Research Article

Evaluation of nephroprotective activity of methanolic extract of *Illicium verum* hook fruits in rodents

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Abstract

Objective: The present study was undertaken to evaluate nephroprotective activity of ethyl acetate and methanolic extract of the fruits of *Illicium verum* against paracetamol and gentamicin induced toxicity using male Wistar rats. **Material and Methods:** Randomly selected animals were divided into five groups of six animals each. The test extracts were administered orally at a dose of 200 mg/kg and 400 mg/kg. Nephrotoxicity was induced in rats by Paracetamol and gentamicin. The effect of extracts of *Illicium verum* at doses 200-400 mg/kg.b.wt compared with standard; was determined using serum urea, creatinine and uric acid. Furthermore, the effect of these extracts on some renal antioxidant enzymes and histopathological examination of kidneys were examined. **Results:** Paracetamol and gentamicin produced significant biochemical (increase in blood urea, serum creatinine, and serum uric acid level) changes, histological (damage to nephrons) changes, induced by paracetamol and gentamicin in kidney parameters. Pretreatment with *Illicium verum* extract significantly ($p<0.005$) prevented the physical, biochemical, and histological changes induced by paracetamol and gentamicin in the kidney**. Conclusion:** The ethyl acetate and methanolic extract of the fruit *Illicium verum* at two different dose level (200 & 400 mg/kg, bd.wt) was found to possess significant nephroprotective activity against paracetamol and gentamicin induced toxicity.

***Keywords*:** *Illicium verum*, nephroprotective, paracetamol, gentamycin, antioxidant

Introduction

Acute renal failure (ARF) is a major complication of kidney, encountered globally. Aminoglycoside induced nephrotoxicity is one of the leading cause of ARF, accounting about 10-15% of total cases of ARF across the world (Kumar et al., 2000). Gentamicin (aminoglycoside antibiotic) was introduced in 1963 and despite of its fatal side effects like, nephrotoxicity and ototoxicity, it has been used successfully for last 4 decades typically against gram-negative infections because of its good bactericidal efficacy and low cost. The exact mechanism of gentamicin induced nephrotoxicity is yet to be elucidated completely. However, the etiology behind gentamicin induced nephrotoxicity is rested on the fact that aminoglycosides (gentamicin) are strong cationic drugs accumulated at biological membranes (especially at S_1-S_2 segments of proximal tubule) causes net increase in oxidative stress and lipid peroxidation leading to necrotic changes in renal tubules and consequently precipitates acute nephrotoxicity (Ramsammy et al., 1986).

Paracetamol is a drug of Para-aminophenol group which is considered one of the commonly used and safe over the counter antipyretic and analgesic drugs, when administered at recommended doses (Ozkaya et al., 2010). The main problem with this medication remains its misuse through intentional or unintentional ingestion of supra- therapeutic dosages which usually lead to hepatic necrosis (Plaa, 2010). Oxidative stress is reported to constitute a major mechanism in the pathogenesis of Paracetamol induced liver and renal damage in experimental animals. Therefore, supplementation with antioxidants is very crucial to delay, prevent or remove oxidative damage (Demirbag et al., 2010).

Illicium verum hook also named star anise is the fruit of a medium sized tree that grows in Asia is native to China and Vietnam. The genus name illicera (allure) probably because of sweet and attractive fragrance. *Illicium verum* fruit is used in traditional system of medicines having both culinary and

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medicinal uses (Chouksey et al., 2013). Its seed oil is used worldwide as medicine. The fruits are sweet, aromatic, carminative, digestive, stomachic, stimulant, diuretic, expectorant, deodorant, constipation and insomnia. It relieves colic and is a common ingredient of cough lozenges and cattle sprays. They are also useful in dyspepsia, flatulence, spasmodic pain, facial paralysis, asthma, bronchitis, halitoris (Chouksey et al., 2010). Scientifically, there is no report on the nephroprotective studies of *Ilicium verum* so far, the objective of the present investigation is a systemic approach to explore the nephroprotective effects of different extracts of *Ilicium verum*.

Materials and methods

Plant material

The plant *Illicium verum hook fruits* was collected from Hyderabad, Ranga reddy district, Telangana state in the month of December 2016, this material was identified and authenticated by botanist.

Preparation of plant extract

The freshly dried hook fruits of the plant *Illicium verum* were collected which was already shade dried and they were pulverized in the laboratory.

