

PROTECTIVE EFFECT OF ABUTILON INDICUM L. (MALVACEAE) AGAINST CISPLATIN INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

Cisplatin (CDDP) is an effective antineoplastic agent in the treatment of solid malignant tumors. But, its clinical use is limited because of various side effects including sensorineural hearing loss. Several agents have been proposed to reduce these side effects. The present study reported that the ethanolic extract of Abutilon indicum scavenge superoxide and hydroxyl radicals, resulting in a reduction of lipid peroxidation. The purpose of the present study was to evaluate EEAI's efficacy as a protective agent against cisplatin-induced ototoxicity. Albino wistar rats were used in this study and were divided into five treatment groups: 1) animals administered 2% v/v aqueous tween 80 solution (5ml/kg, p.o) – control group (Group I), 2) animals administered 2% v/v aqueous tween 80 solution (5ml/kg, p.o) + 6 mg/kg via the i.p route of Cisplatin (Group II), 3) animals received Cystone (5ml/kg, p.o) [Standard] (Group III), 4) animals received 200 mg/kg EEAI suspended in 2% v/v aqueous tween 80 solution, p.o + 6 mg/kg, i.p of cisplatin (Group IV), 5) animals received 400 mg/kg EEAI suspended in 2% v/v aqueous tween 80 solution, p.o + 6 mg/kg, i.p of cisplatin (Group V). The protective effect of EEAI on CDDP-induced nephrotoxicity was evaluated. Nephrotoxicity was evaluated by means of measurement of serum BUN and creatinine and histopathological examination of the kidney. There were significant differences in serum BUN and creatinine levels between control Group and cisplatin treated Groups. The result suggested that EEAI at 200 and 400mg/kg administered 7 days before cisplatin treatment significantly prevented the increase of serum creatinine, blood urea nitrogen, uric acid, total proteins, total cholesterol, alkaline phosphatase, and albumin concentrations and markedly decreased cisplatin-induced renal damage as confirmed by biochemical assays and histopathological studies.

Keywords: Antioxidants, Cisplatin, nephrotoxicity, Abutilon indicum

INTRODUCTION

Cisplatin (cis-diaminedichloroplatinum II) is one of the most effective chemotherapeutic agents and plays a major role in the treatment of a variety of human solid tumors including those of the head, neck, testis, ovary, and breast.¹ The nephrotoxicity of cisplatin limits the efficacy of this important anticancer drug as its pathogenesis induced acute renal failure (ARF).² The molecular mechanisms of its toxicity are poorly understood. Studies have suggested that free radical like superoxide radical play an important role in cisplatin-induced nephrotoxicity.³ It has also been proposed that increase in lipid peroxidation in the kidney is also associated with cisplatin-induced nephrotoxicity.⁴ Cisplatin also induces nephrotoxicity by causing GSH depletion which inhibit antioxidant activity.⁵ Based on the pathophysiological role of cisplatin (CDDP) Nephrotoxicity it has been studied for the last three decades. Oxidative stress, inflammation and apoptosis are the main factors of the CDDP-induced renal injury.⁶⁻⁹

In CDDP-induced nephrotoxicity, platinum-glutathione (GSH) conjugates formed in cells were metabolized through a gamma-glutamyltranspeptidase (GGT) to a reactive thiol, which is a potent nephrotoxin, and depleted GSH impairs regulation of reactive oxygen species (ROS).¹⁰ 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.¹¹ The side effects of cisplatin include nephrotoxicity, gastrointestinal toxicity, neurotoxicity, ototoxicity. Some evidence suggests that the antioxidant DPPD (N,N-diphenylpphenylenediamine) inhibited nephrotoxicity in cisplatin treated rats, by reducing the increase in lipid peroxide level, and also by reducing the inhibition of p-aminohippurate transport in vitro.¹² Thus, our study is carried on screening program to evaluate the protective potentials of natural compounds. The objective of the present study is to explore the protective effect of Abutilon indicum L. (Malvaceae) extracts in Cisplatin induced renal injury in rats.¹³

Materials and methods

Plant material

The plant Abutilon indicum L. (Malvaceae) is widely distributed throughout South India. Abutilon indicum L. was collected from Tirumalahills, Tirupati, India in the month of December and it was identified and authenticated. The taxonomical identification and authentication was done by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati. About 500 g of dried powder of Abutilon indicum was successively extracted with petroleum ether (60-80°C) for 8 hrs. to remove the fatty matter. The defatted marc was then subjected to soxhlet extraction with 95% ethanol to obtain ethanolic extract.¹⁴ After complete exhaustion of the drug it was filtered and the filtrate was concentrated on water bath (45°C) to remove the solvent and to get a sticky brown coloured extract i.e. ethanolic extract of Abutilon indicum. Aqueous extract was prepared by macerating the dried drug powder in double distilled water. The extract was concentrated in a water bath and stored in desiccators.¹⁵

