophylactic group (Group IV) showed a

significant decrease in body weight as compared to the control group (Group I). A significant increase in body weight was seen in curative group (Group V). Animals given *P. niruri* extract alone (Group II) did not differ in weight from the control group. However this group and the curative group showed a highly significant increase in body weight as compared to the test group. Gross weight of the organs namely liver, kidney and testes was significantly lesser in all CCl₄ treated groups as compared to the control. The pattern showed a marked decrease in the test group with gradual betterment from prophylactic to curative groups.

Table 2 gives the MDA and GSH levels in liver homogenates. MDA levels were highest in the test group and least in the curative group. Comparable levels were observed in the prophylactic and control group. In general, a highly significant decrease in

Table 1—Comparison of gross wt. and organ wt. of various groups

[Values are mean ± SD from 6 rats in each group]

Weight (g)	Group I (Control)	Group II (<i>P. niruri</i> only)	Group III (CCl ₄ test)	Group IV (Prophylactic)	Group V (Curative)
Gain in Body wt.	18.33 ± 8.8	19.0 ± 1.1 ^f	-13.16 ± 8.6 ^c	-10.0 ± 1.26 ^c	4.0 ± 6.7 ^{a,e}
Liver	9.06 ± 0.73	8.68 ± 0.67 ^f	6.54 ± 0.84 ^c	7.58 ± 0.64 ^{c,d}	7.2 ± 0.5 ^b
Kidney	1.04 ± 0.13	0.82 ± 0.02 ^{b,d}	0.67 ± 0.13 ^c	0.77 ± 0.07 ^{c,e}	0.79 ± 0.06 ^b
Testes	1.33 ± 0.08	1.27 ± 0.09	1.05 ± 0.09 ^a	1.09 ± 0.15 ^{a,e}	1.26 ± 0.08

P.: ^a ≤ 0.05; ^b ≤ 0.01; ^c ≤ 0.001 (vs control) ^d ≤ 0.05; ^e ≤ 0.01; ^f ≤ 0.001 (vs test)

Table 2—Comparison of oxidative stress between various groups

[Values are mean ± SD from 6 rats in each group]

Parameters	Group I (Control)	Group II (<i>P. niruri</i> only)	Group III (CCl ₄ test)	Group IV (Prophylactic)	Group V (Curative)
MDA (µ mol/gm)	18.04 ± 1.81	13.34 ± 2.88 ^{bf}	65.33 ± 12.99 ^c	18.43 ± 0.78 ^f	12.42 ± 1.9 ^{c,f}
GSH (mg/g of liver)	1.19 ± 0.36	3.22 ± 0.30 ^{c,f}	2.0 ± 0.20 ^c	0.91 ± 0.48 ^f	2.08 ± 0.23 ^c

P.: ^b ≤ 0.01; ^c ≤ 0.001 (vs control) ^f ≤ 0.001 (vs test)

MDA levels was seen in all *P. niruri* treated groups (II, IV, V) in comparison to the test group.

Highest values of GSH were seen in the *P. niruri* group. Likewise the test group and the curative group showed a significant rise in GSH levels as compared to controls. The prophylactic group did not significantly differ from controls but, showed a remarkable decrease in GSH levels in comparison to the test group.

The extent of liver damage was assessed by estimating serum levels of ALT, AST, ALP and TB (Table 3). The test group showed a significant increase in ALT and ALP levels. The prophylactic group showed a significant increase in all enzyme levels as compared to other groups while, the curative group maintained for 14 days showed an improvement in the liver functioning as reflected by decreased enzyme levels. A marginal increase in the liver enzymes was seen in the *P. niruri* treated group (Group II) with associated decrease in TB levels. No significant variation in TB levels was found in any of the other groups.

Histopathology—In the control group (Group I), liver (Fig. 1a), kidney (Fig. 2a) and testes (Fig. 3a) showed normal histological structure. Marked inflammatory changes associated with fatty changes are seen in liver sections of the test group (Group III) (Fig. 1c). The liver section of *P. niruri* treated group (Group II) showed periportal lymphocytic and neutrophilic infiltration without any lesions in the hepatocytes (Fig. 1b). Lesser degree of inflammation

was seen in the prophylactic (Group IV) (Fig. 1d), and least in the curative group (Group V) (Fig. 1e).

Microscopic examination of kidneys also showed marked inflammatory changes in test group (Fig. 2c). Lesser degree of inflammatory infiltrate was found in the *P. niruri* treated (Group II) (Fig. 2b) and curative group (Group V) (Fig. 2e). The prophylactic group (Group IV) did not show obvious inflammatory signs (Fig. 2d). A striking finding was presence of dense eosinophilic casts in kidney tubules of all *P. niruri* treated groups (II, IV, V).

