Research Article

Evaluation of Nephro-protective Effect of Different Fractions of Alcoholic Extract of Root of *Aerva Javanica*

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ABSTRACT

Aerva javanica belonging to family *(*Amaranthaceae) roots and flowers are reported to possess medicinal properties against rheumatism and kidney problems. The present work is to evaluate the nephroprotective effect of *Aerva javanica* by cisplatin induced renal toxicity in adult male albino rats of Sprague Dawley strain. The effect of hexane, ethyl acetate and n-butanol fractions of alcoholic extract were evaluated at 200 mg/kg, 180 mg/kg and 270 mg/kg body weight dose respectively in rats against nephrotoxicity induced by administration of cisplatin through intra peritoneal route. Various serum parameters were studied along with histopathological examination of kidneys in each treatment group. The fractions were compared to the ursolic acid at the dose of 150 mg/kg as a standard drug. Hexane fraction of alcoholic extract was found to have significant nephroprotective activity. The levels of urea, creatinine, albumin and total protein in the serum were normalized after treatment with hexane fraction of alcoholic extract at 200mg/kg body weight dose. The hexane fraction of alcoholic extract at the dose of 200 mg/kg shows similar result as compared to the reference standard ursolic acid. The hexane fraction of alcoholic extract of root of *A. javanica* possesses marked nephroprotective activity and thus can have a promising role in the treatment of acute renal injury.

Keywords: Acute renal failure, *Aerva javanica*, Cisplatin, Nephroprotective, Ursolic acid.

INTRODUCTION

The plant *Aerva javanica* belonging to the family Amaranthaceae is a tall and woolly under shrub found plentiful in rainy season in Bhavnagar district of Gujarat state in India. The drug used as Pasanabheda means which breaks the kidney stone.[1] Roots and flowers of *Aerva javanica* are reported to possess medicinal properties against rheumatism and kidney troubles.[2] It consists of kaempferol, sterol, triterpenes, flavanoids, ß-sitosterol, α-amyrin. palmitic acid, stearic acid, linoleic acid, myristic, oleic acid, palmitoleic acid, aervanone, alkaloids, an acylated iso-rhamnetin glycoside, etc.[3-4]

Cisplatin (*cis* - diamminedichoroplatinium or CDDP) is a potent anticancer drug.[5] The clinical use of cisplatin is often complicated by nephrotoxicity.[6] Cisplatin infusion at a dose of 20-mg/ m^2 over 4 hr: causes an increase in the filtration fraction and decreased glomerular filtration rate.[7] Nephrotoxicity is importantly modulated as a result of biotransformation. Tubular dysfunction has also been demonstrated very early after cisplatin administration.[5-6] There is a continuous search for agent that provide nephroprotection against the renal impairment caused by cisplatin for which allopathy offers no remedial measures. Hence, present study was an attempt to screen the effect of different fractions of alcoholic extract of root of *Aerva javanica* in renal toxicity and compare with ursolic acid as a reference standard drug.

MATERIALS AND METHODS

Plant material

Fresh roots of *A. javanica* collected from Bhavnagar District, Gujarat, India. The authentication of the plant was established and voucher specimen PH/08/002 deposited in the Department of Pharmacognosy and Phytochemistry, KBIPER, Gandhinagar, Gujarat, India. Taxonomist Dr. A.S. Reddy, department of bioscience, S.P. University, V.V. Nagar, Gujarat, India identified this plant. It was shade dried and reduced into coarse powder.

Preparation of fractions

The roots of *Aerva javanica* collected, shade dried. For the preparation of alcoholic extract, the dried plant material extracted by 95% ethanol using soxhlet apparatus. The alcoholic extract root of *Aerva javanica* suspended in distilled water (250 ml) and fractionated successively with n-hexane, toluene, ethyl acetate n-butanol and residue. Remaining aqueous fraction made free from distilled water by evaporation on a water bath. After completion of extraction, the solvent removed by evaporation. Further concentrated in vacuo and evaluated for its therapeutic efficacy.

