Essay: Protective Effect of Abutilon indicum (L.) on Cisplatin & Acetaminophen Induced Nephrotoxic Rats

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Abstract:
Abutilon indicum L. (Malvaceae) is a common Indian medicinal herb traditionally used for curing variety of disorders in ancient systems of medicine. The study aims at evaluating the protective and curative effect of ethanolic extract of Abutilon indicum (EEAI) at 200 & 400 mg doses on cisplatin and acetaminophen induced nephrotoxic rat models. Ethanolic extract of the plant is prepared from the aerial parts of Abutilon indicum by successive solvent extraction using soxhlet apparatus. The extract after preliminary phytochemical studies, was evaluated pharmacologically for nephroprotective role in both cisplatin and acetaminophen induced
nephrotoxic rat models. The drug induced nephrotoxicity is often associated with elevations in serum creatinine, blood urea nitrogen, uric acid, total proteins, total cholesterol, alkaline phosphatase, albumin and acute tubular necrosis. Treatment with ethanolic extract of Abutilon indicum (EEAI) dose dependently attenuated abnormal elevations in serum parameters in both the cisplatin and acetaminophen models when compared to toxic control group. Histopathological results further supported the nephroprotective role of the plant. In the present study, histopathological examination and serum parameters elevation showed a clear evidence of nephrotoxicity following the administration of cisplatin and acetaminophen. EEAI pre-treatment ameliorated both the drug induced renal changes. High dose (400 mg) of the EEAI have shown higher incidence of nephroprotection. Therefore, further establishment of EEAI for nephroprotection may be beneficial for patients undergoing drug therapy with cisplatin and acetaminophen.

Introduction
Abutilon indicum (Malvaceae), referred commonly as ‘Thuthi’, is spread throughout the hot regions of India predominantly in Andhra Pradesh, Karnataka and Maharastra. Plant leaves are stalked, ovate, irregularly crenate or dentate. The flowers are orange-yellow in colour, solitary, axillary. The fruits are hispid, scarcely longer than the calyx and the awns are erect. Seeds are kidney shaped, dark brown or black in colour, tubercled or with minutely stellate hairs 2.

The plant is abundant in fixed oils like gallic acid (roots), linoleic acid, oleic, palmitic, stearic and capric acids. Asparagine, fructose, galactose, vallinic acid, caffeic acid, fumeric acid, alkaloids, flavonoids, triterpenoids, saponins, Abutilin A, ??-sitosterol, scopoletin are also reported. Quercetin and its glycosides have been isolated from flowers. The plant has been described in the Siddha system of medicine as a remedy for jaundice, piles, ulcer and leprosy. The plant is used for various kidney disorders by local traditional healers and abroginal people. A leaf paste is taken orally to cure piles and to relieve leg pains. Bread prepared from the mixture of its leaf powder and wheat flour is used for uterus displacement. The leaf juice when mixed with jaggery is used for the treatment of snakebite as antidote. The fruit is used to treat piles, gonorrhea, and cough. Fruit decoction mixed with ammonium chloride is given orally with water to treat hemorrhagic septicemia. Root infusion is given to cure fever, dry cough and bronchitis. It is traditionally claimed to possess to treat uroliths in the kidneys. The plant is also reported scientifically for analgesic, hepatoprotective, hypoglycaemic, wound healing, and abortifacient properties.

Current day xenobiotics are the agents which may possess many serious acute or chronic toxic effects on the various vital organs of our body. Among them, cisplatin (CP) and acetaminophen (AP) are two drugs belonging to separate categories but with common major adverse effect of causing nephrotoxicity. Cisplatin is one of the most potent antitumor agents and is effective against diverse spectrum of malignancies. Role of reactive oxygen species (ROS) and lipid peroxidation (LP) which develops in response to Cisplatin is the attributory mechanism for causing nephrotoxicity is substantially evidenced. Cisplatin is metabolized in the kidney and liver. In CP-induced nephrotoxicity, platinum glutathione (GSH) conjugates formed in renal cells are metabolized through a gamma-glutamyl transpeptidase (GGT) to a reactive thiol, which is a potent nephrotoxin, and depleted GSH impairs regulation of reactive oxygen species (ROS). In addition, oxidative stress is well known to stimulate transcription factors, including nuclear factor-kappa B (NF-kB). Consequently, NF-kB activation leads to expression of many gene involved in the renal damages such as inducible nitric oxide synthase (iNOS) and proinflammatory cytokine gene, resulting in excessive nitric oxide (NO) generation leading to renal damage.