Cross section of testes showed apparent tubular disorganization and decreased amount of mature spermatozoa in all groups (II, III, IV, V) (Fig. 3b, 3c, 3d, 3e) as compared to controls. In addition to this dense eosinophilic casts similar to that in kidneys were seen in all the *P. niruri* treated groups (II, IV, V) (Fig. 3b, 3d, 3e).

Discussion

CCl₄ toxicity is largely due to free radical mediated damage. As a measure of oxidative damage to the membranes and antioxidant resource MDA and GSH were estimated respectively in various groups. No correlation was established between these parameters in the present study. In contrast, Tirkey *et al*¹⁴ and Kamalakannan *et al*¹⁵ reported decrease in GSH levels with concomitant increase in MDA levels on CCl₄ administration in rats. This difference may be, due to higher dosage¹⁴ administered or longer duration¹⁵ of the study period. Higher GSH values seen in the present test group can be attributed to free radical induced endogenous synthesis.

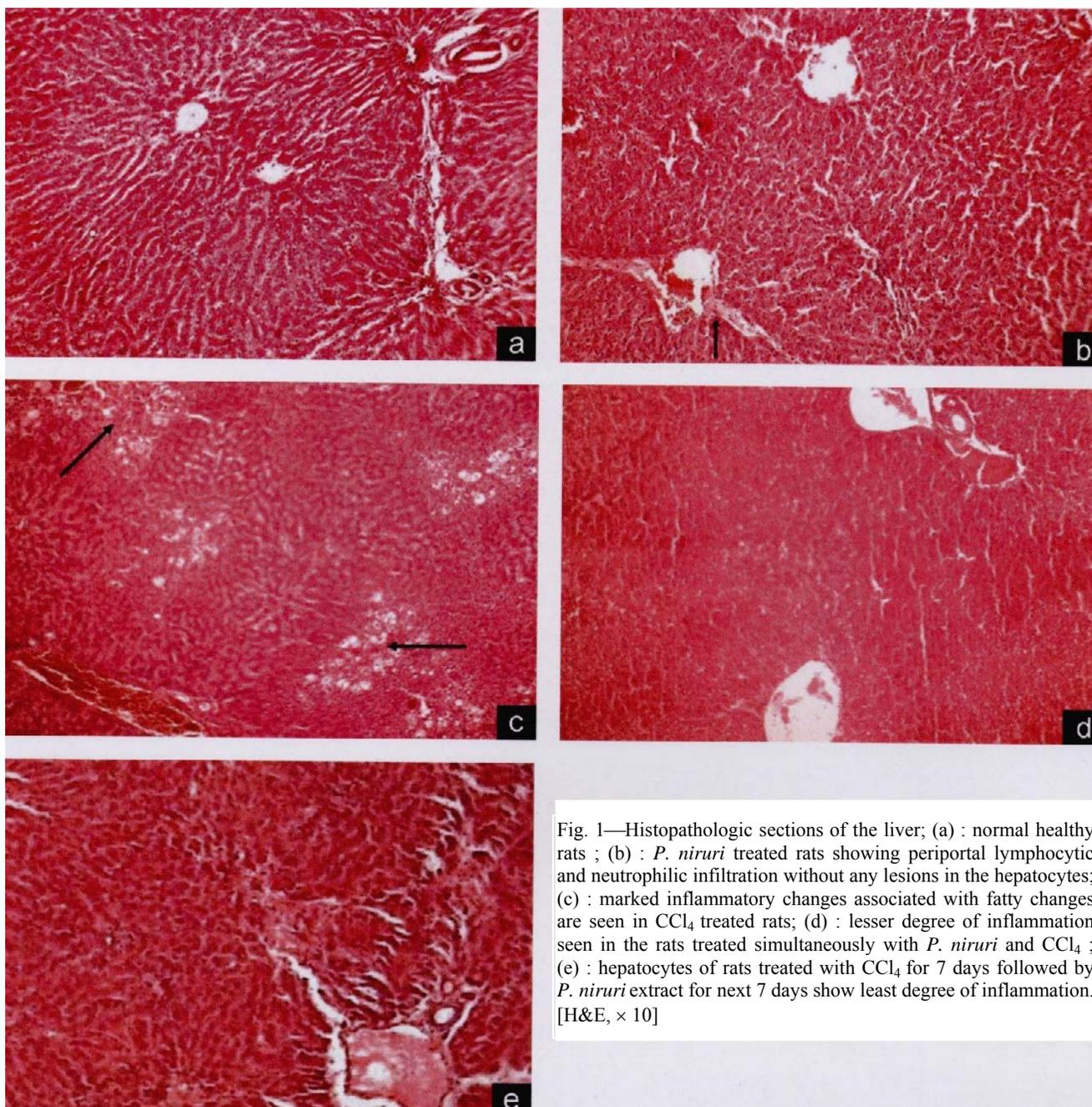
From the results it is evident that, *P. niruri* treatment inhibited lipid peroxidation and improved the GSH status (Table 1). The MDA and GSH levels of the prophylactic group are comparable to the control group indicating that *Phyllanthus* extract has significant free radical scavenging activity resulting in normalization of MDA levels. Also, simultaneous administration of the plant extract with CCl₄ may mask its harmful effects generating lesser free radicals and thus elicit a poorer response in terms of GSH synthesis.