Animals

Healthy adult male albino rats of Sprague Dawley strain weighing between 200 – 250g aged 60 - 90 days used for the study. The rats were housed two in a cage, maintained in a temperature regulated and humidity controlled environment. The rats were fed with standard food pellets and water.[8] Study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee of K.B.I.P.E.R. with approval no. KBIPER/13/469.

Drug and Chemicals

Cisplatin injection (Fresenius kabi oncology Ltd., Baddi), ursolic acid (Yucca lab, Mumbai), Urea estimation kit, Creatinine estimation kit, Protein estimation kit and Albumin estimation kit were procured from Lab care diagnostics Ltd., Valsad, Gujarat, India.

Effect of different fractions of alcoholic extract of root of *Aerva javanica* in cisplatin-induced renal damage[9]

Group A administered with equivalent volumes of 2 % gum acacia (5 ml/kg) for 15 days orally.

The extent of renal damage was determined by treating group B with cisplatin 5-mg/kg-body weight, single dose intraperitoneally.

Group C, group D and group E treated with hexane, ethyl acetate and n-butanol fractions of alcoholic extract of root of *Aerva javanica* respectively for 10 days orally followed by cisplatin administration.

Group F treated daily with 150 mg/kg body weight of ursolic acid from 6th day for 10 days after cisplatin administration. Cisplatin was administered 5 mg/kg body weight single dose intraperitoneally.

Blood withdrawn and kidney was isolated on the 16th day from all the groups to assess the renal function. Complete protocol given in Table 1**.**

Table 1: Experimental protocol of treatment of different fractions of alcoholic extract of root of *A. javanica* to cisplatin-induced renal toxicity

Group no.	Drug treatment	Route & dose	Duration (in days)	Days of withdrawal of blood & kidney	Purpose
A	Gum acacia (2%)	5 ml/kg p.o.	1st – 15th	16th	Normal Control
B	Cisplatin	5 mg/kg i.p.	1st	16th	Renal toxicity control
C	Cisplatin + hexane fraction	5 mg/kg i.p. + 200 mg/kg p.o.	1st 6th – 15th	16th	Curative effect
D	Cisplatin + ethyl acetate fraction	5 mg /kg i.p. + 180 mg/kg p.o.	1st 6th – 15th	16th	Curative effect
E	Cisplatin + n-butanol fraction	5 mg /kg i.p. + 270 mg/kg p.o.	1st 6th – 15th	16th	Curative effect
F	Cisplatin + Ursolic acid	5 mg /kg i.p. + 150 mg/kg p.o.	1st 6th – 15th	16th	Curative effect reference standard

Table 2: Effect of different fractions of alcoholic extract of root of *Aerva javanica* in cisplatin induced renal damage

Gr.	% change in body weight	Serum urea (mg/dL)	Serum creatinine (mg/dL)	Total protein (gm/dL)	Serum Albumin (gm/dL)
A	02.96 ± 0.51	23.81 ± 1.11	0.64 ± 0.93	7.45 ± 0.67	4.98 ± 0.27
B [a]	-12.45 ± 2.30	41.35 ± 0.91	8.27 ± 0.74	3.80 ± 0.82	3.06 ± 0.43
C [b]	13.18 ± 1.36	23.88 ± 0.96	0.59 ± 0.65	7.40 ± 0.62	4.96 ± 0.71
D [c]	08.30 ± 0.90	34.99 ± 2.06	4.99 ± 0.72	5.65 ± 0.58	4.05 ± 0.52
E [d]	06.39 ± 1.20	38.91 ± 1.23	6.80 ± 0.83	5.21 ± 0.78	3.63 ± 0.42
F [e]	12.74 ± 7.79	25.64 ± 1.25	0.62 ± 0.57	7.39 ± 0.47	4.86 ± 0.53

Values are expressed in the terms of Mean ± S.E.M., P<0.05 (n=6) b and e vs. a

Assessment of renal function of cisplatin induced toxicity in different fractions of alcoholic extract of root of *Aerva javanica*

Body weight

The weight (in grams) of the animals noted on the first and last day of treatment and the percentage change in body weight was calculated.