Acetaminophen commonly called paracetamol, is known to cause hepatic necrosis and renal failure in both humans and animals when administered in over-doses. Acetaminophen undergoes deacetylation to p-aminophenol and bind to kidney proteins which turn is distributed to mitochondria, microsomes, cytosol and associated proteins DNA, mitochondrial enzymes and Glucose-6-phosphatase. Renal tubular damage and acute renal failure can occur even in absence of liver injury can even lead to fatality in humans and experimental animals.

The serious adverse effects of the drugs made their usage limited. Attempts were made to prevent the nephrotoxic effects of the drugs by using various antioxidants and other drugs. Days have arrived where we need to rely on herbal medicine. Many herbal extracts were studied for reducing renal injury in acetaminophen and Cisplatin induced nephrotoxicity. Flavonoids, an important constituent of Abutilon indicum has been investigated for its antioxidant activities. Several other plants containing antioxidant
properties exhibited nephro-protective activity against cisplatin and acetaminophen. Utilizing the review of literature of the plant Abutilon indicum, along with traditional claim for renal protection, the present research study was carried out to pharmacologically evaluate the potential of the extract of the Abutilon indicum on experimental renal damage induced by acetaminophen and cisplatin models.

Nephrotoxicity can be defined as renal dysfunction that arises as a direct result due to external agents such as drugs and environmental chemicals. Many therapeutic agents of current era have been shown to induce clinically significant renal damage due to Nephrotoxicity. The term renal failure or dysfunction implies failure of the excretory mechanism of kidney, leading to accumulation of nitrogenous byproducts of metabolism in blood. In addition, there is also deviation in control of fluid electrolytes and along with endocrine dysfunction.

The renal failure is fundamentally categorized as acute and chronic renal failure23.

Acute Renal Failure (ARF) usually refers to sudden reversible loss of kidney function, which arises over a period of days or weeks. There are many causes for acute renal failure, which could be pre-renal (55%), renal (40%), or post renal (5%). Renal causes constitute acute renal failure and acute tubular necrosis which is more common accounting for 85% of incidence. Acute tubular necrosis occurs due to ischemia or toxins. The toxin can be either exogenous or endogenous. The exogenous agents are radiocontrast agents, organic solvents, cyclosporine, antibiotics, chemotherapeutic agents, acetaminophen and illegal abortifacients24. Chronic Renal Failure (CRF) leads to irreversible change in kidney function, which classically develops over a period of years, leading to loss of excretory metabolic and endocrine functions. Various causes of renal failure has been attributed to hypertension, diabetes mellitus, antineoplastic agents like cyclophosphamide, vincristin and Cisplatin25, 26

Materials and methods:

Collection and authentication of the plant material:
The plant was collected from the Hills of Tirumala, Tirupati, India in the month of December 2011 and authenticated by Dr. K. Madhava Chetty, Asst. Professor, Dept of Botany, S.V. University, Tirupati, India. The plant was evaluated for its physicochemical parameters like determination of foreign matter, moisture content, ash and extractive values to obtain the qualitative information about the purity and quality of Abutilon indicum.

Extraction & Phytochemical analysis:
The plant is extracted to explore the phytochemical constituent’s present in it. Plant material (400 grm) is subjected to successive extraction by petroleum ether, chloroform and ethanol as solvents. The ethanolic extract obtained was concentrated to a thick paste on the water bath, maintained at 50oC. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. These extracts were stored in airtight at refrigeration temperature. The extracts were examined for their colour and consistency and their percentage yield of extract was calculated. Extracts are stored in airtight containers and were reconstituted for various concentrations of the extracts.

Preliminary phytochemical screening:
The preliminary phytochemical investigations were performed for extracts of Abutilon indicum Linn for qualitative identification of phytoconstituents present by following standard methods27. All the chemicals and reagents used were of analytical grade.