Animals: Adult Albino Wistar rats weighing about 150-180 g of either sex were procured from the animal house of SreeVidyanikethan College of Pharmacy, Tirupati. The animals were maintained in a well-ventilated animal house approved by Committee For The Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with 12:12 hour light/dark cycle in propylene cages with 27± 2°C temperature. The animals were given standard pellet.

Acute oral toxicity study: The study was followed by using OECD-423 (Acute Toxic Class Method). Adult Wistar rats weighing 150-180g were used for the study. The starting dose level of EEAI was 2000mg/kg body weight p. o. volume was administered to overnight fasted rats with ad libitum. Food was withheld for further 3- 4 hours after administration of EEAI and observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory,

circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted.

Nephroprotective study

Method: Five groups of six rats in each were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control, received 2% v/v aqueous tween 80 solution (5ml/kg, p.o); the second group received 2% v/v aqueous tween 80 solution (5ml/kg, p.o) along with 6 mg/kg/i.p; the third group received cystone (5ml/kg, p.o); the fourth, fifth groups received the EEAI at the doses of 200 mg/kg and 400 mg/kg body weight orally daily for seven days respectively for six days, starting 3 days before cisplatin injection (Table 1). The biochemical parameters were estimated 24 hrs following the last dose.

Table 1: Experimental design

Groups	Treatment
Group I	2% v/v aqueous tween 80 solution (5ml/kg, p.o) [Control group]
Group II	2% v/v aqueous tween 80 solution (5ml/kg, p.o) + 6 mg/kg/i.p cisplatin
Group III	Cystone (5ml/kg, p.o) [Standard]
Group IV	200 mg/kg EEAI suspended in 2% v/v aqueous tween 80 solution, p.o + 6 mg/kg, i.p of cisplatin
Group V	400 mg/kg EEAI suspended in 2% v/v aqueous tween 80 solution, p.o + 6 mg/kg, i.p of cisplatin

Biochemical analysis: Blood samples were collected by cardiac puncture under diethyletheranaesthesia, using 21 guage (21 G) mounted on a 5ml syringe (Hindusthan syringes and medical devices ltd, Faridabad, India.). Serum samples were analyzed for creatinine, blood urea nitrogen (BUN), uric acid, total protein, albumin, alkaline phosphatase, total cholesterol.16

Histopathological analysis: After the animals were sacrificed, the kidney samples were excised from the control and treated groups of animals and washed them with normal saline.17 They were fixed in 10% buffered formalin for 24 h and embedded in paraffin wax. Cross- sections of the kidney tissue (5-6µm thick) were prepared and stained with haematoxylin-eosin dye. The sections were evaluated by microscopical examination.18

Statistical analysis: Values are expressed as mean \pm SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values were considered statistically significant if the P- value is less than 0.05.19

RESULTS AND DISCUSSION

Cisplatin is an extensively used anti-cancer agent for the management of germ cell tumours, head and neck cancers, bladder cancer, cervical cancer and as a slavage in the treatment of other solid tumours.20 Although higher doses of cisplatin are more efficacious for the suppression of cancer, high dose therapy manifests irreversible renal damage. A number of therapeutic agents have been evaluated experimentally and clinically against cisplatin-induced nephrotoxicity, but none of them proved to be clinically effective as a complete protective agent.21 Cisplatin therapy induces oxidative stress, principally involving ROS, in renal proximal tubular cells.22 Oxidative stress caused by various free oxygen radicals including superoxide anion, hydrogen peroxide and hydroxyl radical. The interaction of ROS with cellular components may result in damage to DNA, proteins and lipids.23 The protective effects of EEAI may be partially mediated by preventing the cisplatin-induced decline of renal anti oxidant status.24 In this 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.

The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. Acute toxicity studies showed that the body weight of the rats before and after administration was noted and the changes in the body weight were not so prominent. No changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity were also not observed. In further study there was no toxicity / death observed at these levels. Serum analysis for creatinine, blood urea nitrogen, uric acid, total proteins, total cholesterol, alkaline phosphatase, and albumin were analyzed after cisplatin induced toxicity and the protective potential of Abutilon indicum extract in rats. The histopathology study for kidney was also carried out to assess the protective effect of Abutilon indicum.