Blood Urea

Urea concentration in blood estimated by NED Dye method (colorimetric Fix Time test) using Urea (NED) kit.[10]

Serum creatinine

Creatinine level in serum estimated without deproteinisation method using creatinine estimation kit.[11]

Serum total protein

Protein level estimated by colorimetric assay with modified biuret end point method using protein estimation kit.[12]

Serum albumin

Albumin level estimated by BCG method using albumin estimation kit.[13]

Histopathological examination

Two animals from each group sacrificed on the day of blood withdrawal and their kidneys were isolated. It washed with saline and preserved in 10% formaldehyde solution for histopathological studies. The kidney were processed and embedded in paraffin wax. The sections were stained with Hematoxylin and Eosin and observed under light microscope.[14] Photomicrographs of kidney slides were taken.

Statistical analysis

Results are given as mean ± SEM. Data were analyzed using one-way ANOVA followed by Tukey's test to determine the significant difference in hexane, ethyl acetate and n-butanol fractions of alcoholic extract of root of *A. javanica*. The statistical significance of difference was taken as $P < 0.05$.

In-vivo antioxidant studies

Animals sacrificed by cervical dislocation and kidneys were dissected out. The kidneys perfused with an ice-cold saline. The whole kidney was removed, blot-dried, weighed and a 10 % homogenate was prepared with an ice-cold 1.15 % potassium chloride to make a 10 % homogenate using homogenizer (Yamato LSG LH-21, Japan). The homogenate was centrifuged at 3^0C for 30 minutes at 10000 rpm and used for the following estimations.[15]

Tissue protein

1 ml of Liver homogenate was taken and made upto 10 ml with 0.5-M sodium hydroxide. From the above solution, 1 ml was pipetted out. 1ml of 10 % trichloroacetic acid (TCA) was added and centrifuged for 10 minutes at 4^0C at 4000 rpm. The supernatant was discarded and the precipitate was dissolved in 1 ml 0.5 N sodium hydroxide in boiling water bath at 50^0C for 10 minute. Then 4 ml alkaline copper tartarate reagent was added and kept for 10 minute at room temperature. Finally, 0.5 ml Folin's reagent added and absorbance recorded at 540 nm. Blank was performed in the same manner but without homogenate.[16]

Glutathione (GSH)

Proteins were precipitated by 5 % TCA, centrifuged and the supernatant was collected. 0.5 ml supernatant was mixed with 3 ml 0.2 M sodium phosphate buffer pH 8.0 and 0.5 ml 0.6 mM DTNB (5, 5- di thio bis (2-nitro benzoic acid) and incubated for 10 minutes at room temperature. The absorbance of the samples was recorded against the blank at 412 nm in a UV-Visible spectrophotometer and the GSH concentration was calculated from the standard curve.[17]

Lipid peroxidation

0.5 ml of 10 % homogenate was pipetted into centrifuging tube. 2.5 ml (TBA-TCA-BHT) reagent was added and shaken well. The mixture was incubated for 5 minutes. The mixture heated at 80 ^{0}C for 10 min on a boiling water bath and centrifuged the mixture at 2000 rpm for 20 minutes. Absorbance was measured at 532 nm. The difference was used as the TBARS (thiobarbituric acid reactive substance) value.[18]

RESULTS

The roots of *Aerva javanica* were collected (20 kg) from Bhavnagar District, Gujarat. The voucher specimen (PH/08/002) was deposited in the department of pharmacognosy and phytochemistry, KBIPER, Gandhinagar, Gujarat, India. The extractive value of hexane, ethyl acetate and n-butanol fractions of alcoholic extract found to be 0.97 % w/w, 0.44 w/w and 4.31 % w/w respectively.

Blood serum examination

Successive fractions of alcoholic extract of root of *Aerva javanica* (hexane, ethyl acetate and n-butanol fractions) were investigated with reference to ursolic acid for its nephroprotective activity. Ursolic acid and alcoholic extract of root of *A. javanica* showed significant improvement in the % change in body weight, serum urea, serum creatinine, serum total protein and serum albumin levels as compared to cisplatin induced group as mentioned in table 2.