Paracetamol induced nephroprotective model in rats

A total of 42 animals were taken and were divided into 7 groups of 6 animals each (n=6 / group). Group I (control) received normal saline orally for 7 days. Group II (Disease control) received a Paracetamol at a dose of 500 mg/kg,bd.wt *p.o* for 7 days. Group III & Group IV (Test) were administered with ethyl acetate extract *Illicium verum* hook fruits at a dose of 200 and 400 mg/kg,bd.wt *p.o* from 4th day to 7th along with Paracetamol at a dose of 500 mg/kg,bd.wt *p.o.* for 7 days. Group V & VI animals were administered with methanolic extract of *Illicium verum* hook fruits at a dose of 200 & 400 mg/kg,bd.wt *p.o*. from 4th day to 7th followed by Paracetamol at a dose of 500 mg/kg,bd.wt *p.o*. Group VII animals were treated with Standard hepatoprotective drug silymarin from 4th day to 7th at a dose of 250 mg/kg,bd.wt *p.o* followed by Paracetamol - 500 mg/kg,bd.wt *i.p*. for 7 days. On 8th day, the animals were anaesthetized using isoflurane anaesthesia and blood was collected by retro-orbital plexus. Serum was separated by centrifugation of blood at 3,000 rpm for 10 min and the separated serum was used for further biochemical analysis and kidney tissues were isolated and subjected for histopathological studies (Alqasoumi, 2014).

Gentamicin induced nephroprotective model in rats

A total of 42 animals were taken and were divided into 7 groups of 6 animals each (n=6 / group). Group I (control) received normal saline orally for 21 days. Group II (Disease control) received a gentamicin at a dose of 80 mg/kg,bd.wt *i.p.* for 21 days. Group III & Group IV (Test) were administered with ethyl acetate extract *Illicium verum* hook fruits at a dose of 200 and 400 mg/kg,bd.wt *p.o.* from 9th day to 21st along with gentamicin at a dose of 80 mg/kg,bd.wt *i.p.* for 21 days. Group V & VI animals were adiministered with methanolic extract of *Illicium verum* hook fruits at a dose of 200 & 400 mg/kg,bd.wt *p.o*. from 9th day to 21st followed by gentamicin at a dose of 80 mg/kg,bd.wt *i.p.* Group VII animals were treated with Standard hepatoprotective drug Silymarin from 4th day to 7th at a dose of 100 mg/kg,bd.wt *p.o* followed by gentamicin at a dose of 80 mg/kg,bd.wt *i.p*. for 21 days. On 22nd day, the animals were anaesthetized using isoflurane anaesthesia and blood was collected by retro-orbital plexus. Serum was separated by centrifugation of blood at 3,000 rpm for 10 min and the separated serum was used for further biochemical analysis and kidney and liver tissues were isolated and subjected for histopathological studies (Kannappan et al., 2010).

Results and discussions

Preliminary phytochemical screening

Preliminary phytochemical analysis of *Illicium verum* fruit extracts was performed.

Gentamicin induced nephrotoxicity

In gentamicin induced nephrotoxicity, gentamicin treated group showed a significant ($p<0.05$) increase serum creatinine, uric acid and urea as compared to control group was significantly ($p<0.05$) lower in gentamicin treated group. Administering the ethyl acetate and methanolic extract of *Illicium verum* at a dose of (200 & 400 mg/kg, bd.wt *p.o*) significantly ($p<0.05$) lowered the creatinine, uric acid and urea when compared to control group (Table 1).

The mechanism of Gentamicin (GM) induced nephrotoxicity is not completely known. However, proposed pathological mechanism includes induction of oxidative stress, apoptosis, necrosis, elevation of endothelin I and increase of monocyte/macrophages infiltration (Balakumar et al., 2010). GM-induced nephrotoxicity is characterized functionally by increased serum creatinine, increased blood urea nitrogen, and decreased glomerular filtration rate (Romero et al., 2009), and morphologically characterized by proximal tubule epithelial desquamation, tubular necrosis, epithelial edema, and glomerular hypertrophy (Lakshmi et al., 2009).

