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NEPHROPROTECTIVE ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *PHYSALIS ALKEKENGI* (SOLANACEAE) FRUIT AGAINST CISPLATIN INDUCED NEPHROTOXICITY IN RATS

Aslam M[a], Siddiqui WA*[b], Sabahatullah[c], Javed K[d], Khan AH[e]

[a]Department of Ilmul Advia, Faculty of Medicine(U), Jamia Hamdard, New Delhi 110062, India.

[b]Department of Chemistry, Faculty of Science, Jamia Hamdard, New Delhi 110062, India.

[c]Department of Ilmul Advia, Faculty of Medicine(U), Jamia Hamdard, New Delhi 110062, India.

[d]Department of Biochemistry, Faculty of Science, Jamia Hamdard, New Delhi 110062, India.

[e]Department of Tashreeh and Munafeul-Aza, Faculty of Medicine(U), Jamia Hamdard, New Delhi 110062, India.

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***Correspondence for Author**
Siddiqui WA
Department of Chemistry, Faculty of Science, Jamia Hamdard, New Delhi 110062, India.

ABSTRACT

Physalis alkekengi (L.) (Unani name: *Kaknaj*) Fam. *Solanaceae* has been used in Unani system of medicine for long time to treat various kidney disorders. In this study, hydroalcoholic extract of *Physalis alkekengi* (PAHE) was studied for its nephroprotective activity against cisplatin induced acute renal injury in albino rats of either sex. In the experimental regimen, the animals were administered with PAHE (p.o.) at dose levels of 420mg/kg (equivalent to 3 gm of the traditional therapeutic crude dose), and 980 mg/kg (equivalent to 7g) for 10 days. Cisplatin (7mg/kg, i.p.) was administered in a single dose on 4th day of the experiment. The results showed significant ($p<0.01$) reduction in the elevated blood urea, serum creatinine, uric acid, TBARS level and also normalized the histopathological changes. However, the results were comparatively better at 420mg/kg dose level. The findings suggest that the PAHE possesses marked nephroprotective activity with minimal toxicity and could offer a promising role in the treatment of acute renal injury caused by nephrotoxins like cisplatin.

KEYWORDS: *Physalis alkekengi*; Cisplatin; Nephrotoxicity.

1. INTRODUCTION

Physalis alkekengi (*Kaknaj*) belongs to the family Solanaceae. It is a perennial herb and considered to be a native of the region extending from Japan to South East Europe. Fruits are available in Indian city markets (Nadkarni., 1989). Many wild and domestic solanecious species have been associated with human culture from antiquity, as medicinal, ritual or magical herbs and/ or food crops in the world (Daunay et al., 2007). The dried ripe fruits of *Kaknaj* are widely used in various ailments of kidney and other organs since the ancient period. In Unani system of medicine it has been described as *Mudir-e-Bol* (Diuretic), *Mufatit-e-Hissat* (Lithotriptic), *Mohallil-e-warm* (Anti-inflammatory) and *Qatil-e-kirmeshikam* (Anthelmintic) (Hakim., 1311; Ghani., 1920; Kabiruddin., 1951; Razi., 2002). It is widely used in *Sang-e-gurda wa masana* (Renal & vesicular calculi), *Qurooh-e-gurda wa masana* (Ulcers of kidney and bladder), *Warm-e-gurda wa masana* (Nephritis & Cystitis), *Yarqan* (Jaundice), *Warm-e-Jigar* (Hepatitis), *Niqras* (Gout) and various other ailments (Ali., 1301; Hakim., 1311; Ghani., 1920; Sina I., 1927; Arzani., 1952; Razi., 2002).

Cisplatin is used as anti-cancerous drug. The cytotoxic effects of cisplatin are found to occur via several mechanisms, including inhibition of protein synthesis, DNA damage and mitochondrial injury which lead ultimately to the activation of apoptotic cell death in both tumor cells and renal tubular cells (Huang et al., 1995; Ueda et al., 2000). The present work consists of the evaluation of efficacy of *Physalis alkekengi* (PA) in two different doses for its nephroprotective effects against cisplatin-induced nephrotoxicity.

2. METHODS AND MATERIALS

2.1 *Plant Material*

Kaknaj (dried fruits of *Physalis alkekengi*) were purchased from Shamsi Dawakhana, Kucha Cheylan Delhi-110006. The identity of the purchased drug was established as fruit of *Physalis alkekengi* by the scientist working at NISCAIR (National Institute of Science Communication and Information Resources), Dr. K.S. Krishnan Marg, Pusa Gate, New Delhi, 110012.