The disease-control group induced with cisplatin (5 mg/kg, i.p.) was showed definite signs of nephrotoxicity on 16th day after cisplatin administration, as compared to the normal control group, as evidenced by significant decrease in % change in body weight, total protein and serum albumin level and elevation in serum urea and serum creatinine as shown in table 2.

Hexane faction of alcoholic extract of root of *A. javanica* and ursolic acid showed significant improvement in the % change in body weight, serum urea, serum creatinine, serum total protein and serum albumin levels as compared to cisplatin induced group as mentioned in table 2.

Effect of different fractions of alcoholic extract of root of *A. javanica* on % change in body weight

Ursolic acid and hexane fraction of alcoholic extract of root of *A. javanica* showed a significant increase in body weight when compared to cisplatin-induced group of rats. Hexane fraction of alcoholic extract of root of *A. javanica* were significantly recovered the cisplatin induced decrease in body weight as shown in figure 1. Hexane faction of alcoholic extract of root of *A. javanica* showed almost similar result as compared to ursolic acid reference standard.

Effect of different fractions of alcoholic extract of root of *A. javanica* on serum urea level and serum creatinine level

On 16th day of study, hexane fraction of alcoholic extract of root of *A. javanica* at the dose of 200 mg/kg body weight and ursolic acid were significantly recovered the cisplatin induced elevation of serum urea and serum

creatinine level as compared to disease control group and other fractions as shown in figure 2 and figure 3 respectively.

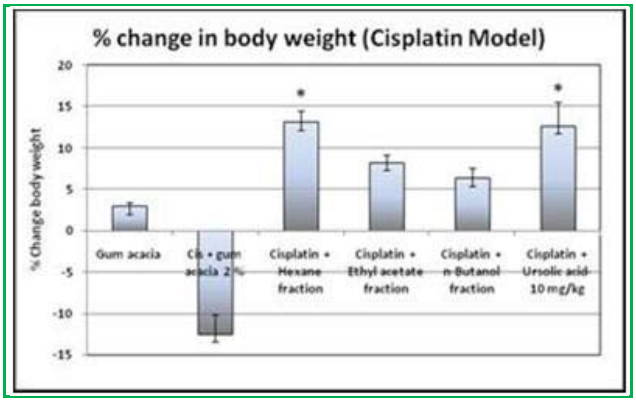

Figure 1: Effect of different fractions of alcoholic extract of root of *Aerva javanica* on % change in body weight in cisplatin induced renal injury

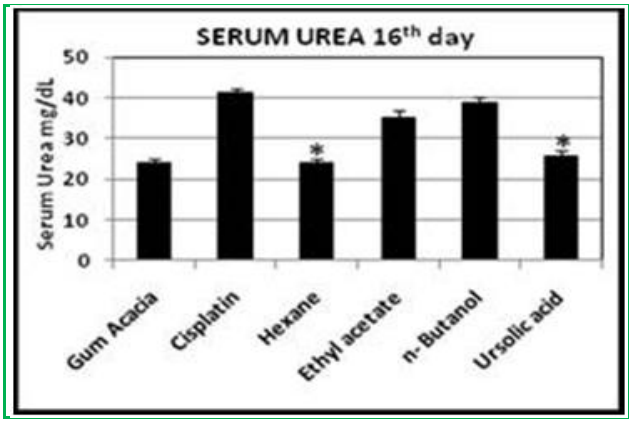

Figure 2: Serum urea level

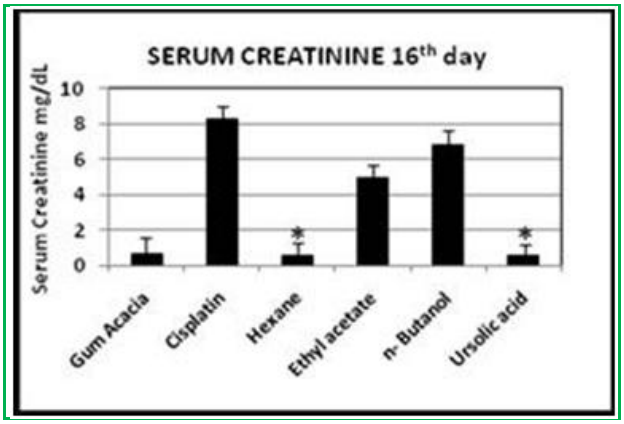

Figure 3: Serum creatinine level

Effect of different fractions of alcoholic extract of root of *A. javanica* on serum total protein level and serum albumin level

On 16th day of study, hexane fraction of alcoholic extract of root of *A. javanica* and ursolic acid were significantly improve the reduced serum total protein level and serum albumin level as compared to disease control group and other fractions as mentioned in figure 4 and figure 5 respectively.