Several nephrotoxicants have been shown to induce an inflammatory response, which participated in the organ injury (Araujo et al., 2012). It is believed that during kidney toxicity, the initial insult by the toxicant results in tissue

Table 1. Effect of *Illicium verum* extracts in gentamicin induced nephrotoxicity

S. No	Groups	Creatinine	Uric acid	Urea
1	Normal Control	2.566 ± 0.1060	5.066 ± 0.0271	19.750 ± 0.074
2	Disease Control	$6.933 \pm 0.0578^{b,B}$	$10.39 \pm 0.0580^{b,B}$	$30.533 \pm 0.037^{b,A}$
3	EAIV 200 mg/kg, bd.wt.	$1.3 \pm 0.0523^{b**,B}$	$4.202 \pm 0.0196^{b,**,B}$	$23.600 \pm 0.0391^{b,**,A}$
4	EAIV 400 mg/kg, bd.wt.	$1.899 \pm 0.0978^{b,**,A}$	$4.308 \pm 0.0824^{**,B}$	$15.10 \pm 0.8761^{b,**,A}$
5	MEIV 200 mg/kg, bd.wt.	$1.896 \pm 0.0678^{b,**,A}$	$4.265 \pm 0.0336^{b,**,B}$	$21.633 \pm 0.0946^{b,**,A}$
6	MEIV 400 mg/kg, bd.wt.	$1.899 \pm 0.0363^{**,A}$	$3.233 \pm 0.0188^{b,**,B}$	$23.6 \pm 0.8200^{b,**,A}$
7	Standard (Silymarin) 100 mg/kg, bd.wt.	$1.59 \pm 0.0121^{b,**}$	$3.0188 \pm 0.0188^{b,**}$	$17.33 \pm 0.0494^{b,**}$

Values are expressed as Mean ± SEM, (n=6) followed by Dunnett's tests. All the groups were compared with control group, disease control group and standard group. Significant values are expressed as control group (a=p<0.01, b=p<0.05), disease control group (**= p<0.01, *= p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.

Table 2. Effect of *Illicium verum* extracts in paracetamol induced nephrotoxicity

S. No	Groups	Creatinine	Uric acid	Urea
1	Normal Control	$1.566 \pm 0.106^{b,**,A}$	$5.866 \pm 0.20^{b,**,A}$	$15.75 \pm 0.0529^{b,**,A}$
2	Disease Control	$3.95 \pm 0.0554^{b,A}$	$8.250 \pm 0.0547^{b,A}$	$28.85 \pm 0.0438^{b,A}$
3	EAIV 200 mg/kg, bd.wt.	$1.92 \pm 0.055^{b,**,A}$	$3.05 \pm 0.0191^{b,**,A}$	$22.016 \pm 0.066^{b,**,A}$
4	EAIV 400 mg/kg, bd.wt	$1.233 \pm 0.0626^{b,**,A}$	$3.330 \pm 0.0950^{b,**,A}$	$21.65 \pm 0.4826^{b,**,A}$
5	MEIV 200 mg/kg, bd.wt	$1.197 \pm 0.0217^{b,**,A}$	$3.016 \pm 0.468^{b,**,A}$	$23.06 \pm 0.0511^{b,**,A}$
6	MEIV 400 mg/kg, bd.wt	$1.92 \pm 0.0385^{b,**,A}$	$3.833 \pm 0.0193^{b,**,A}$	$21.05 \pm 0.0381^{b,**,A}$
7	Standard (Silymarin) 100 mg/kg, bd.wt	$0.80 \pm 0.0131^{b,**}$	$4.733 \pm 0.0167^{b,**}$	$19.316 \pm 0.0567^{b,**}$

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group, disease control group and standard group. Significant values are expressed as control group (a=p<0.01, b=p<0.05), disease control group (**= p<0.01, *= p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.

damage, which leads to generation of inflammatory mediators by the injured cells as well as by immune cells. Subsequently, these inflammatory mediators induce migration and infiltration of leukocytes into the injured organs and aggravate the primary injury induced by the toxicant (Luster et al., 2001; Akcay et al., 2009). This evidence is supported by the histological results of the present study which revealed the presence of inflammatory cells infiltration in kidney sections of gentamicin administered rats. For kidneys, the proinflammatory cytokine TNF-α is the main orchestrator of this inflammatory response and in several cases has been shown to aggravate the toxicant-induced pathophysiological responses (Piao et al., 2012). Results from many studies have shown that intraperitoneal injections of gentamicin resulted in development of destructive renal injury that was associated with significant elevation in serum urea and creatinine levels (Soliman et al., 2007).

In addition, the findings of histopathological examinations confirmed the biochemical data and showed the clear signs of nephrotoxicity in the form of marked glomerular and tubular degenerative changes and necrosis, tubulointerstitial nephritis and dilatation of the tubular lumen. These biochemical and histopathological observations of GM-induced nephrotoxicity run in consistency with those reported earlier in human patients (Baciewicz et al., 2003) and experimental animals (Silan et al., 2007). On the other hand, methanolic and ethyl acetate extracts of *Illicium verum* concurrently administered with GM efficiently protected the rat kidneys from the serious nephrotoxic effects of GM.