Table 2: Effect of EEAI on serum parameters with/without Cisplatin treatment

Treatment	Creatinine (mg/dl)	Blood urea nitrogen (mg/dl)	Uric acid (mg/dl)	Total protein (g/dl)	Total cholesterol (mg/dl)	Alkaline phosphate (IU)	Albumin (g/dl)
Group I	0.42 \pm 0.02	18.35 \pm 0.03	0.65 \pm 0.04	5.8 \pm 0.03	120.94 \pm 0.30	73.5 \pm 0.07	3.9 \pm 0.02
Group II	1.2 \pm 0.01	31.64 \pm 0.07	3.54 \pm 0.09	10.45 \pm 0.09	154 \pm 0.45	131.4 \pm 0.90	7.64 \pm 0.03
Group III	0.65 \pm 0.02	18.5 \pm 0.09	0.85 \pm 0.04	6.4 \pm 0.15	98.2 \pm 0.54	82.9 \pm 0.06	4.15 \pm 0.06
Group IV	0.81 \pm 0.01	21.46 \pm 0.06	1.25 \pm 0.06	7.05 \pm 0.06	102 \pm 0.04	87.34 \pm 0.54	4.75 \pm 0.07
Group V	0.72 \pm 0.04	20.3 \pm 0.09	1.04 \pm 0.09	6.68 \pm 0.02	99.4 \pm 0.06	85.4 \pm 0.07	4.24 \pm 0.02

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

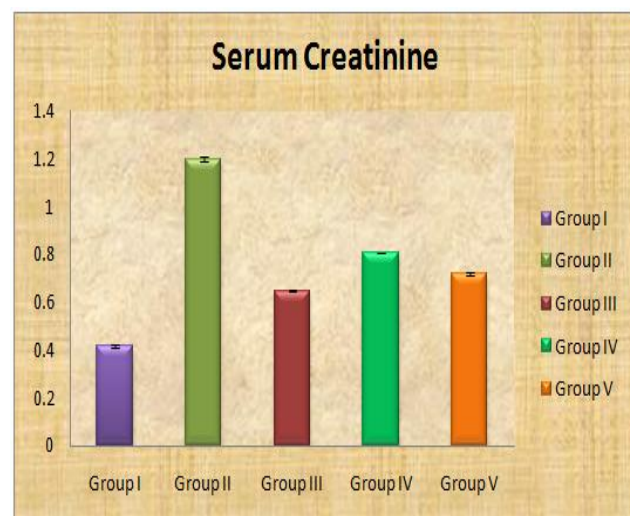


Fig 1: Effect of EEAI on serum creatinine levels in cisplatin induced nephrotoxic rats

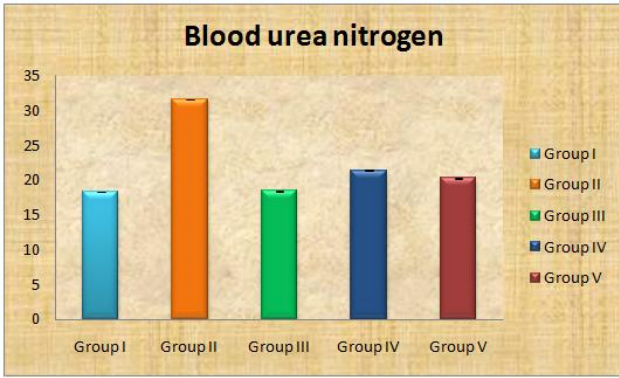


Fig 2: Effect of EEAI on blood urea nitrogen levels in cisplatin induced nephrotoxic rats

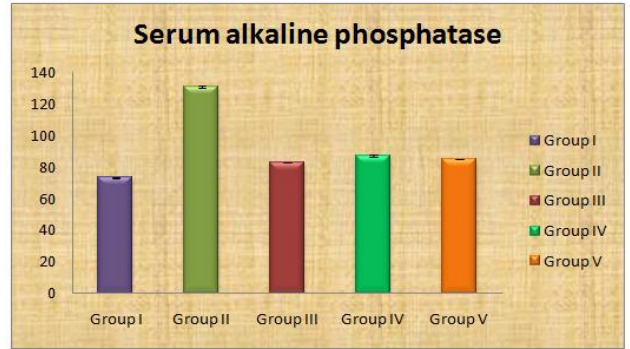


Fig 6: Effect of EEAI on serum alkaline phosphate levels in cisplatin induced nephrotoxic rats

