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COMPARISON OF ALCOHOLIC AND AQUEOUS EXTRACT OF ROOT OF *AERVA JAVANICA* FOR ITS NEPHRO-PROTECTIVE EFFECT IN CISPLATIN-INDUCED RENAL TOXICITY

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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 Wistar strain. The effect of alcoholic extract and aqueous extract were evaluated at 200 mg/kg and 400 mg/kg, 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. Both the extracts were compared to the ursolic acid at the dose of 150 mg/kg as a standard drug. 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 alcoholic extract at both the doses. The alcoholic extract at the dose of 400 mg/kg shows similar result as compared to the reference standard ursolic acid. The alcoholic extract of *A. javanica* possesses marked nephroprotective activity and thus can have a promising role in the treatment of acute renal injury.

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 is used as Pasanabheda means one 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].

Cisplatin (*cis* - diamminedichoroplatinium or CDDP) is a potent anticancer drug [4]. The clinical use of cisplatin is often complicated by nephrotoxicity [5]. 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 [6]. Nephrotoxicity is importantly modulated as a result of biotransformation. Tubular dysfunction has also been demonstrated very early after cisplatin administration [6].

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There is a continuous search for agents which 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 alcoholic and aqueous extracts of *Aerva javanica* in renal toxicity and also compare with ursolic acid as a reference standard drug.

MATERIALS AND METHODS:

Plant material: Fresh roots of *A. javanica* were 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. Identification of this plant was done by taxonomist Dr. A.S. Reddy, department of bioscience, S.P. University, V.V. Nagar, Gujarat, India. It was shade dried and reduced into coarse powder.

Preparation of extracts: The roots of *Aerva javanica* were collected, shade dried. For the preparation of alcoholic extract the dried plant material was extracted by 95% ethanol using soxhlet apparatus. For the preparation of aqueous extract the dried plant material was extracted with distilled water by maceration process for 7 days. After completion of extraction, the solvent was removed by evaporation and 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 were 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. 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.

Acute toxicity study: Oral acute toxicity studies were carried out with Sprague Dawley rat, weighing 200-250 g, with 2 rats per dose group. The extracts were administered in a staircase method [7]. The rats were fed with alcoholic and aqueous extract of root of *A. javanica* suspended in 2 % gum acacia at dose 2000 mg/kg body weight. The animals were observed continuously for 2 hours for the gross behavioral changes and then intermittently once in every 2 hours and finally at the end of 24 and up to 72 hours to observe for any signs of toxicity including mortality.

Effect of alcoholic and aqueous extract of root of *Aerva javanica* in cisplatin-induced renal damage:

Cisplatin induced renal damage: [7] Nine groups of six rats were used. Ist group treated with 2% gum acacia (5ml/kg) for 15 days orally. Groups V to Group IX were treated with cisplatin single dose 5 mg/kg body weight. After 6 days IIrd group was treated with gum acacia. Group III and IV were treated with 400 mg/kg body weight of alcoholic and aqueous extract of root of *Aerva javanica* for 10 days respectively to check any side effect.

Curative group: Groups V and VI after initial treatment of cisplatin, 200, 400 mg/kg body weight alcoholic extract was administered respectively. Groups VIII and IX after initial treatment of cisplatin, 200, 400 mg/kg body weight aqueous extract was administered respectively.

Reference standard group: Group VII was treated daily with 150 mg/kg body weight of ursolic acid from 6th day for 10 days orally after cisplatin administration. Cisplatin was administered 5mg/kg body weight single dose intraperitoneally. The blood was withdrawn on the 16th day to assess renal function. Complete protocol was mentioned in **table 1**.

TABLE 1: EXPERIMENTAL PROTOCOL FOR EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACT OF ROOT OF *AERVA JAVANICA* IN CISPLATIN-INDUCED RENAL DAMAGE:

Group no.	Drug treatment	Route and dose	Duration (in days)	Days of withdrawal of blood and kidney	Purpose
I	Gum acacia (2%)	5 ml/kg p.o.	$1^{st} – 15^{th}$	16^{th}	Vehicle Control
II	Cisplatin + Gum acacia	5 mg/kg i.p. (single dose) equivalent volume	1^{st} $6^{th} – 15^{th}$	16^{th}	Induce kidney damage & to check regeneration
III	Alcoholic Extract	400 mg/kg p.o.	$1^{th} – 10^{th}$	11^{th}	Normal Control
IV	Aqueous Extract	400 mg/kg p.o.	$1^{th} – 10^{th}$	11^{th}	Normal Control
V	Cisplatin + AL Extract	5 mg/kg i.p.(single dose) + 200 mg/kg p.o.	1^{st} $6^{th} – 15^{th}$	16^{th}	Curative effect
VI	Cisplatin + AL Extract	5 mg /kg i.p.(single dose) + 400 mg/kg p.o.	1^{st} $6^{th} – 15^{th}$	16^{th}	Curative effect
VII	Cisplatin + Ursolic acid	5 mg /kg i.p.(single dose) + 150 mg/kg p.o.	1^{st} $6^{th} – 15^{th}$	16^{th}	Reference standard
VIII	Cisplatin + AQ Extract	5 mg/kg i.p.(single dose) + 200 mg/kg p.o.	1^{st} $6^{th} – 15^{th}$	16^{th}	Curative effect
IX	Cisplatin + AQ Extract	5 mg /kg i.p.(single dose) + 400 mg/kg p.o.	1^{st} $6^{th} – 15^{th}$	16^{th}	Curative effect

The dose of the extract was calculated as 1/10th of the maximum tolerated dose (2000 mg/kg body weight).

Assessment of renal function of cisplatin induced toxicity in alcoholic and aqueous extract of root of *Aerva javanica*:

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

2. **Blood Urea:** Urea concentration in blood was estimated by NED Dye method (colorimetric Fix Time test) using Urea (NED) kit [8].

3. **Serum creatinine:** Creatinine level in serum was estimated without deproteinisation method using creatinine estimation kit [9].

4. **Serum total protein:** Protein level was estimated by colorimetric assay with modified biuret end point method using protein estimation kit [10].

5. **Serum albumin:** Albumin level was estimated by BCG method using albumin estimation kit [11].

6. **Histopathological examination:** Two animals from each group were sacrificed on the day of blood withdrawal and their kidneys were isolated. It was 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 [12]. Photomicrographs of kidney slides were taken.

Statistical analysis: Results are given as mean ± SEM. Data were analyzed using one-way ANOVA followed by post hoc Sheffe's test using SPSS computer software version 7.5. The statistical significance of difference was taken as $P < 0.05$ in case of alcoholic and aqueous extract of root of *Aerva javanica*.

***In-vivo* antioxidant studies:** Animals were sacrificed by cervical dislocation and kidneys were dissected out. The kidneys were 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 [13].

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 was added and absorbance was recorded at 540 nm. Blank was performed in the same manner but without homogenate [14].

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 [14].

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 was heated at 80^0C 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 [14].

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 alcoholic extract and aqueous extract were found to be 9.3 % w/w and 7.0 % w/w respectively.

Acute toxicity study: 2000 mg/kg of alcoholic and aqueous extract of root of *Aerva javanica* were found to be safe in rats. Based on acute toxicity study 200 mg/kg and 400 mg/kg dose were selected.

Blood serum examination: 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**.

TABLE 2: EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACTS OF *AERVA JAVANICA* IN CISPLATIN INDUCED RENAL DAMAGE