Paracetamol induced nephrotoxicity

In paracetamol induced nephrotoxicity, paracetamol treated group showed a significant (p<0.05) increase serum creatinine, uric acid and urea as compared to control group was significantly (p<0.05) lower in gentamicin treated group. Administering the ethyl acetate and methanolic extract of *Illicium verum* (200 & 400 mg/kg, bd.wt *p.o*) significantly (p<0.05) lowered the creatinine, uric acid and urea (Table 2).

Results of present study indicate that fruits of *Illicium verum* reduce the toxicity of paracetamol and also restores paracetamol induced histopathological impairment of

kidneys. Toxicity of paracetamol in mice is an established fact. Several earlier reports in human and in animal studies have cemented this fact. Due to this reason paracetamol is used as experimental toxin to induce kidney damage in experimental studies. Pre-treatment i.e., prophylactic administration of EAIV & MEIV at two different doses (200 mg/kd, bd.wt & 400 mg/kg, bd.wt) for 07 days to rats could provide appreciable protection against acetaminophen (paracetamol) challenge on 8th day in sub lethal experiments. *Illicium verum* hook fruits a natural antioxidant might have enhanced endogenous antioxidant system of rats, however, it could have also played protective role via other routes which are discussed subsequent paragraphs.

Constituents enhance detoxification and excretion of medicines including acetaminophen in rat liver. Star anise constituents stabilize integrity of hepatic lysosomes and mitochondria. Pre-treatment to rats orally with EAIV and MEIV decreased hepatic microsomal lipid peroxidation and increase in the level of GSH content and its dependent enzymes (glutathione-reductase, glutathione-s-transferase and glutathione peroxidase) and lowered DNA synthesis.

Increased levels of serum creatinine and urea have been considered as index of assessing nephrotoxicity. The elevated values in experimental rats indicate the severity of kidney damage by the paracetamol. Necrosis of kidney cells observed in the present study might be responsible for elevation of these biomarker enzymes (Mandal et al., 2015). Values of serum creatinine and urea and uric acid in group III, IV, V & VI rats treated with *Illicium verum* methanolic and ethyl acetate extracts of *Illicium verum* hook fruits showed significant ($p<0.05$) improvement and serum creatinine values of group II rats were statistically comparable with the values of serum creatinine and urea and uric acid with (healthy control) group rats. Previous studies reported the significant decrease in serum creatinine level on treatment with extracts which was increased in paracetamol induced nephrotoxic rats which support the findings of present study.

Histopathological study of kidney in gentamicin and paracetamol induced nephrotoxicity

Histopathological study revealed the normal renal architecture in control group. Paracetamol treated rats showed sever damage in the kidney cells appeared as variable size and atrophic cellular glomeruli, marked cloudy swelling in tubules and narrow lumens. Kidneys of animal treated with 200 mg/kg bd.wt EAIV showed less protective effect than that exerted on the liver cells. Marked congestion, tubular dilation, chronic inflammatory exudates in the cortex, haemorrhage and blood casts in the tubules, cellular glomeruli with variable sizes (few of them atrophic) were all observed. Treatment with 400 mg/kg bd.wt EAIV prior to Paracetamol intoxication showed cells with cortical vascular dilation and congestion, chronic inflammation and destruction of glomeruli, focal cortical degeneration, glomerular atrophy and chronic inflammatory exudates in the cortex around glomeruli. Treatment with 200 mg/kg bd.wt MEIV was effective in improving the histopathological appearance of the renal cells. Congestion and haemorrhage at corticomedullary area, glomerular changes, cloudy swelling in tubules, vessels congestion and dilation. Best histopathological nephroprotection was observed in subgroup treated with 400 mg/kg bd.wt MEIV where cells showed normal medulla and few small atrophic glomeruli with mild cloudy swelling. The protective standard drug Silymarin at 250 mg/kg helped in decreasing the cellular damage induced by Paracetamol. Cellular appearance showed mostly nearly normal glomeruli with few variable size atrophic glomeruli, mild tubular degeneration, necrosis and cloudy swelling.