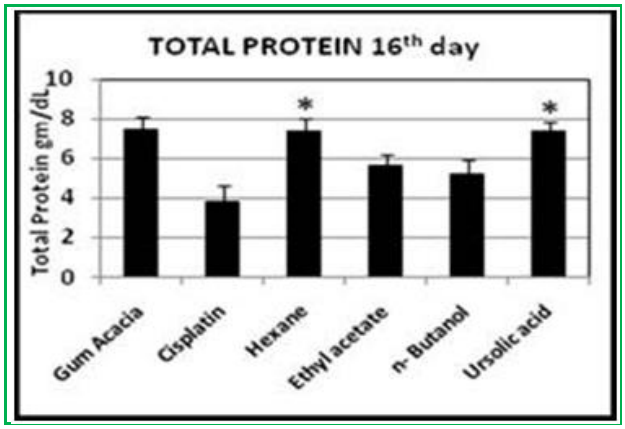

Figure 4: Serum total protein level

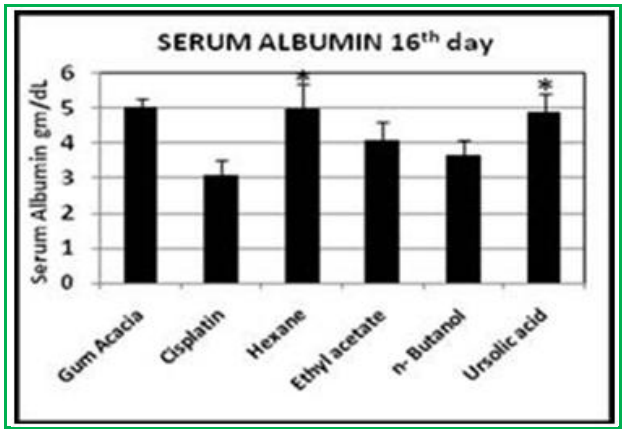

Figure 5: Serum albumin level

Hexane fraction of alcoholic extract of root of *A. javanica* and ursolic acid showed significant improvement in selected biochemical variables indicative of oxidative stress in cisplatin induced renal damage as compared to disease control group as mentioned in table 3.

Table 3: Effect of different fractions of alcoholic extract of root of *Aerva javanica* in few selected biochemical variables indicative of oxidative stress in cisplatin induced renal damage

Groups	Protein mg/ml	GSH µmol/mg Protein	TBARS nM/mg protein
A	189.12 ± 9.87	11.94 ± 0.590	139.69 ± 8.678
B [a]	088.32 ± 7.98	06.45 ± 0.876	458.05 ± 9.695
C [b]	179.93 ± 8.81	09.87 ± 0.965	176.07 ± 7.257
D [c]	137.76 ± 8.96	07.52 ± 0.896	328.65 ± 8.327
E [d]	097.54 ± 9.85	06.89 ± 0.768	389.87 ± 9.256
F [e]	182.02 ± 7.79	10.85 ± 0.675	168.80 ± 8.579

Values are expressed in the terms of Mean ± S.E.M., (n=6) P<0.05 b and e vs. a

Histopathological examination

Histopathology of healthy rat kidney

Histological section of healthy rat kidney showed normal glomeruli figure 6 A1 and tubules mentioned in figure 6 A2.

Histopathology of rat kidney of cisplatin induced group

The presence of glomerular congestion, tubular casts, blood vessel congestion, which are features of acute tubular necrosis, were observed in the histopathological sections of the kidneys in these groups. The sections of cisplatin treated group on the 16th day showed marked congestion of the glomeruli with numerous tubular casts associated with epithelial desquamation. Marked peritubular and blood vessel congestion was observed. The interstitium showed infiltration and congestion. The glomerular congestion and tubular casts suggest that cisplatin induces acute tubular necrosis as shown in figure 6 B1 and figure 6 B2 respectively.

