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PROTECTIVE EFFECT OF *CARICA PAPAYA* L. SEED EXTRACT IN GENTAMICIN INDUCED HEPATOTOXICITY AND NEPHROTOXICITY IN RATS

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ABSTRACT

A study was conducted to evaluate the protective effect of aqueous seed extract of *Carica papaya* L. on gentamicin induced hepatotoxicity and nephrotoxicity in Wistar rats. A control group (Group I, n=12) was compared with rats administrated with 40 mg/kg gentamicin, once daily for 14 days (Groups II, III and IV, n=12 each). The effect of aqueous extract of *Carica papaya* L. at a dose level of 200 mg/kg (Group III) and taurine @ 1000 mg/kg body weight (Group IV) was compared with gentamicin treated group (Group II). The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine and uric acid values were significantly increased in rats exposed to gentamicin (Group II). Moreover, administration of gentamicin resulted in damage to liver and kidney structures. Administration of aqueous extract of *Carica papaya* L. before gentamicin exposure prevented severe alterations of biochemical parameters and disruptions of liver and kidney structures. In conclusion, this study obviously demonstrated that pretreatment with aqueous extract of *Carica papaya* L. significantly attenuated the physiological and histopathological alterations induced by gentamicin. Also, the present study identifies new areas of research for development of better therapeutic agents for liver, kidney, and other organs dysfunctions and diseases.

KEYWORDS: ***Carica papaya*** **L, Gentamicin, Hepatotoxicity, Nephrotoxicity, Wistar rats.**

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INTRODUCTION

Aminoglycoside antibiotics have long been used in antibacterial therapy. Gentamicin is an aminoglycoside antibiotic derived from *Micromonospora purpurea*. It is effective against most of the life threatening Gram negative bacterial infections (Kaloyanides *et al*., 1991). The incidence of nephrotoxicity from aminoglycosides has increased from 2 to 3 % in 1969 to 20 % in the past decade (Leehey, 1993). Despite nephrotoxicity and ototoxicity, the aminoglycosides are being continuously used in clinical practice because of their bactericidal efficacy, synergism with β lactam agents, low cost, limited bacterial resistance and a post-antibiotic effect.

Carica papaya L. is an evergreen, tree-like herb, 2-10 m tall; usually un- branched, although sometimes branched due to injury, containing white latex in all parts. It is a herbaceous plant, belonging to the family "Caricaceae" originated in Central America (Beckstrom-Sternberg *et al.,* 1994). Different parts of the plant are employed for the treatment of different human and veterinary diseases around the world. *Carica papaya* has the great potential against a number of health problems viz. tissue burns, bacterial, fungal, helminthic and protozoan infections. It has antioxidant, immunomodulatory, insecticidal and molluscicidal activity. Recently it has shown some hypoglycemic as well as hypolipidemic effects in its seed preparations (Singh *et al.* 2010). Therefore an attempt is made to study the effect of seed extract of *Carica papaya* L. in gentamicin induced hepatotoxicity and nephrotoxicity in Wistar rats

MATERIALS AND METHOD

Collection of Plant Material

Carica papaya L. seeds (Plate 1) from unripe mature papaya fruits (Plate 2) were procured from local areas of Shirwal village. Seeds were dried under shade and then pulverized into fine powder by using mixer grinder.

Preparation of extract of Carica papaya L. seeds

An aqueous extract of seeds of *Carica papaya* L. (CPE) was used for study. The 20 grams of seed powder was added into 200 ml of distilled water and boiled for 20-30 minutes. After boiling, the contents of the flask were cooled and filtered through muslin cloth. The filtrate so obtained was subsequently filtered through Whatman no.1 filter paper. Final filtrate thus obtained was subsequently transferred to sterilized evaporating bowels and placed under fan for evaporation of the solvent. The residue left in the bowels was collected and stored in refrigerator for subsequent studies.

Experimental Design

Forty eight Wistar rats of either sex were divided randomly into four equal groups (n= 12) with different treatments (Table 1). The rats were housed in clean polypropylene cages, under controlled environmental conditions (25±2^{0}C) and 12 hour dark and light cycles with sterilized dried clean, autoclaved rice husk was used as bedding material, which was changed on alternate days. The rats were maintained on standard balanced diet with clean de ionized drinking water *ad libitum* throughout the experimental period. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and were in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Table 1
Experimental design with group wise treatment protocol

Group	No. of rats	Treatment
I	12	Control (Negative control)
II	12	Gentamicin sulphate @ 40 mg/kg b.wt. I/P (Positive control)
III	12	Gentamicin sulphate @ 40mg/kg b.wt. I/P + aqueous extract of seeds of *Carica papaya* Linn.*(CPSE)* @ 200 mg/kg b.wt. orally
IV	12	Gentamicin sulphate @ 40mg/kg b.wt. I/P + Taurine @ 1000 mg/kg b.wt. orally

Table 2
Results of Phytochemical Analysis of CPE

Sr No	Phytoconstituents	Aqueous seed extract of *Carica papaya* L.
1	Alkaloids	++
2	Saponins	+
3	Tannins	-
4	Glycosides	+
5	Flavonoids	++
6	Resins	-
7	Sterols	-

All above treatments were given for 14 days, after which all the rats were sacrificed and blood samples for biochemical investigations were collected. Liver and kidneys were collected for relative organ weight and for histopathological evaluation.