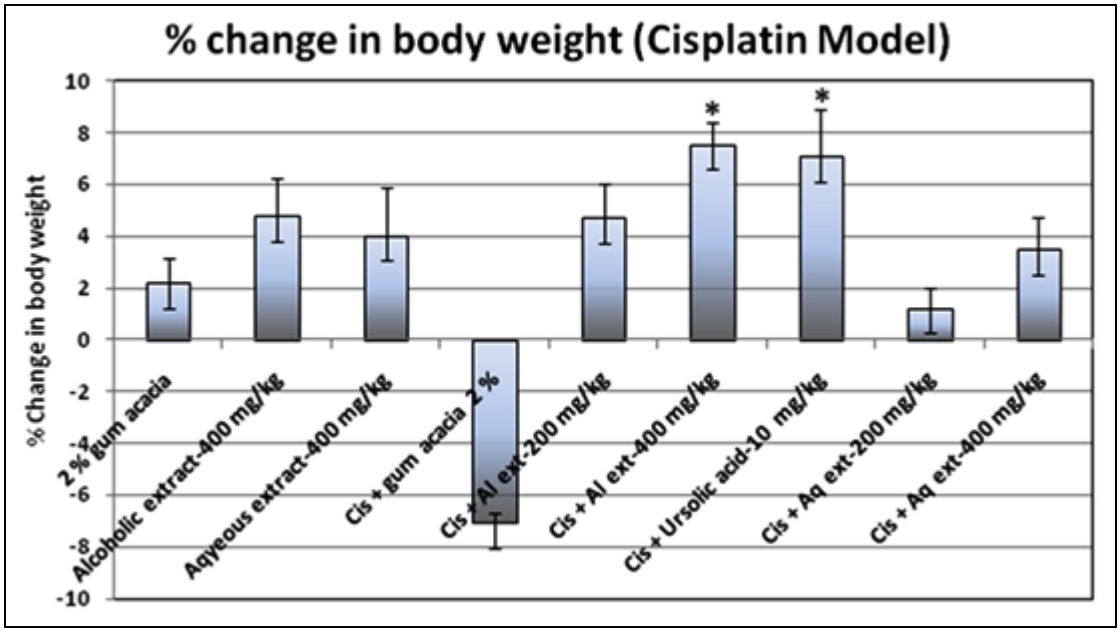
Group	% change in body weight	Serum urea (mg/dL)	Serum creatinine (mg/dL)	Total protein (gm/dL)	Serum Albumin (gm/dL)
1	02.31 ± 0.89	038.68 ± 19.54	0.70 ± 0.52	6.60 ± 0.25	4.05 ± 0.20
2	04.79 ± 1.40	037.94 ± 18.46	0.65 ± 0.63	6.65 ± 0.15	4.15 ± 0.25
3	04.15 ± 1.75	038.12 ± 16.05	0.65 ± 0.45	6.70 ± 0.35	4.10 ± 0.30
4 [a]	-07.10 ± 0.54	337.40 ± 16.78	5.30 ± 0.42	4.25 ± 0.41	2.45 ± 0.25
5 [b]	04.62 ± 1.83	063.50 ± 10.48	1.25 ± 0.58	6.45 ± 0.32	3.50 ± 0.45
6 [c]	07.85 ± 0.76	039.80 ± 18.65	0.60 ± 0.67	7.15 ± 0.36	3.80 ± 0.35
7 [d]	07.20 ± 1.81	041.20 ± 17.02	0.65 ± 0.45	6.75 ± 0.35	3.90 ± 0.31
8 [e]	01.51 ± 0.68	190.60 ± 19.13	3.45 ± 0.51	4.65 ± 0.10	2.75 ± 0.50
9 [f]	03.48 ± 1.31	146.50 ± 12.34	2.70 ± 0.57	5.70 ± 0.15	3.20 ± 0.25

Values are expressed in terms of Mean ± S.E.M., $p<0.05$ b, c and d V/S a in each parameter

Effect of alcoholic and aqueous extract of root of *Aerva javanica* on % change in body weight: The curative regimen, alcoholic extract of root of *A. javanica* at the dose 400 mg/kg body weight was significantly recovered the cisplatin induced decrease in body weight as shown in **graph 1**.

Alcoholic extract of root of *Aerva javanica* at the dose of 400 mg/kg body weight showed better result as compared to aqueous extract.

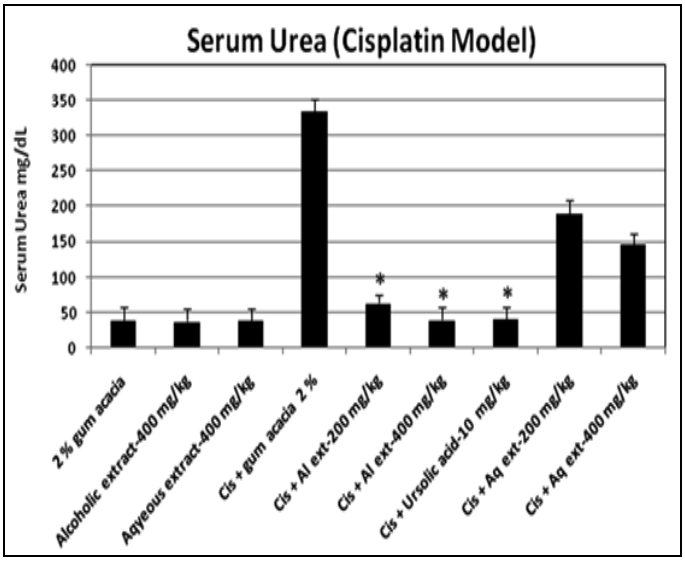
The alcoholic extract of root of *Aerva javanica* at the dose of 400 mg/kg bodyweight showed almost similar result as compared to ursolic acid reference standard.