2.2 *Preparation of extract*

The crushed dried fruits of PA were extracted by refluxing with aqueous Methanol (Methanol: Water, 80:20 V/V) in distillation flask over boiling water bath for 3 hrs. It was removed from the water bath and allowed to cool at room temperature and filtered. The plant material obtained after filtration was re-extracted twice by the same procedure. All three

extracts were podded together (Hydroalcoholic extracts). Solvent was recovered by distillation method under reduced pressure. The dark brown residue left after removal of the solvent was coded as hydroalcoholic extract of *Physalis alkekengi* (PAHE). The yield of PAHE was 21.2% w/w in the terms of starting materials.

2.3 Experimental Animal

All the experiments were carried out on Wistar strain (150-250 gm) of either sex, supplied by the Central Animal House facility of Jamia Hamdard, New Delhi (Registration no. 173/CPSEA). All animals were housed in groups in polypropylene cage and maintained on a standard pellet diet (Amrut Laboratory rat and mice feed, New Maharashtra chakan oil mills Ltd., Mumbai) and water *ad libitum*. The animals were kept under standard laboratory conditions at 25 ± 1^0C temperature.

The experiments were performed in accordance with the guidelines for the care and use of laboratory animals, laid down by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India, Jan. 2000. In all experiments 1% CMC in water was used as vehicle and given in volume of 10 ml/kg. All the treatments were given in the form of suspension in the vehicle and given in volume of 10 ml/kg. The animals were anaesthetized with ether and sacrificed.

2.4 *Treatment Regimen*

The animals were assigned to 4 different groups of 6 animals each (n = 6). The first group was named as control and was given normal saline only. The second group was served as toxicant and was given cisplatin 7 mg/kg body weight. The 3rd and 4th groups were given PAHE in the doses of 420mg/kg (equivalent to 3 gm of the traditional therapeutic crude dose), and 980 mg/kg (equivalent to 7g) for 10 days. On 11th day, before collecting the blood samples, the animals were anaesthetized and sacrificed. The blood samples were collected from retro-orbital plexus and left at room temperature for two hrs. The blood samples were centrifuged for 30 minuets at 5000 rpm to separate the serum from coagulated blood.

2.5 *Biochemical Analysis*

The following parameters were assessed in this study

- **Blood Urea Nitrogen (BUN):** Blood urea level was estimated by the urease enzymatic method and the absorbance were measured at 520 nm (Godkar., 1994) using UV -VIS Spectrophotometer, Model UV- 1201, Schimadzu, Japan.

- **Serum creatinine:** Serum creatinine level was estimated by alkaline picrate method and the absorbance were measured at 520 nm (Godkar., 1994) using UV -VIS Spectrophotometer, Model UV- 1201, Schimadzu, Japan.
- **Serum uric acid** (Godkar., 1994)
- **Thiobarbituric acid-Reacting substances:** The kidneys were removed to determine the levels of Thiobarbituric acid-Reacting substances (TBARS) (Okhawa et al., 1979)

2.6 Histopathological analysis

After the collection of blood for the estimation of the hematological parameters, the rats were anesthetized under deep ether anesthesia then sacrificed and their kidneys were removed, weighed and washed with normal saline. Kidney tissues were collected in 10% formalin saline for proper fixation followed by routine Hematoxylin & Eosin (H & E) staining.

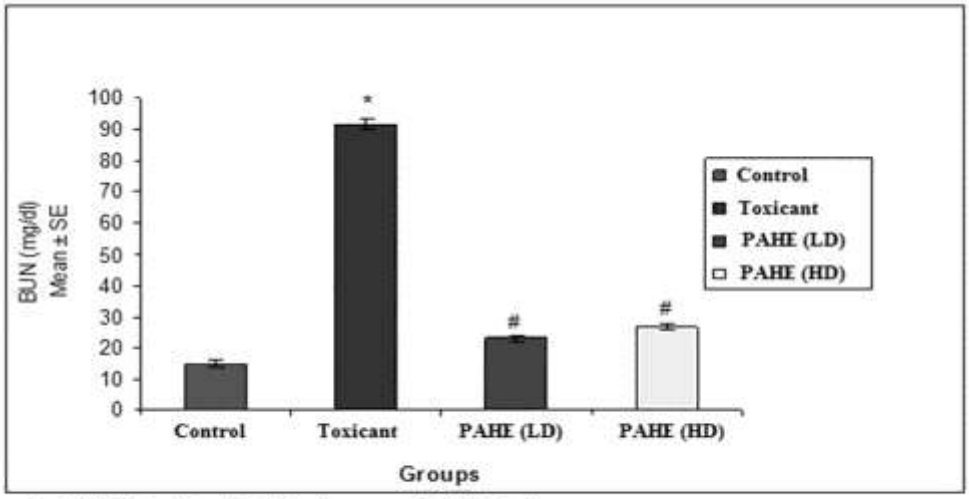
2.7 *Statistical analysis*

Results of blood urea nitrogen and serum creatinine estimation are presented in the form of mean and percentage inhibitions by the test drug against nephrotoxin induced changes. The percentage inhibition was calculated by considering the difference in BUN/serum creatinine /TBARS between rats treated with nephrotoxin and control. A 100% inhibition indicates that there is complete inhibition of toxin induced increase in the level of the biochemical parameters, while a 0% inhibition indicates that there is no reduction in the toxin induced elevation in the level of biochemical parameters. Statistical evaluation was done by one way analysis of variance (ANVOVA) followed by Dunnett's post hoc test.