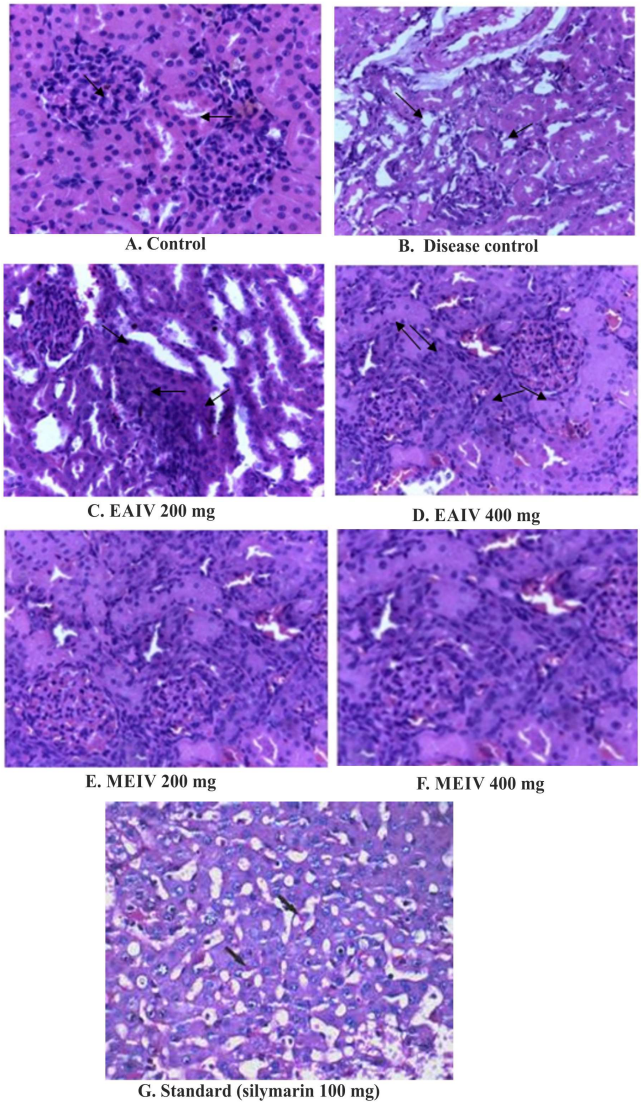

Figure 2. Histopathology of kidney tissues (paracetamol induced nephrotoxicity)

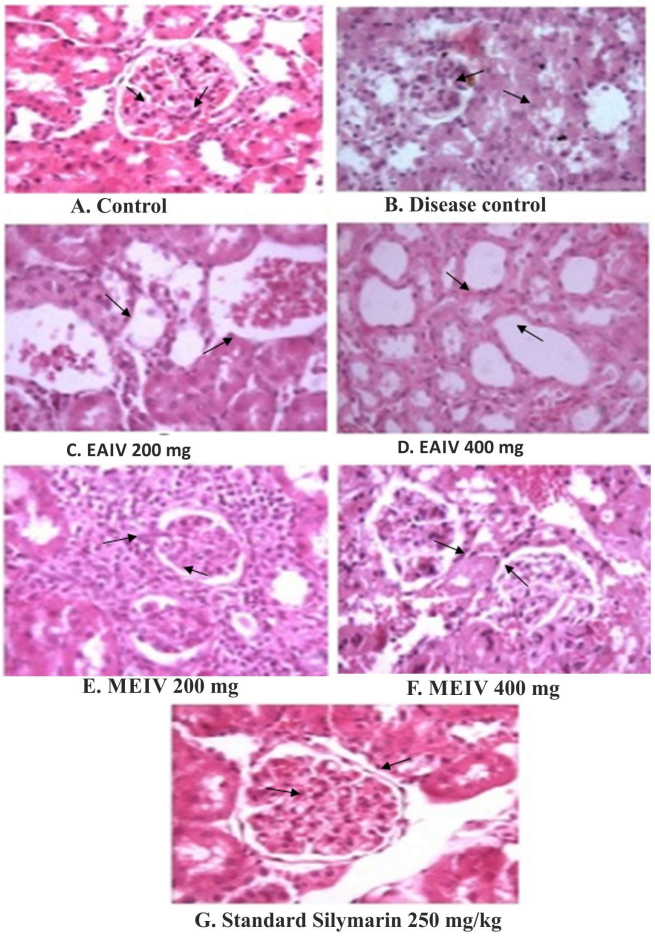

Figure 1. Histopathology of the kidney tissues (gentamicin induced nephrotoxicity)

Conclusion

The present study revealed that extracts of *Illicium verum* ethylacetate and methanol showed significant nephroprotective activity.

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Conflict of interest

The authors have no conflict of interest

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