Histopathology of kidney after treatment of ursolic acid

Ursolic acid was showed almost complete normalization of kidney section with few inflammatory cells. Ursolic acid was showed an improvement in histopathological parameters more significantly compared to disease control group as shown in figure 6 C1 and figure 6 C2.

Histopathology of kidney after treatment of different fractions of alcoholic extract of root of Aerva javanica

Hexane fraction of alcoholic extract of root of *A. javanica* showed complete normalization of kidney section with few inflammatory cells as shown in figure 7 He 1 and He 2. However, glomerular as well as peritubular congestion and inflammatory cells were noted in ethyl acetate fraction of alcoholic extract of root of *A. javanica* following cisplatin administration as shown in figure 7 EA 1 and EA 2. Also, histopathological sections showed significant recovery from glomerular congestion and tubular casts in hexane fraction treated group as compared to disease control group. The hexane fraction of alcoholic extract of *A. javanica* showed better results than ethyl acetate fraction of alcoholic extract of *A. javanica* in histopathological examinations.

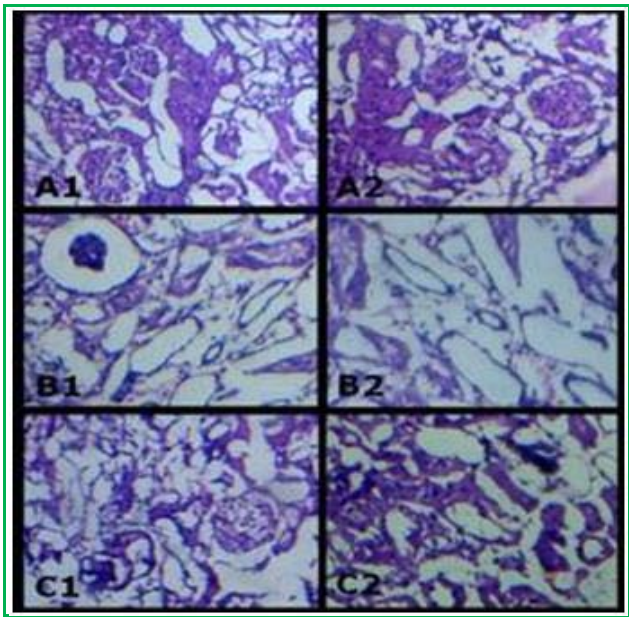

Figure 6: Histopathology in different groups

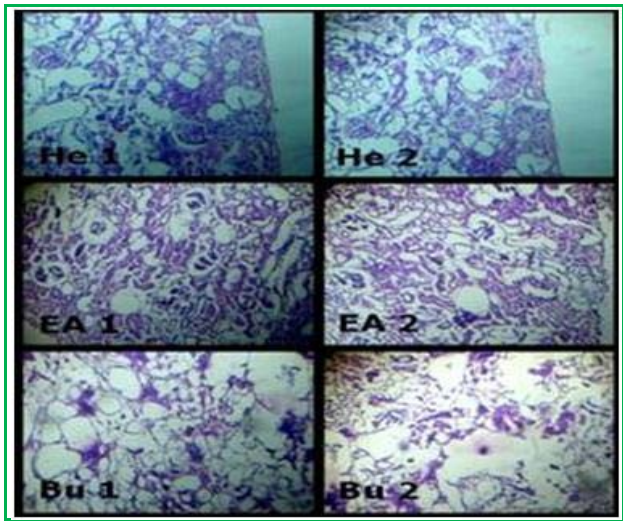

Figure 7: Histopathology in different fractions

From our study, it was observed that cisplatin induced renal injury was evidenced by the elevated biochemical markers (blood urea, serum creatinine, total protein and serum albumin) and was confirmed by the histopathological features of acute tubular necrosis (Figure 6). The hexane fraction of alcoholic extract of root of *Aerva javanica* was showing significant recovery. Based on our histopathological evidence, 200 mg/kg dose of hexane fraction of alcoholic extract of root of *Aerva javanica* showed significant recovery of the kidney tissue as shown in figure 7.