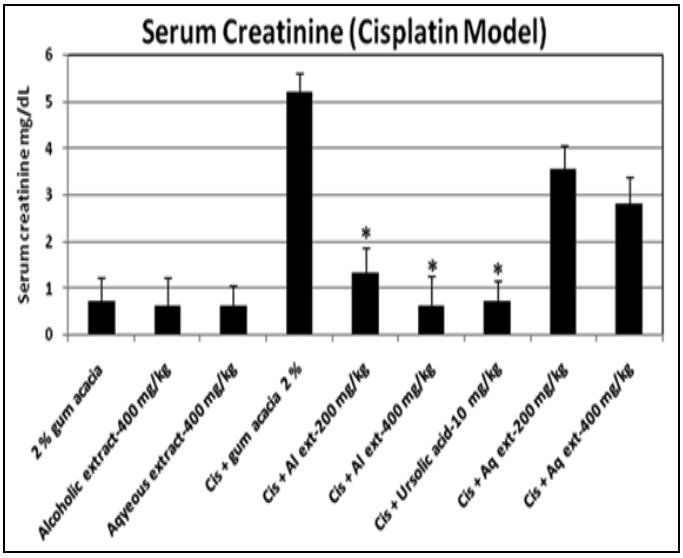
GRAPH 1: EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACT OF ROOT OF *AERVA JAVANICA* ON % CHANGE IN BODY WEIGHT IN CISPLATIN INDUCED RENAL INJURY

Effect of alcoholic and aqueous extract of root of *Aerva javanica* on serum urea level: The curative regimen, alcoholic extract of root of *A. javanica* at the both dose level were significantly recovered the cisplatin induced elevation of serum urea as compared to aqueous extract as shown in **graph 2**. Alcoholic extract of root of *A. javanica* at the dose of 400 mg/kg body weight showed almost similar result as compared to ursolic acid reference standard.

Effect of alcoholic and aqueous extract of root of *Aerva javanica* on serum creatinine level: The curative regimen, alcoholic extract of root of *A. javanica* at the both dose level were significantly recovered the cisplatin induced elevation of serum creatinine as compared to aqueous extract as shown in **graph 3**. Alcoholic extract of root of *A. javanica* at the dose of 400 mg/kg body weight showed almost similar result as compared to ursolic acid reference standard.

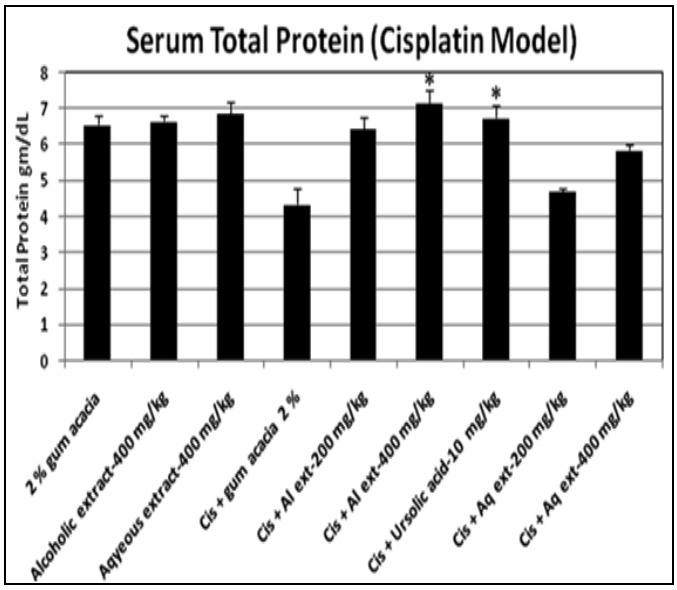


GRAPH 2: EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACT OF ROOT OF *AERVA JAVANICA* ON SERUM UREA LEVEL IN CISPLATIN INDUCED RENAL INJURY



GRAPH 3: EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACT OF ROOT OF *AERVA JAVANICA* ON SERUM CREATININE LEVEL IN CISPLATIN INDUCED RENAL INJURY