Pharmacological and Toxicological evaluation:

Experimental Animals:
The animals used for experimental study were procured from Sri Venkateswara enterprises, Bengaluru, India and were acclimatized for 7 days under standard husbandry conditions (25 ?? 20 C, 45-55% relative humidity, 12:12 Light/ dark cycle). All the experimental design, procedures and protocol of the study were reviewed, approved and monitored by the Institutional Animal Ethics Committee (IAEC) of Sree Vidyanikethan College of Pharmacy, Tirupati, India. (Ref no: SVCP/IAEC/1-027/2011-12 dated 21/01/2012).

The acute toxicity of ethanol extract of Abutilon indicum (EEAI) was determined in albino mice of either sex weighing between 18-22 g those maintained under standard husbandry conditions. The animals were fasted 3 h prior to the experiment and 2000 mg of the extract was administered orally as per OECD test guidelines (425) and acute oral and mortality was observed for 7 days.

Experimental Procedure:

Cisplatin induced renal damage:
Five groups of six rats in each were fasted and deprived of water for 18 hrs prior to the experiment. The first group (control), received 2% v/v aqueous tween 80 solution (5 ml/kg, p.o); the second group (negative control), received 2% v/v aqueous tween 80 solution (5 ml/kg, p.o) along with Cisplatin (750 mg/kg), per orally; the third group (standard) received cystone (5 ml/kg, p.o); the fourth & fifth groups received the EEAI (Ethanolic extract of Abutilon indicum) at the doses of 200 mg/kg and 400 mg/kg p.o for seven days respectively following Cisplatin injection for last days. Various biochemical parameters were estimated after 24 hrs following the last dose 27, 28.

Acetaminophen induced renal damage:
Five groups of six rats in each were fasted and deprived of water for 18 hrs prior to the experiment. The first group (control), received 2% v/v aqueous tween 80 solution (5 ml/kg, p.o); the second group (Negative control) received 2% v/v aqueous tween 80 solution (5 ml/kg, p.o) along with 750 mg/kg via oral route of acetaminophen; the third group (standard) received cystone (5 ml/kg, p.o); the fourth and fifth groups received the EEAI (Ethanolic extract of abutilon indicum) at the doses of 200 mg/kg and 400 mg/kg p.o for seven days respectively. On the seventh day, Acetaminophen suspension is administered orally at dose of 750 mg/kg to all the groups except to Group-I. Various biochemical parameters were estimated after 24 hrs following the last dose 29.

Biochemical analysis:
Blood samples were collected by cardiac puncture under diethyl ether anesthesia using a 5 ml syringe (Hindusthan syringes & medical devices, Faridabad, India). Serum samples were analysed for creatinine blood urea nitrogen (BUN), Uric acid, total protein, albumin, alkaline phosphate and total cholesterol in both acetaminophen and cisplatin induced nephrotoxic rats.

Histopathological analysis:
Kidney samples were excised from sacrificed animals of control and treated groups and washed with normal saline. They were fixed in 10% buffered Formalin for 24 h and embedded in paraffin wax. Cross sections of the kidney tissue (5-6 ??m) were prepared and stained with haematoxylin-eosin dye and evaluated microscopically.

Statistical analysis: All results will be expressed as mean ?? SEM from 6 animals. Statistical difference in mean will be analyzed using one-way ANOVA (analysis of variance) followed by Duncan’s multiple range test (DMRT). If ‘P’ value < 0.05*, 0.01** and 0.001*** will be considered as statistically significant.

Results:
The plant was authenticated and identified by physicochemical parameters. The preliminary phytochemical analysis of Abutilon indicum L. (Malvaceae) revealed presence of alkaloids, glycosides, flavonoids, carbohydrates, terpenoids, phenolic groups in ethanolic extract. The acute toxicity results revealed no signs of acute toxicity (Table No: 01)
The toxic control treated rats in both models, i.e., cisplatin and acetaminophen caused significant increase in biological parameters like creatinine, blood urea nitrogen (BUN), Uric acid, total protein, albumin, alkaline phosphate and total cholesterol. Pretreatment with EEAI dose dependently attenuated the elevations in serum parameters in both the cisplatin and acetaminophen models (P<0.05) which are presented in table no 2 & 3. Histological analysis of kidneys revealed severe and widespread necrosis with dilation of proximal tubules, vacuolization, tubular cell desquamation and intraluminal cast formation in the cisplatin toxic control group. The kidney histopathology results of Acetaminophen treated toxic control animal group revealed multiple focal tubulonephritis with marked lymphocytic infiltration when compared to normal renal architecture. The EEAI treated animals had minimal renal changes compared to toxic control groups in both the Cisplatin & Acetaminophen models