DISCUSSION

The plant *Aerva javanica* belonging to the family Amaranthaceae is used as Pasanabheda means which breaks the kidney stone.[1] Roots are reported to possess medicinal properties against rheumatism and kidney troubles.[2] The nephroprotective activity of alcoholic extract and aqueous extract of root of *Aerva javanica* were separately reported by Suttee A. *et al.*[19] and Movaliya V. *et al.*[20] respectively. In addition, Isolation of ursolic acid from *Aerva javanica* plant was reported by Khan *et al.*[21] Also, comparison of the alcoholic and aqueous extract of root of *Aerva javanica* for its nephroprotective activity with reference standard ursolic acid were reported.[22-In press] The plant *Aerva javanica* was authenticated by macroscopic and microscopic studies as per reported work.[23]

Cisplatin induced renal toxicity study

Relevant perhaps to the nephrotoxicity of cisplatin are the observations that the kidney accumulates and retains platinum largely than other organs. The changes in renal function correlate well with the nephrotoxic effects of cisplatin.[24] Change in creatinine clearance and serum creatinine levels taken as indications of an abnormal glomerular function.[25] Cisplatin caused a marked reduction in the glomerular filtration rate, indicating induction of acute renal failure.[26] Various mechanism proposed for the cisplatin cytotoxicity, Which include direct DNA damage[27], activation of caspase[28] and

mitochondrial dysfunction.[29] The formation of reactive oxygen species(ROS)[30] effects on the endoplasmic reticulum[31] and activation of TNF-α-mediated apoptotic pathways are considered important for cisplatin cytotoxicity. Cisplatin-induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney. There is a large body of evidence on the chemo protective activities of vitamin C, curcumin, selenium, bixin and other dietary components that scavenge free radicals induced by exposure to cisplatin.[32] Cisplatin-induced nephrotoxicity is considered sensitive and significant[33] that showed increase in serum creatinine and urea level and reduced the protein and albumin level on 6th day of the study. The disease-control group showed definite sign of nephrotoxicity, as evidenced by significant decrease in % change in body weight. The reduction in bodyweight may possibly due to the injured renal tubules and the subsequent loss of tubular cells to reabsorb water, leading to dehydration and loss of body weight.[34] The alleviation of cisplatin induced body weight reduction is a reflection of the general palliative effect of hexane fraction of alcoholic extract of root of *Aerva javanica* on the nephrotoxicity. The hexane fraction of alcoholic extract of root of *Aerva javanica* showed almost similar result as compared to ursolic acid as reference standard. Elevation of serum creatinine and serum urea considered as the most important manifestation of severe tubular necrosis of kidney.[35] Cisplatin-induced nephrotoxicity showed decrease serum protein and serum albumin level. Based on results, hexane fraction of alcoholic extract of root of *A. javanica* showed significant recovery in serum protein level as compared to other fractions and disease-control group. Available evidence suggests that cisplatin exerts its nephrotoxic effects by the generation of reactive free radicals.[36-37] Reasonable cellular-protective agents against cisplatin toxicity may have at least some antioxidant properties to prevent GSH depletion and/or scavenge the intracellular ROS. Hence, antioxidants and free radical scavengers of natural and synthetic origin might provide nephroprotection in cisplatin-induced renal injury.[38] Treatment with hexane fraction of alcoholic extract of *Aerva javanica* root decrease the TBARS and increase the antioxidant enzymes levels of GSH and tissue protein as compared to the cisplatin treated group, which indicate its nephroprotective activity. Hence, the significant effect is mainly due to the ability of the hexane fraction of alcoholic extract of root of *Aerva javanica* to restore renal antioxidant defence system as compared to other fractions. To conclude, the hexane fraction of alcoholic extract of root of *A. javanica* possesses marked nephroprotective activity as compared to other fractions and thus can have a promising role in the treatment of acute renal injury induced by cisplatin. Further isolation of active components and its nephroprotective activity in chronic renal failure model need to be evaluate.

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