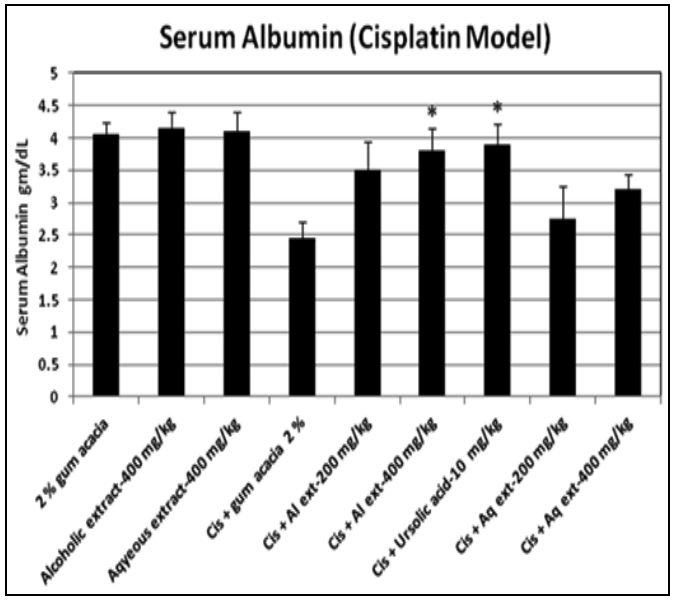
Effect of alcoholic and aqueous extract of root of *A. javanica* on serum total protein level: The curative regimen, alcoholic extract of root of *A. javanica* at the dose of 400 mg/kg body was significantly recovered the cisplatin induced reduction of serum total protein level as compared to aqueous extract as shown in **graph 4**. Alcoholic extract of root of *A. javanica* at the dose of 400 mg/kg body weight showed better result as compared to ursolic acid reference standard.



GRAPH 4: EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACT OF ROOT OF *AERVA JAVANICA* ON SERUM TOTAL PROTEIN LEVEL IN CISPLATIN INDUCED RENAL INJURY

Effect of alcoholic and aqueous extract of root of *Aerva javanica* on serum albumin level: The curative regimen, alcoholic extract of root of *A. javanica* at the dose of 400 mg/kg body was significantly recovered the cisplatin induced reduction of serum albumin level as compared to aqueous extract as shown in **graph 5**.

Alcoholic extract of root of *A. javanica* at the dose of 400 mg/kg body weight showed better result as compared to ursolic acid as a reference standard.



GRAPH 5: EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACT OF ROOT OF *AERVA JAVANICA* ON SERUM ALBUMIN LEVEL IN CISPLATIN INDUCED RENAL INJURY

Administration of cisplatin caused a significant decrease in tissue protein levels and GSH level in disease control group when compared to vehicle control group. Also there was a significant elevation of lipid peroxides in disease control group compared to vehicle control group.

Alcoholic extract of root of *A. javanica* and ursolic acid showed significant improvement as compared to aqueous extract of root of *Aerva javanica* in selected biochemical variables indicative of oxidative stress in cisplatin induced renal damage as mentioned in **table 3**.

TABLE 3: EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACTS 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
1	198.15 ± 4.65	11.95 ± 0.36	150.62 ± 12.83
2	210.62 ± 8.35	12.45 ± 0.354	143.37 ± 13.92
3	190.38 ± 9.43	12.65 ± 0.789	144.15 ± 14.33
4 [a]	090.45 ± 5.87	06.25 ± 0.675	475.24 ± 10.73
5 [b]	140.61 ± 8.75	09.12 ± 0.703	264.12 ± 12.67
6 [c]	178.25 ± 3.76	10.96 ± 0.459	185.67 ± 17.78
7 [d]	182.75 ± 5.98	11.57 ± 0.927	168.25 ± 15.54
8 [e]	109.16 ± 8.81	06.76 ± 0.903	378.53 ± 10.57
9 [f]	123.35 ± 3.54	07.65 ± 0.762	324.76 ± 11.42