3. RESULTS AND DISCUSSION

3.1 Effect of PAHE on BUN in Cisplatin induced nephrotoxicity in rats

PAHE at a dose of 420 mg/kg significantly inhibited the rise of BUN level. The mean score were 14.96 ± 1.09 in control group, 91.86 ± 1.65 in toxicant group, 23.10 ± 0.84 in group III PAHE (420 mg/kg) and 26.75 ± 0.94 in group IV PAHE (980 mg/kg). The percentages of toxicity inhibition were 74.84, 70.87 in groups III and IV respectively. PA inhibited the rise of BUN level by 74.84% at a dose of 420 mg/kg and 70.87% at a dose of 980 mg/kg. The results are shown in Table I/Figure 1.



n= 6; statistically significant: *p<0.01, when compared with Control group,
#p<0.01, when compared with Toxicant group,
Group II was compared with all the other groups by one way ANOVA followed by Dunnet's post hoc test.

Fig. 1 Effect of PAHE on BUN in Cisplatin induced nephrotoxicity in rats

Table 1: Effect of PAHE on BUN in Cisplatin induced nephrotoxicity in rats

Groups	Treatment	Dose	BUN (mg/dl) Mean ± SE	% Inhibition
I (Control)	Vehicle	10ml/kg	14.96 ± 1.09	-
II (Toxicant)	Cisplatin	7 mg/kg *i.p.*	91.85 ± 1.65*	-
III	PAHE(LD)	420 mg/kg	23.10 ± 0.84#	74.84
IV	PAHE(HD)	980 mg/kg	26.75 ± 0.94#	70.87

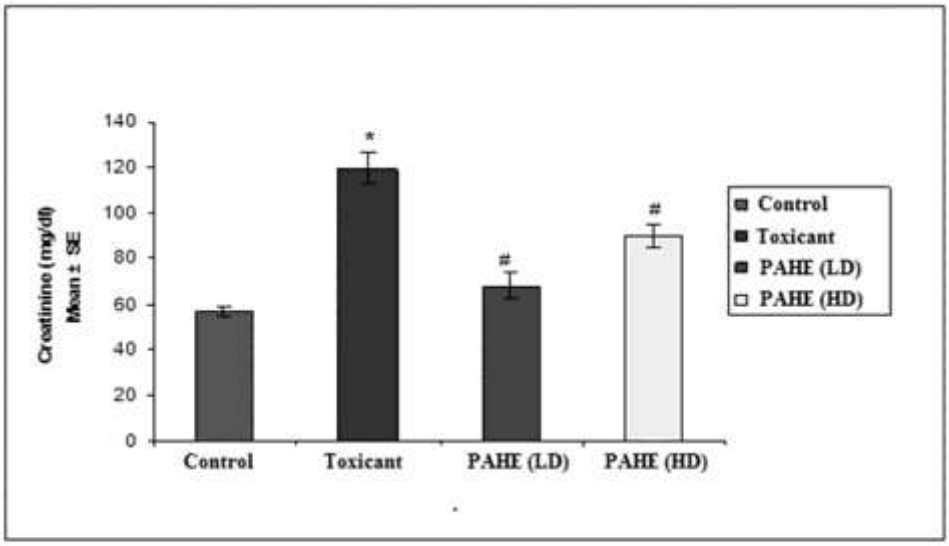
n= 6; statistically significant: *p<0.01, when compared with Control group,

#p<0.01, when compared with Toxicant group,

Group II was compared with all the other groups by one way ANOVA followed by Dunnet's post hoc test.

3.2 Effect of PAHE on serum creatinine in Cisplatin induced nephrotoxicity in rats

The results show significant inhibition of raised serum creatinine level in test drugs. Hydroalcoholic extract of *Kaknaj* (*Physalis alkekengi*) at a dose of 420 mg/kg significantly inhibited the rise of creatinine level. The mean score were 56.54 ± 2.27 in control group, 119.64 ± 6.74 in toxicant group, 68.05 ± 5.44 in group III PAHE (420 mg/kg) and 90.07 ± 5.04 in group IV PAHE (980 mg/kg). The percentages of toxicity inhibition were 43.11, 24.70 in groups III and IV respectively. *Kaknaj* (*Physalis alkekengi*) inhibited the rise of creatinine level by 43.11% at a dose of 420 mg/kg and 24.70% at a dose of 980 mg/kg. The results are shown in Table II/Figure 2.



n= 6; statistically significant: *p<0.01, when compared with Control group,
#p<0.01, when compared with Toxicant group,
Group II was compared with all the other groups by one way ANOVA followed by Dunnet's post hoc test.