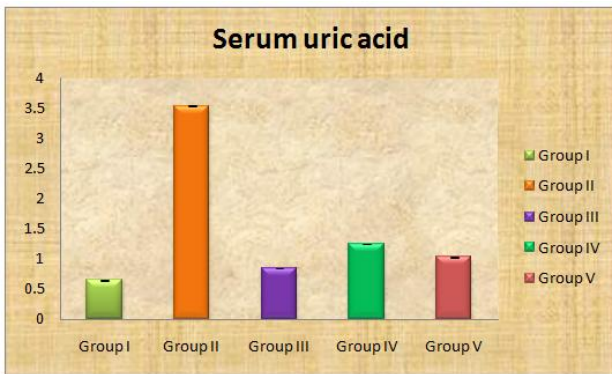


Fig 3: Effect of EEAI on serum uric acid levels in cisplatin induced nephrotoxic rats

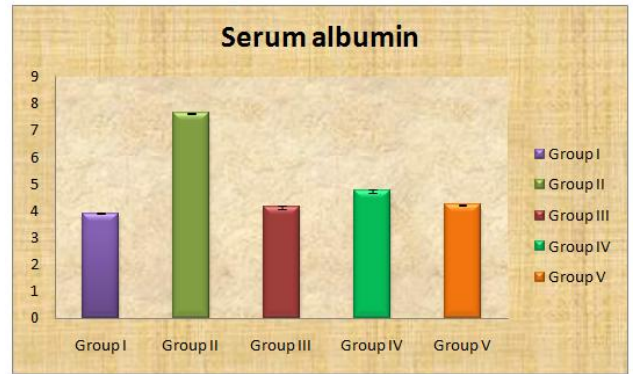


Fig 7: Effect of EEAI on serum albumin levels in cisplatin induced nephrotoxic rats

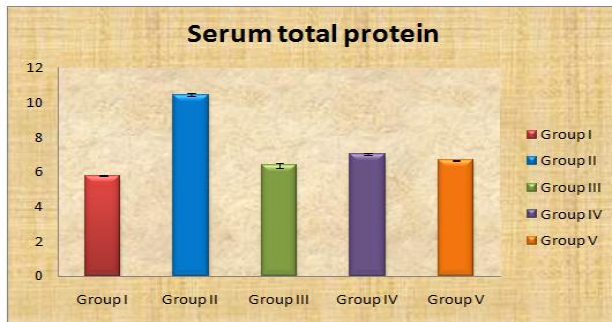


Fig 4: Effect of EEAI on serum total protein levels in cisplatin induced nephrotoxic rats

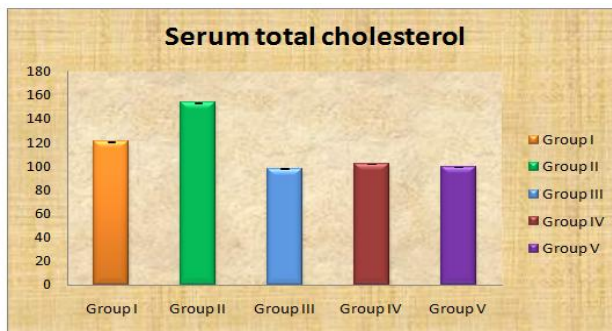


Fig 5: Effect of EEAI on serum total cholesterol levels in cisplatin induced nephrotoxic rats

Histopathological studies of the kidney in cisplatin induced nephrotoxic rats

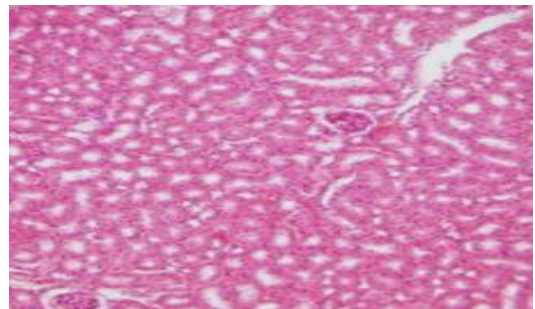


Fig 8: Histopathology of rat kidney in control group (10X)