Histopathological Examination
The tissue samples viz. kidneys and liver from all animals of each group were collected in 10% Neutral Buffered Formalin (NBF) and processed by routine paraffin embedding method. The tissue sections of 3 to 5 microns size were stained by routine Haematoxylin and Eosin staining as suggested by Chauhan (1995).

STATISTICAL ANALYSIS
The data collected for various parameters were statistically analyzed by using analysis of variance (ANOVA) using WASP 2 computer software. All the values in the text are expressed as mean ±SE.

RESULTS AND DISCUSSION

The various experimental investigations recorded were Phytochemical analysis of aqueous seed extract, Group wise mean body weight, relative organ weights, biochemical investigations for liver and kidney function assessment and detailed histopathological evaluation of liver and kidney.

Body weight and organ weight changes

Significant reduction in body weight was observed in gentamicin sulphate treated rats (group II) as compared with control (group I). Rats treated with CPSE (group III) showed highest per cent increase in body weight when compared with other groups (Table 3). The results showed that rats treated with CPSE and taurine (group IV) did not caused any alterations in liver and kidneys weight which was observed by rats administered with only gentamicin (Table 3).

Table 3

Group wise mean body weight and relative liver and kidney weights

Group	Body weight (g)	Relative liver wt. (%)	Relative kidney wt. (%)
Group I	280.42 ± 21.61^{a}	3.55 ± 0.059^{b}	0.35 ± 0.011^{c}
Group II	230.42 ± 18.71^{b}	4.28 ± 0.077^{a}	0.52 ± 0.013^{a}
Group III	282.17 ± 18.77^{a}	3.59 ± 0.071^{b}	0.37 ± 0.012^{bc}
Group IV	273.17 ± 20.03^{a}	3.65 ± 0.040^{b}	0.39 ± 0.015^{b}

Means bearing different superscripts within the same column differ significantly (P<0.05)

Biochemical parameters

AST, ALT, serum creatinine, blood urea nitrogen and serum uric acid were found to be significantly increased in rats treated with only gentamicin; whereas treatment with the CPE and taurine was found to protect the rats from such effects of gentamicin (Table 4). Total protein level of gentamicin treated rats showed significant reduction as compared to healthy control rats. The mean total protein level was significantly increased in rats treated with preventive regimen of *C. papaya* L. seed extract as compared to taurine treated rats.

Table 4

Evaluation of biochemical parameters using CPE

Parameter	Group I	Group II	Group III	Group IV
1. AST(IU/L)	69.01 ± 1.918^{c}	118.89 ± 3.504^{a}	70.58 ± 2.750^{c}	78.68 ± 2.900^{b}
2. ALT(IU/L)	28.99 ± 0.945^{c}	38.96 ± 1.588^{a}	31.23 ± 0.589^{c}	34.62 ± 0.939^{b}
3. BUN (mg/dl)	17.49 ± 0.475^{c}	24.32 ± 0.531^{a}	18.17 ± 0.501^{bc}	19.38 ± 0.605^{b}
4. Serum creatinine (mg/dl)	0.71 ± 0.040^{b}	1.21 ± 0.064^{a}	0.68 ± 0.045^{b}	0.80 ± 0.027^{b}
5. Serum uric acid(mg/dl)	2.83 ± 0.094^{b}	4.21 ± 0.183^{a}	3.15 ± 0.186^{b}	3.21 ± 0.168^{b}
6. Total Protein (g/dl)	7.15 ± 0.276^{a}	4.95 ± 0.199^{b}	6.93 ± 0.182^{a}	6.79 ± 0.141^{a}

Means bearing different superscripts within the same row differ significantly (P<0.05)

Histopathological examination

Light microscopic examination of the liver and kidneys in control rats showed the normal histological structure. Histopathological findings of liver and kidneys in rats of different experimental groups are depicted in Fig 1 to 8.