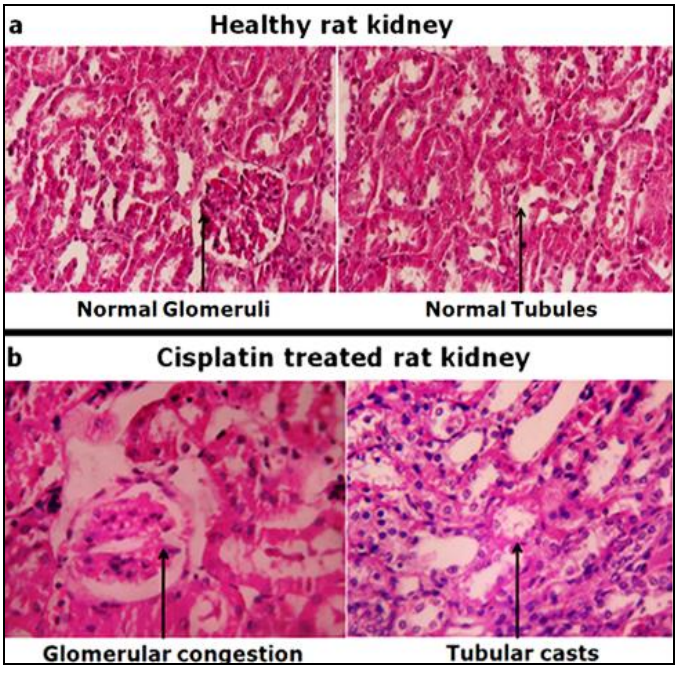
Values are expressed in the terms of Mean ± S.E.M., P<0.05 b, c and d vs. a

Histopathological examination of root of *Aerva javanica* in cisplatin induced renal damage:

- **Histopathology of healthy rat kidney:** Histological section of healthy rat kidney showed normal glomeruli and tubules mentioned in **figure 1a**.

- **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 cisplatin induced groups. The sections of cisplatin treated rat kidney of disease control group on the 16th day were showed marked congestion of the glomeruli with numerous tubular casts associated with epithelial desquamation. Marked peritubular and blood vessel congestion was observed. The interstitium was showed infiltration and congestion. These features suggest that cisplatin induces acute tubular necrosis as shown in **figure 1b.**

- **Histopathology of kidney after treatment of ursolic acid:** Ursolic acid showed almost complete normalization of kidney section with few inflammatory cells. Ursolic acid showed an improvement in histopathological parameters more significantly compared to disease control group shown in **figure 1c**.



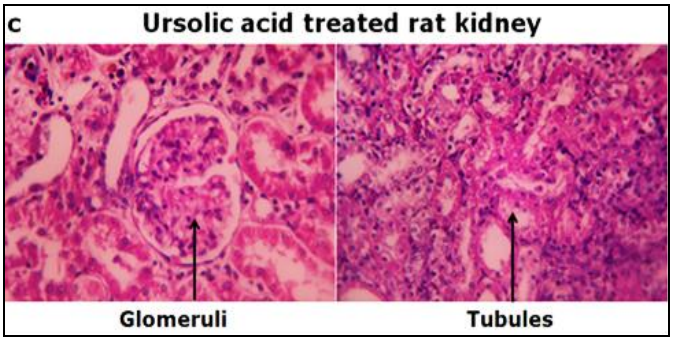


FIGURE 1: HISTOPATHOLOGY OF RAT KIDNEY IN DIFFERENT GROUP. (A) HEALTHY RAT KIDNEY; (B) CISPLATIN TREATED RAT KIDNEY; (C) URSOLIC ACID TREATED RAT KIDNEY

- **Histopathology of kidney after treatment of alcoholic and aqueous extract of root of *Aerva javanica*:** Alcoholic extract of root of *A. javanica* showed a dose dependent improvement in histopathological parameters more significantly compared to 400 mg/kg body weight of aqueous extract. Alcoholic extract of root of *A. javanica* at dose of 400 mg/kg body weight showed complete normalization of kidney section with few inflammatory cells. However, mild glomerular as well as peritubular congestion and inflammatory cells were noted in curative group of 200 mg/kg body weight of alcoholic extract and aqueous extract of root of *Aerva javanica* following cisplatin administration as shown in figure 2 and figure 3.