The findings of the curative group (Group V) suggest that CCl₄ administration during the first 7 days induced endogenous synthesis of GSH which was sustained through the next 7 days. A decrease in MDA levels may be due to the dual effect of the antioxidants of plant extract as well as the raised GSH status. It could also be partly attributed to hepatic regeneration following withdrawal of CCl₄.

Table 3—Assessment of liver damage in various groups
[Values are mean ± SD from 6 rats in each group]

Parameters	Group I (Control)	Group II (<i>P. niruri</i> only)	Group III (CCl ₄ test)	Group IV (Prophylactic)	Group V (Curative)
ALT (IU / L)	59.5 ± 6.53	73.5 ± 12.21 ^{a,d}	185.16 ± 84.12 ^b	206.0 ± 40.7 ^c	70.0 ± 6.31 ^{a,d}
AST (IU / L)	254.67 ± 68.32	281.5 ± 79.01	314.8 ± 68.11	420.5 ± 57.08 ^{b,d}	246.8 ± 67.6
ALP (IU / L)	409 ± 61.94	503 ± 83.9 ^a	663 ± 231.6 ^a	811.16 ± 101.5 ^c	561 ± 92.56 ^b
TB (mg /dl)	0.37 ± 0.08	0.25 ± 0.05 ^a	0.4 ± 0.09	0.3 ± 0.1	0.33 ± 0.08

P.: ^a ≤ 0.05; ^b ≤ 0.01; ^c ≤ 0.001 (vs control) ^d ≤ 0.05 (vs test)



Significantly low MDA levels and high GSH levels seen in *P. niruri* group (Group II) further confirm that the extract is rich in antioxidants^{2,8, 16-18}.

Of the liver enzymes estimated, a marked increase in ALT and ALP levels was observed in the CCl₄ test group which is in agreement with previous studies¹⁵. Although the prophylactic group showed an improvement in MDA levels from the test group, no significant difference between their enzyme levels was seen since the enzyme levels require longer duration to

return to baseline levels. Values of the curative group were comparatively lesser than the test and prophylactic group but still significantly higher than the control group. This further substantiates the above argument that, enzyme levels take longer to normalize. A marginal increase in liver enzymes associated with marked decrease of TB was seen in the *P. niruri* treated group. This suggests that *P. niruri* extract when given alone may increase bilirubin excretion explaining its usage in folklore medicine to treat “jaundice”.