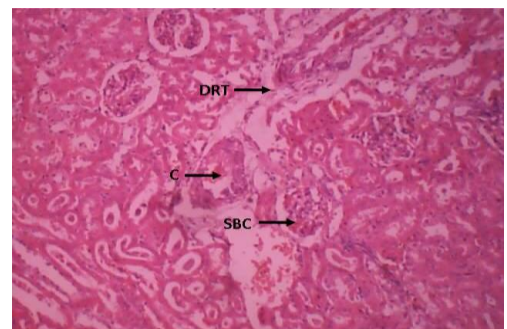


Fig 9: Histopathology of rat kidney in cisplatin (6mg/kg) treated group (10X)

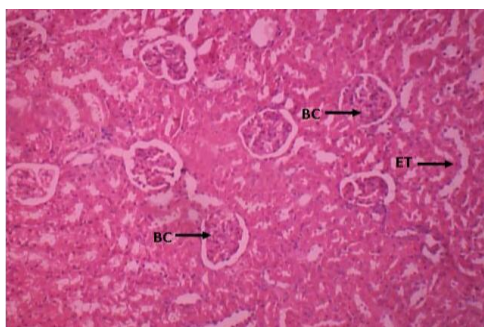


Fig 10: Histopathology of rat kidney in standard (cystone-5ml/kg) treated group (10X)

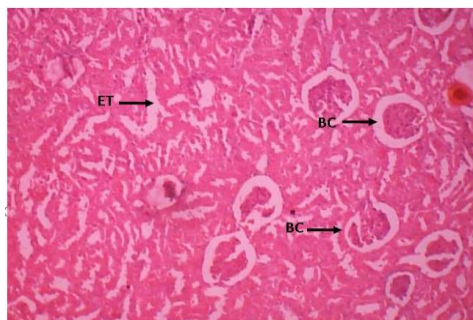


Fig 11: Histopathology of rat kidney in EEAI (200mg/kg) + cisplatin (6mg/kg) treated group (10X)

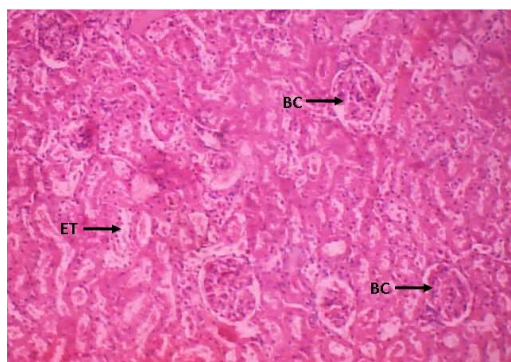


Fig 12: Histopathology of rat kidney in EEAI (400mg/kg) + cisplatin (6mg/kg) treated group (10X)

The administration of Cisplatin to control rats caused significant increase in serum creatinine, blood urea nitrogen, Serum uric acid, serum total proteins, total cholesterol, Serum alkaline phosphatase and serum albumin concentrations. EEAI pretreatment dose-dependently attenuated these cisplatin induced elevations in serum ($p < 0.05$) Histological analysis of the kidneys showed that cisplatin administration caused severe and widespread necrosis with dilatation of proximal tubules, vacuolization, tubular cell desquamation and intraluminal cast formation. Cisplatin induced histopathological renal changes were minimal in animals received EEAI.

CONCLUSION

In this 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 serum creatinine, blood urea nitrogen, uric acid, total proteins, total cholesterol, alkaline phosphatase, and albumin 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.