DISCUSSION & CONCLUSION
Cisplatin is an extensively used anti-cancer agent for the management of germ cell tumors, head and neck cancers, bladder cancer, cervical cancer and as a salvage in the treatment of other solid tumours. Although higher doses of cisplatin are more efficacious for the suppression of cancer, high dose therapy manifests irreversible renal damage. Cisplatin therapy induces oxidative stress, principally involving ROS, in renal proximal tubular cells. The interaction of ROS with cellular components may result in damage to DNA, proteins and lipids. The protective effects of EEAI may be partially mediated by preventing the cisplatin-induced decline of renal anti oxidant status.
In the study, single intraperitoneal administration of 5mg/kg of cisplatin to rats induced significant increase in serum creatinine, blood urea nitrogen, uric acid, total proteins, total cholesterol, alkaline phosphatase, and albumin concentrations compared to control animals, suggesting an acute renal failure. EEAI at 200 and 400mg/kg administered 7 days before Cisplatin treatment significantly prevented the increase of various serum constituent abnormal concentrations and markedly decreased cisplatin-induced renal damage as confirmed by biochemical assays and histopathological studies. In the present study, EEAI revealed potent protective activity against cisplatin-induced nephrotoxicity, therefore, renal protective action of EEAI may be beneficial for patients undergoing chemotherapy of cisplatin.

Acetaminophen is an effective analgesic and antipyretic drug alternative to aspirin. Over doses or prolonged use is commonly associated with hepatotoxicity and nephrotoxicity in humans and in experimental animals. Acetaminophen nephrotoxicity results from the toxic effects of its reactive intermediate metabolite, N-acetyl-para-aminobenzoquinoneimine (NAPQI), which arylates proteins in the S3 segment of proximal tubule, initiating cell death of renal tubular cells. These drug induced nephrotoxicities are often associated with marked elevations in serum constituents like creatinine, blood urea nitrogen, uric acid, total proteins, total cholesterol, alkaline phosphatase, albumin causing acute tubular necrosis.

Acetaminophen induced renal damage is consistent with acute tubular necrosis. In the present study, the results of histopathological examination showed a clear evidence of nephrotoxicity following the administration of acetaminophen in an overdose. Acute tubular necrosis was the most relevant histopathological change. These results are in agreement with those of the previous investigation describing the renal histological alterations following the administration of acetaminophen in an overdose. EEAI pretreatment ameliorated the drug-induced histopathological renal changes. Both biochemical and histopathological evaluations reveal that the combined therapy of Abutilon indicum with current generation xenobiotics like cisplatin and acetaminophen could be promisingly nephroprotective.

Acknowledgements:
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RESULTS

Table No: 1. Acute Toxicity studies of EEAI

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Animals</th>
<th>Dose/kg p.o</th>
<th>Weight of Rats</th>
<th>Signs of Toxicity</th>
<th>Onset of Toxicity</th>
<th>Duration of Study</th>
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<tbody>
<tr>
<td>1</td>
<td>No. 1</td>
<td>2000 mg</td>
<td>155</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>7 days</td>
</tr>
<tr>
<td>2</td>
<td>No. 2</td>
<td>2000 mg</td>
<td>162</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>7 days</td>
</tr>
<tr>
<td>3</td>
<td>No. 3</td>
<td>2000 mg</td>
<td>172</td>
<td>No signs of Toxicity</td>
<td>Nil</td>
<td>7 days</td>
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Table No: 2 Effect of EEAI on serum parameters with/without acetaminophen treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Creatinine (mg/dl)</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Total protein (g/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Alkaline phosphate</th>
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<tr>
<td>EEAI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
(IU) Albumin
(g/dl)
Group I
Control 0.42 ±0.01 18.35±0.32 0.65±0.04 5.8±0.03 120.94±0.13 73.5±0.07 3.9±0.02
Group II
Neg. control 1.25±0.01 33.06±0.4 3.4±0.08 10.6±0.25 152±0.38 132.4±0.02 7.45±0.07
Group III
Standard
(5ml/kg) 0.63±0.02 19.75±0.03 0.65±0.04 5.8±0.03 100.4±0.6 84±0.10 4.05±0.09
Group IV
EEAI 200mg/kg 0.75±0.02 24.42±0.09 1.35±0.02 7.02±0.09 103.24±0.5 89±0.07 4.65±0.1
Group V
EEAI 400mg/kg 0.68±0.02 21.05±0.07 1.14±0.06 6.95±0.04 101.52±0.74 86.4±0.04 4.43±0.03