Liver

Histopathological effects on liver of gentamicin treated rats are presented in Fig 1 and 2. Rats treated with gentamicin (group II) showed severe histopathological alterations. The microscopic examination of liver showed derangement of hepatic cords with granular changes in cytoplasm. Multifocal swelling of hepatocytes was observed with congestion of central and portal blood vessels with sinusoidal congestion. Focal degenerative and necrotic changes along with mononuclear cell infiltration were also evident. Similar findings were observed by Noorani *et al.,* (2011) who noticed the administration of gentamicin for 7 days resulted in damage of liver structure with disarrangement of hepatic strands.

Fig. 1

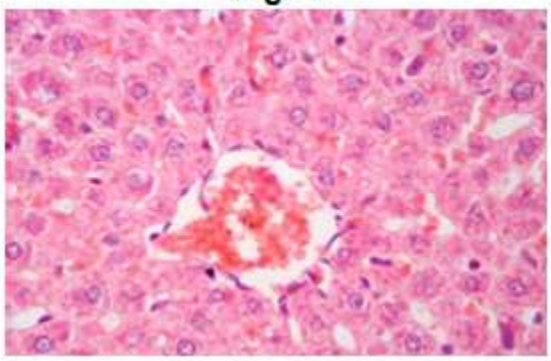
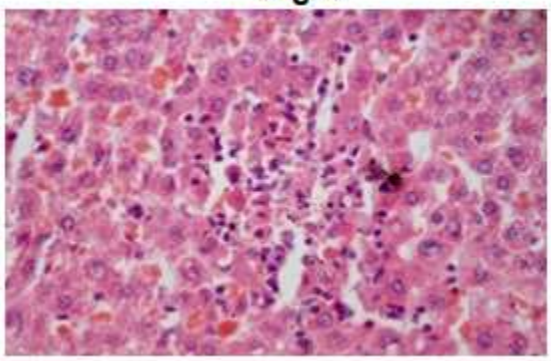


Fig. 2



The histopathological changes in Groups III and IV were only of mild nature that consisted of focal congestion of central vein with sinusoidal congestion with focal cellular swelling of hepatocytes. (Fig.3 & 4). These restorative changes of hepatocytes might be due to *Carica papaya* L. and taurine treatment given during the experimental period.

Fig. 3

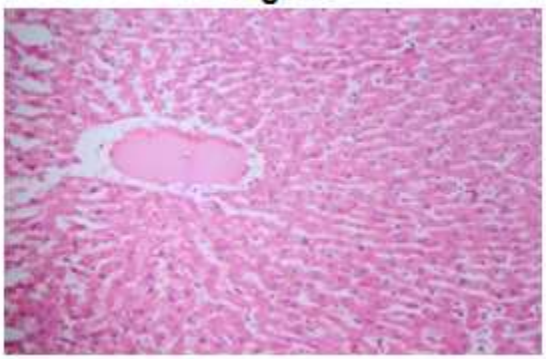
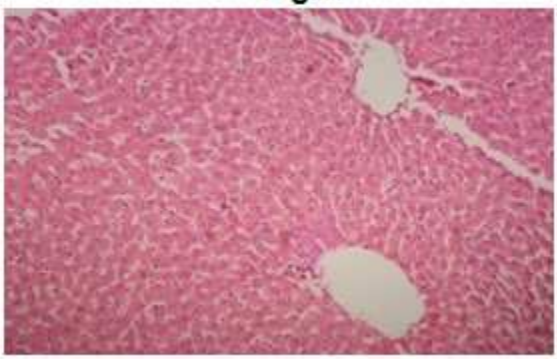


Fig. 4



Kidneys

Histological evaluation of kidneys treated with gentamicin (Group II) showed moderate to severe nephropathic changes with distinct cellular alterations (Fig.5 & 6). Marked degenerative and necrobiotic changes with inflammatory reaction were observed in kidneys of rats of Group II. Tubular changes like diffuse tubular swelling and loss of tubular epithelium was prominent at many foci in both, proximal and distal tubules. Focal areas showed degenerative changes in glomeruli

with hyper-cellularity and also atrophy of glomeruli. Few areas showed interstitial hemorrhages and infiltration of mononuclear cells. Similar changes were also noticed by Mouedden *et al.,* (2000), Bibu and Joy (2010) and Dehghani *et al.,* (2011).