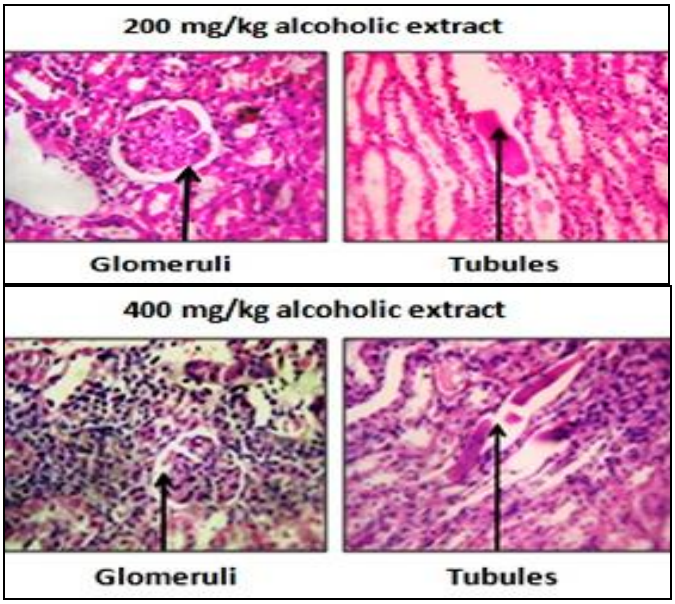


FIGURE 2: HISTOPATHOLOGY OF KIDNEY AFTER TREATMENT OF ALCOHOLIC EXTRACT OF ROOT OF *AERVA JAVANICA* IN CISPLATIN INDUCED RENAL INJURY

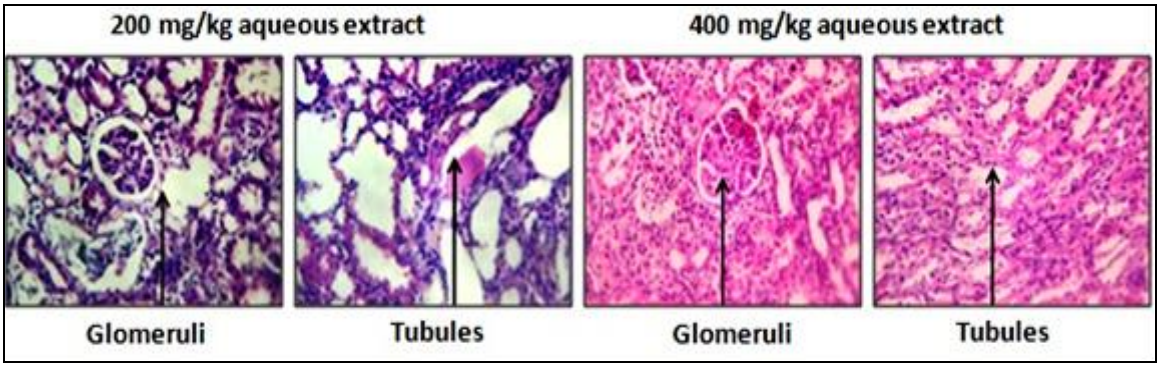


FIGURE 3: HISTOPATHOLOGY OF KIDNEY AFTER TREATMENT OF AQUEOUS EXTRACT OF ROOT OF *AERVA JAVANICA* IN CISPLATIN INDUCED RENAL INJURY

TABLE 3: EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACT OF *AERVA JAVANICA* IN HISTOPATHOLOGICAL FEATURES AS SEEN IN THE KIDNEY BY CISPLATIN MODEL

Histopathological features	GP-I	GP-II	GP-III	GP-IV	GP-V	GP-VI	GP-VII	GP-VIII	GP-IX
Glomerular congestion	-	-	-	+++	-	-	-	+	-
Tubular Casts	-	-	-	+++	+	-	-	+	+

- Absent, + present, ++ more, +++ most

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 1).

The alcoholic extract of root of *Aerva javanica*, at both dose levels was showing recovery. Based on our histopathological evidence, 400 mg/kg dose of alcoholic extract of root of *Aerva javanica* showed significant recovery of the kidney tissue as shown in figure 2 and table 3.

DISCUSSION: The plant *Aerva javanica* belonging to the family Amaranthaceae is used as Pasanabheda means one 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.* [13] and Movaliya V. *et al.* [14] respectively. In addition, Isolation of ursolic acid from *Aerva javanica* plant was reported by Khan *et al* [15].

Based on these literature data we compared the alcoholic and aqueous extract of root of *Aerva javanica* for its nephroprotective activity with reference standard ursolic acid.

The plant *Aerva javanica* was authenticated by macroscopic and microscopic studies as per reported work [16].