Values are expressed as mean ± SEM of six different samples; P<0.05 compared with control by Duncan’s Multiple Range Test (DMRT).

Table No: 3. Effect of EEAI on serum parameters with/without Cisplatin treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Creatinine (mg/dl)</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Total protein (g/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Alkaline phosphate (IU)</th>
<th>Albumin (g/dl)</th>
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<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>0.42 ±0.01</td>
<td>18.35±0.32</td>
<td>0.65±0.04</td>
<td>5.8±0.03</td>
<td>120.94±0.13</td>
<td>73.5±0.07</td>
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<td></td>
<td>Neg. control</td>
<td>1.25±0.01</td>
<td>33.06±0.4</td>
<td>3.4±0.08</td>
<td>10.6±0.25</td>
<td>152±0.38</td>
<td>132.4±0.02</td>
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<tr>
<td>Group III</td>
<td>Standard</td>
<td>(5ml/kg) 0.63±0.02</td>
<td>19.75±0.03</td>
<td>0.65±0.04</td>
<td>5.8±0.03</td>
<td>100.4±0.6</td>
<td>84±0.10</td>
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<tr>
<td></td>
<td>EEAI 200mg/kg</td>
<td>0.75±0.02</td>
<td>24.42±0.09</td>
<td>1.35±0.02</td>
<td>7.02±0.09</td>
<td>103.24±0.5</td>
<td>89±0.07</td>
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<tr>
<td></td>
<td>EEAI 400mg/kg</td>
<td>0.68±0.02</td>
<td>21.05±0.07</td>
<td>1.14±0.06</td>
<td>6.95±0.04</td>
<td>101.52±0.74</td>
<td>86.4±0.04</td>
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Values are expressed as mean ± SEM of six different samples; P<0.05 compared with control by Duncan’s Multiple Range Test (DMRT).

Fig No: 2. (a, b, c, d). Effect of EEAI on serum creatinine, blood urea nitrogen, serum uric acid levels in acetaminophen induced nephrotoxic rats

Fig No: 3 (e, f, g). Effect of EEAI on serum total cholesterol, serum alkaline phosphate & serum albumin levels in acetaminophen induced nephrotoxic rats

Fig No: 4 (a,b,c,d,e). Histopathological studies of the kidney in acetaminophen induced nephrotoxic rats

Fig 4a. Control group (10X) Fig 4b. Acetaminophen treated group (750mg/kg) (10X)
Fig 4c. Standard (cystone) (5ml/kg) (10X) Fig 4d EEAI (200mg/kg) + acetaminophen treated
Fig 4e EEAI (400mg/kg) + acetaminophen treated group (10X)

Fig No: 5 Effect of EEAI on various serum parameter levels in Cisplatin induced nephrotoxic rats

Fig 5a. Histopathology of rat kidney in control group (10X) 5b. Histopathology of rat kidney in Cisplatin (6mg/kg) treated group (10X)

Fig 5c. Histopathology of rat kidney in standard (cystone- 5ml/kg) 5d. Histopathology of rat kidney in EEAI (200mg/kg) +
treated group (10X) Cisplatin (6mg/kg) treated group (10X)

5e. Histopathology of rat kidney in EEAI (400mg/kg) + Cisplatin (6mg/kg) treated group (10X)

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