Fig. 5

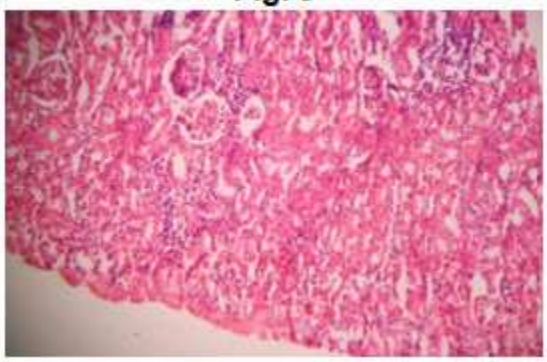
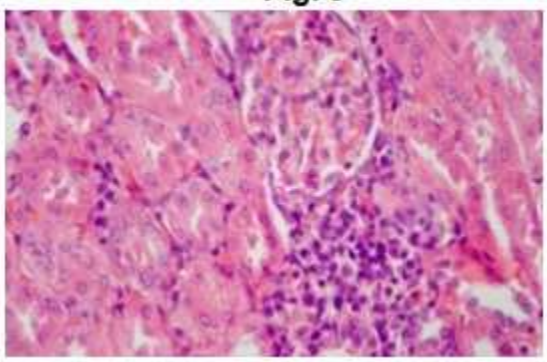


Fig. 6



As compared to group II, the histopathological changes in Groups III and IV were only of mild nature that consisted of tubular swelling and focal degenerative changes with occasional mild inflammatory reaction (Fig.7 & 8). Tubular changes observed were of mild nature with focal tubular swelling and desquamation of epithelium while focal areas showed degenerative changes in glomeruli with hyper-cellularity and atrophy of glomeruli. Also the interstitial hemorrhages were present focally. Intensity of histopathological changes in kidneys of rats from Group III was found to be less pronounced as compared to Group II. This might be due to restoration of architecture of an organ in terms of its histology possibly due to nephroprotective effect exerted by *Carica papaya* L. and taurine.

Fig. 7

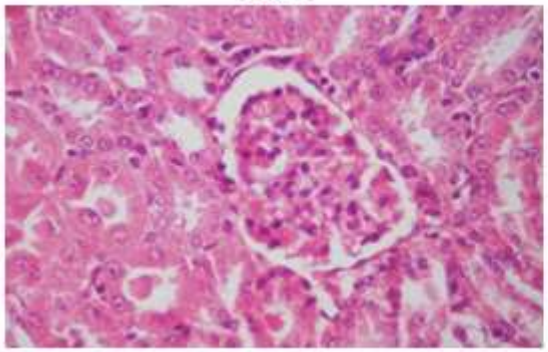
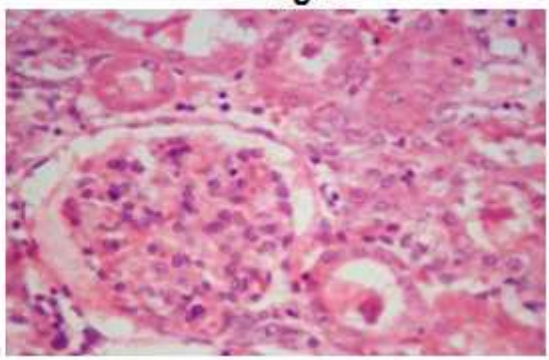


Fig. 8



CONCLUSION

Results of this study confirmed that gentamicin at a dose of 40 mg/kg resulted into significant hepatotoxicity and nephrotoxicity as evidenced by significantly increased levels of AST, ALT, BUN, serum creatinine, uric acid and decreased total protein level. In addition, gentamicin induced severe hepatic and renal damages as shown in histopathological examination which coupled with markedly elevated levels of liver biochemical markers (AST, ALT and Total Protein) and significant changes of kidney biochemical indices including statistically increased levels of BUN, serum creatinine, uric acid and decreased total protein level. In gentamicin treated rats, there was a significant increase in oxidative

stress suggesting the liver and kidney damage.

The present study concludes that aqueous extract of seeds of *Carica papaya* L. has nephroprotective activity. The protective effects of aqueous extract of *Carica papaya* L. seeds may be due to the presence of Phytochemical like Flavanoids, tannins which act as antioxidants individually or synergistically. This study obviously demonstrated that pretreatment (or concurrent administration of) with aqueous extract of *Carica papaya* L. seeds significantly attenuated the physiological and histopathological alterations induced by gentamicin. Also, the present study identifies new areas of research for development of better therapeutic agents for liver, kidney, and other organs dysfunctions and diseases.

Although the antioxidant status of the extract in the liver and kidney was not investigated in the current study, the biochemical and histological findings thus obtained were suggestive of that the extract may be mediating its protective effects either by decreasing the metabolic activation of gentamicin, or by acting as a chain-breaking antioxidant for scavenging free radicals or by a combination of these effects. In an earlier study, the presence of alkaloids, flavonoids, saponin, tannin, anthraquinones, and anthacyanosides in CPSE was reported (Adeneye and Olagunju, 2009). The presence of these active biological principles may thus be accounting for the biological effect of *CPSE* and could be via antioxidant and/or free radicals scavenging activities. However, further studies are required to substantiate these mechanisms.

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