Acute toxicity study: Acute systemic toxicity was assessed by the administration of a single dose of compound, typically to rats and mice, orally, dermally or by inhalation. For pharmaceuticals, the main aims of these studies were to determine the nature (including delayed toxicity) and duration of any acute toxic response. They also determine the maximum non-lethal dose and provide preliminary information relevant to single exposure or over-dosage in humans [17].

Oral administration of the alcoholic and aqueous extracts of root of *Aerva javanica* up to 2 g/kg body weight in rats were produced no toxic effects and mortality even after 24 hr and observed up to 72 hr.

Cisplatin induced renal toxicity study: Acute renal failure can occur with a single dose of cisplatin and repeated exposure causes chronic renal failure.

Relevant perhaps to the nephrotoxicity of cisplatin are the observations that the kidney accumulates and retains platinum to a greater extent than other organs. The renal accumulation of platinum *in-vivo* is a rapid process which is completed 1 to 6 h after its administration.

Twenty four hours after administration of cisplatin at a dose of 5 mg/kg body weight to rats, its kidney contains nearly 1 % of the administered dose and concentrates platinum eightfold above the plasma concentration. Platinum can be found in many sub cellular sites with most of it present in the cytosolic compartment.

The changes in renal function observed in the rat system correlate well with the nephrotoxic effects of cisplatin [18]. Alterations in values of creatinine clearance and serum creatinine levels observed in treatment with cisplatin are taken as indications of an abnormal glomerular function [19]. Single injection of cisplatin at a dose of 5 mg/kg body weight in rats caused a marked reduction in the glomerular filtration rate, which was accompanied by an increase in the serum creatinine level, serum urea and decrease serum protein and serum albumin level indicating induction of acute renal failure. A single dose of cisplatin induced a significant increase in serum creatinine level within three to seven days after its administration [20].

Cisplatin causes functional alterations including inhibition of protein synthesis, reduced glutathione (GSH) depletion, lipid peroxidation and mitochondrial damage. Several distinct mechanisms have been proposed for cisplatin cytotoxicity in renal tubule cells, including direct DNA damage, activation of caspase, mitochondrial dysfunction, formation of reactive oxygen species (ROS), effects on the endoplasmic reticulum and activation of TNF-α-mediated apoptotic pathways [21].

In addition, it has been reported that cisplatin-induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney. In addition, cisplatin has been found to lower the activities of antioxidant enzymes and to induce depletion of GSH.

Much attention has been given to the possible role of dietary antioxidants in protecting the kidney against cisplatin-induced nephrotoxicity. There is a large body of evidence on the chemo protective activities of vitamin C, E, curcumin, selenium, bixin and other dietary components that scavenge free radicals induced by exposure to cisplatin [22].

Cisplatin-induced nephrotoxicity is considered sensitive and significant [23] 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 [24]. Treatment with alcoholic extract of root of *Aerva javanica* antagonized this reduction significantly as compared to aqueous extract. This alleviation of cisplatin induced body weight reduction is a reflection of the general palliative effect of alcoholic extract of root of *Aerva javanica* on the nephrotoxicity. The 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 has been considered as the most important manifestation of severe tubular necrosis of kidney [25]. Cisplatin-induced nephrotoxicity showed decrease serum protein and serum albumin level.

Treatment with aqueous extract and alcoholic extract of root of *Aerva javanica*, observed significant recovery in alcoholic extract in elevated serum urea level as compared to aqueous extract of root of *A. javanica.*

Based on results, alcoholic extract of root of *A. javanica* showed significant recovery in serum protein level as compared to aqueous extract and disease-control group. Also alcoholic extract of root of *A. javanica* showed almost similar results as compared to ursolic acid reference standard.

Available evidence suggests that cisplatin exerts its nephrotoxic effects by the generation of reactive free radicals [26-27]. 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 [28].

Treatment with alcoholic and aqueous extracts 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 possible mechanism of nephroprotection of *Aerva javanica* against cisplatin induced renal damage may be attributed to its antioxidant and free radical scavenging properties.

The significant effect is mainly due to the ability of the alcoholic extract of root of *Aerva javanica* to restore renal antioxidant defence system as compared to aqueous extract.

To conclude, the alcoholic extract of root of *A. javanica* possesses marked nephroprotective activity as compared to aqueous extract 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 evaluated.

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