REFERENCES

1. Ravi R., Ritesh J., Narendra M., Prshant A., Khadbadi S.S. Anti-inflammatory Action of Abutilon indicum (L.) Sweet Leaves by HRBC Membrane Stabilization. *Research J Pharm and Tech.* 2009; 2(2): 415-416.
2. Finley R.S, Fortner C.L, Grove W.R. Cisplatin nephrotoxicity: a summary of preventative interventions. *Drug Intell Clin Pharm.* 1985; 19: 362-367.
3. Adelstein, D.J, Lavertu P, Saxton J.P, Secic M, Wood B.G, Wanamaker J.R, Eliachar I, Strome M, Larto M.A. Mature results of a phase III randomized trial comparing concurrent chemoradiotherapy with radiation therapy alone in patients with stage III and IV squamous cell carcinoma of the head and neck. *Cancer* 2000; 88: 876-883.
4. Jiang M, Wei Q, Pabla N, Dong G, Wang C.Y, Yang T, Smith S.B, Dong Z. Effects of hydroxyl radical scavenging on cisplatin-induced p53 activation, tubular cell apoptosis and nephrotoxicity. *Biochem Pharmacol.* 2007; 73: 1499-1510.
5. Sammia Y, Muhammad A.K, Muhammad N.A, Mushtaq A, Ayesha Mohy-ud-Din. Antioxidant potential and radical scavenging effects of various extracts from Abutilon indicum and Abutilon muticum. *Pharmaceutical Biology* 2010; 48(3): 282-289.
6. Ghaisas MM, Navghare VV, Takawale AR, Zope VS, Phanse MA. Antidiabetic and Nephroprotective effect of Tectonagrandis Linn. In Alloxan induced Diabetes. *Ars Pharm.* 2010; 51(4): 195-206.
7. Golwala D.K, Patel L.D, Vaidyal S.K, Bothara S.B, Munesh M, Patel P. Anticonvulsant activity of Abutilon indicum leaf. *International Journal of Pharmacy and Pharmaceutical Sciences* 2010; 2(1): 66-71.
8. Kakasaheb J. Kore, Dr. Rajkumar V. Shete, Apsari J. Patel. Effect of Abutilon indicum Extract in Gentamicin Induced Nephrotoxicity. *International Journal of Pharmaceutical Research & Development* 2011; 3(7): 73-79.
9. Yasmin S, Kashmiri MA, Asghar MN, Ahmad M, Mohy-ud-Din A., Antioxidant potential and radical scavenging effects of various extracts from Abutilon indicum and Abutilon muticum. *Pharm Biol.* 2010; 48(3): 282-9.
10. Gaiind KN, Chopra KS, Phytochemical investigation of Abutilon indicum; *Planta Med.* 1976; 30(2): 174-88.
11. Alden W.W, Repta, A.J. Exacerbation of cisplatin-induced nephrotoxicity by methionine. *Chem Biol Interact.* 1984; 48: 121-124.
12. Chandrashekar V.M, Nagappa A.N, Channesh T.S. Antidiarrhoeal activity of Abutilon indicum Linn leaf extract in rats. *J Nat Remedies* 2004; 4(10): 12-16.
13. Tripathi P, Chauhan NS, Patel JR., Anti-inflammatory activity of Abutilon indicum extract. *Nat Prod Res.* 2011 Oct 14.
14. Ahmed M, Amin S, Islam M, Takahashi M, Okuyama E, Hossain CF., Analgesic principle from Abutilon indicum. *Pharmazie.* 2000; 55(4): 314-6.
15. Antunes L.M.G, Darin J.D.C, Bianchi, M.L.P, Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. *Pharmacol. Res.* 2001; 43: 145-150.
16. Corcostegui R, Labeaga L, Arteché JK, Orjales A. Protective effect of hidrosmin against cisplatin induced acute nephrotoxicity in rats. *J Pharm Pharmacol.* 1998; 4: 465-467.
17. Murthy R.L.N, Nataraj H.N, Ramachandra Setty S. Nephroprotective activity of Cyanotis Fasciculata var., Fasciculata against cisplatin induced Nephrotoxicity. *IJRP.* 2011; 2(9): 137-142.

18. Bartelink H, Schellens J.H.M, Verheij M. The combined use of radiotherapy and chemotherapy in the treatment of solid tumours. *Eur J Cancer* 2002; 38: 216-222.
19. Matławska I, Sikorska M. Flavonoid compounds in the flowers of *Abutilon indicum* (L.) Sweet (Malvaceae). *Acta Pol Pharm.* 2002;59(3):227-9.
20. Nishanta R, Cory S, Harris, G.H.N. Towers. Antimicrobial Activity of Plants Collected from Serpentine Outcrops in Sri Lanka. *Pharmaceutical Biology* 2002;40(3): 235-244.
21. E. Porchezian, Ansari S.H. Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats. *Phytomedicine: International Journal of Phytotherapy & Phytopharmacology* 2005; 12: 62-64.
22. Yang J, Meyers K, Van der Heide J & Liu RH. Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *J Agri and Food Chem.* 2004; 52: 6787-6973.
23. Yao, X., Panichpisal, K., Kurtzman, N., Nugent, K., Cisplatin nephrotoxicity: a review. *Am. J. Med. Sci.* 2007; 334:115-124.
24. Chutwadee Krisanapun, Seong-Ho Lee, Penchom Peungvicha, Rungravi Tamsiriririkkul, and Seungjoon Baek. Antidiabetic Activities of *Abutilon indicum* (L.) Sweet Are Mediated by Enhancement of Adipocyte Differentiation and Activation of the GLUT1 Promoter. *Evid Based Complement Alternat Med.* 2011; 167- 684.