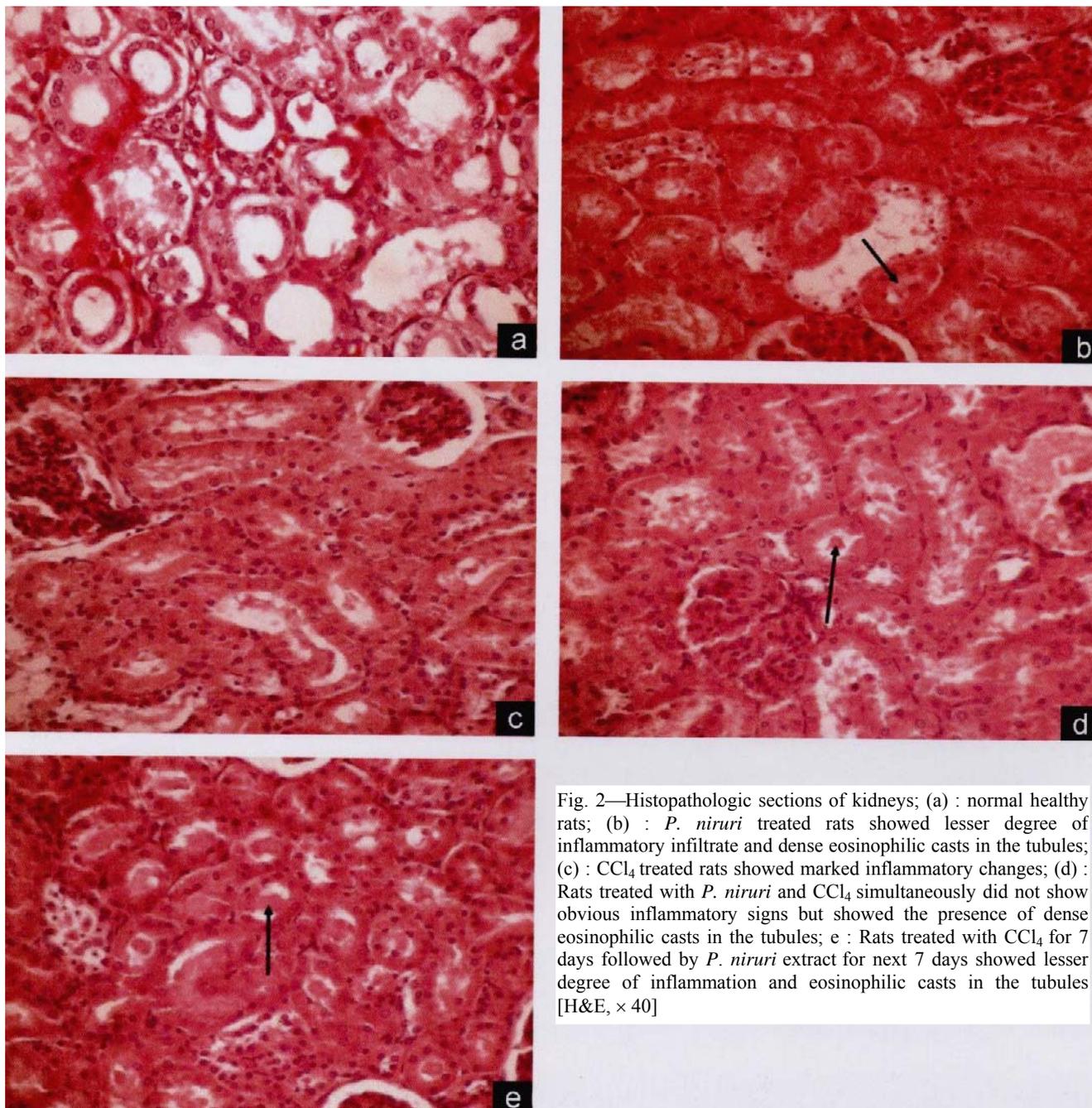


Fig. 2—Histopathologic sections of kidneys; (a) : normal healthy rats; (b) : *P. niruri* treated rats showed lesser degree of inflammatory infiltrate and dense eosinophilic casts in the tubules; (c) : CCl_4 treated rats showed marked inflammatory changes; (d) : Rats treated with *P. niruri* and CCl_4 simultaneously did not show obvious inflammatory signs but showed the presence of dense eosinophilic casts in the tubules; e : Rats treated with CCl_4 for 7 days followed by *P. niruri* extract for next 7 days showed lesser degree of inflammation and eosinophilic casts in the tubules [H&E, $\times 40$]

The histopathological findings in liver correlate with the biochemical estimations (Table 3) done in various groups. The changes seen in *P. niruri* group are supportive of the studies by Adedapo *et al*¹⁹ who have described this inflammatory response in liver as tissue reaction to the presence of plant extract. This also explains the mild elevation in enzymes shown by this group in present study.

The kidney sections show that the plant extract has anti-inflammatory properties more manifested in the

prophylactic group (Fig. 2d). The findings of the present study were similar to the findings of Adedapo *et al*^{19, 20} and Kapur *et al*²¹ who have also described identical damage and disorganization of seminiferous tubules. The presence of dense eosinophilic protein casts in the tubules of kidney and testes have been attributed in their studies to cardiac glycosides present in the plant extract^{19,20}.

The results of the present study confirm the hepatoprotective and antioxidant effects of *P. niruri*

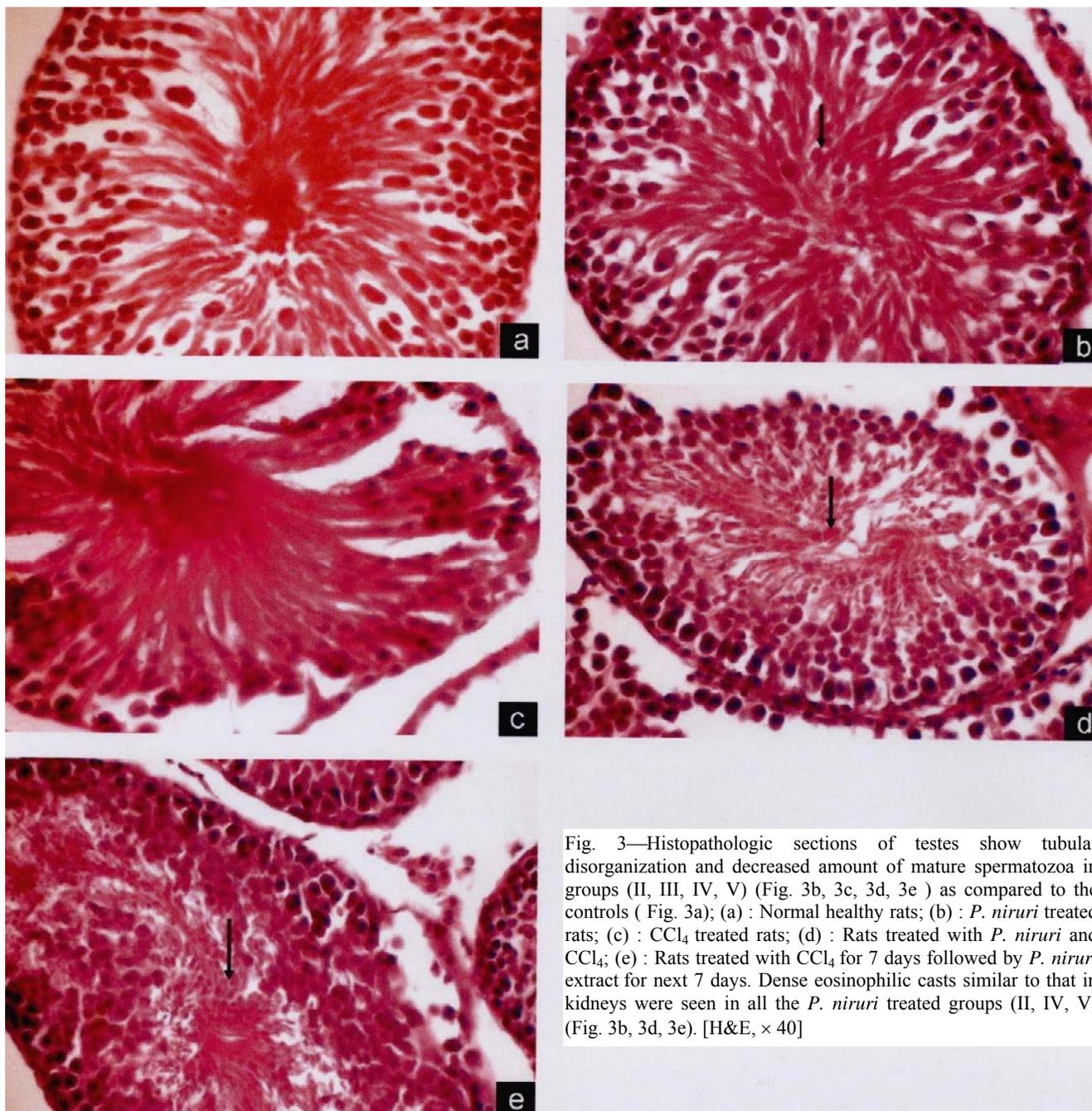


Fig. 3—Histopathologic sections of testes show tubular disorganization and decreased amount of mature spermatozoa in groups (II, III, IV, V) (Fig. 3b, 3c, 3d, 3e) as compared to the controls (Fig. 3a); (a) : Normal healthy rats; (b) : *P. niruri* treated rats; (c) : CCl_4 treated rats; (d) : Rats treated with *P. niruri* and CCl_4 ; (e) : Rats treated with CCl_4 for 7 days followed by *P. niruri* extract for next 7 days. Dense eosinophilic casts similar to that in kidneys were seen in all the *P. niruri* treated groups (II, IV, V) (Fig. 3b, 3d, 3e). [H&E, $\times 40$]

extract as seen by inhibition of lipid peroxidation and improved Glutathione status. Enhanced hepatic integrity is reflected by betterment in the enzyme levels. Among all the organ sections studied, there was gradual betterment from prophylactic to curative group. Despite the hepatoprotective effects, the plant extract shows potentially toxic effects on the kidneys and testes.

The presence of eosinophilic casts in kidneys and testes seen on *P. niruri* treatment demands further

analysis of the same regarding its composition and effects on chronic exposure. Until such a time cautious use of this plant for medicinal purposes is recommended. Alternatively the plant may be exploited for use as an 'antifertility drug'.

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