Fig. 2 Effect of PAHE on serum creatinine in Cisplatin induced nephrotoxicity in rats

Table 2: Effect of PAHE on serum creatinine in Cisplatin induced nephrotoxicity in rats

Groups	Treatment	Dose	Creatinine (mg/dl) Mean ± SE	% Inhibition
I (Control)	Vehicle	10ml/kg	56.54 ± 2.27	-
II (Toxicant)	Cisplatin	7 mg/kg *i.p.*	119.64 ± 6.74*	-
III	PAHE(LD)	420 mg/kg	68.05 ± 5.44#	43.11
IV	PAHE(HD)	980 mg/kg	90.07 ± 5.04#	24.70

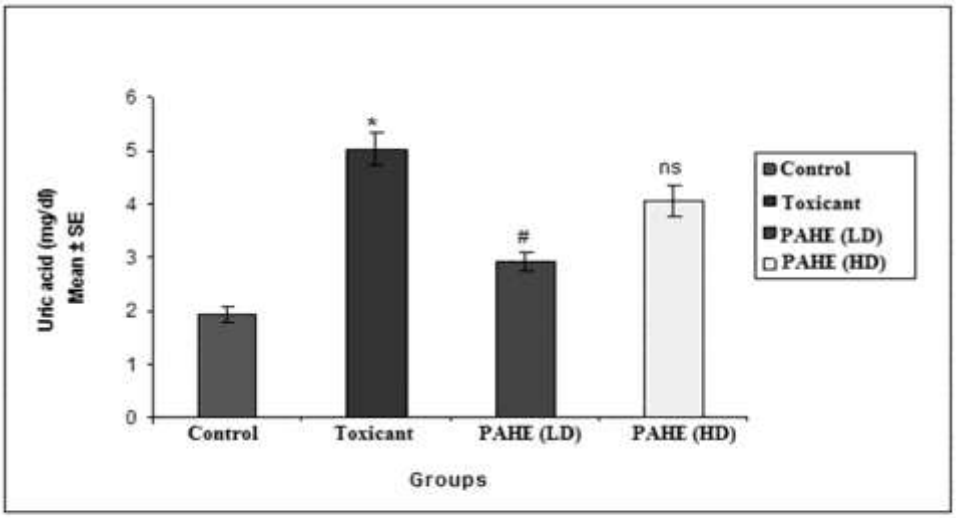
n= 6; statistically significant: *p<0.01, when compared with Control group,

#p<0.01, when compared with Toxicant group,

Group II was compared with all the other groups by one way ANOVA followed by Dunnet's post hoc test.

3.3 Effect of PAHE in serum uric acid on Cisplatin induced nephrotoxicity in rats

The result show significant inhibition of raised serum uric acid level at the lower dose while it was insignificant at higher dose. Hydroalcoholic extract of *Kaknaj* (*Physalis alkekengi*) at a dose of 420 mg/kg significantly inhibited the rise of serum uric acid level. The mean score were 1.94 ± 0.17 in control group, 5.02 ± 0.31 in toxicant group, 2.92 ± 0.18 in group III PAHE (420 mg/kg) and 4.07 ± 0.28 in group IV PAHE (980 mg/kg). The percentages of toxicity inhibition were 41.71, 18.83 in groups III and IV respectively. *Kaknaj* (*Physalis alkekengi*) inhibited the rise of creatinine level by 41.71% at a dose of 420 mg/kg and 18.83% at a dose of 980 mg/kg. The results are shown in Table III/Figure 3.



n= 6; statistically significant: *p<0.01, when compared with Control group,
#p<0.01, nsp>0.05, when compared with Toxicant group,
Group II was compared with all the other groups by one way ANOVA followed by Dunnet's post hoc test.

Fig. 3 Effect of PAHE on serum uric acid in Cisplatin induced nephrotoxicity in rats

Table 3: Effect of PAHE on serum uric acid in Cisplatin induced nephrotoxicity in rats

Groups	Treatment	Dose	Uric acid (mg/dl) Mean ± SE	% Inhibition
I (Control)	Vehicle	10ml/kg	1.93 ± 0.16	-
II (Toxicant)	Cisplatin	7 mg/kg *i.p.*	5.02 ± 0.31*	-
III	PAHE(LD)	420 mg/kg	2.92 ± 0.18$^{\#}$	41.71
IV	PAHE(HD)	980 mg/kg	4.07 ± 0.28^{ns}	18.83

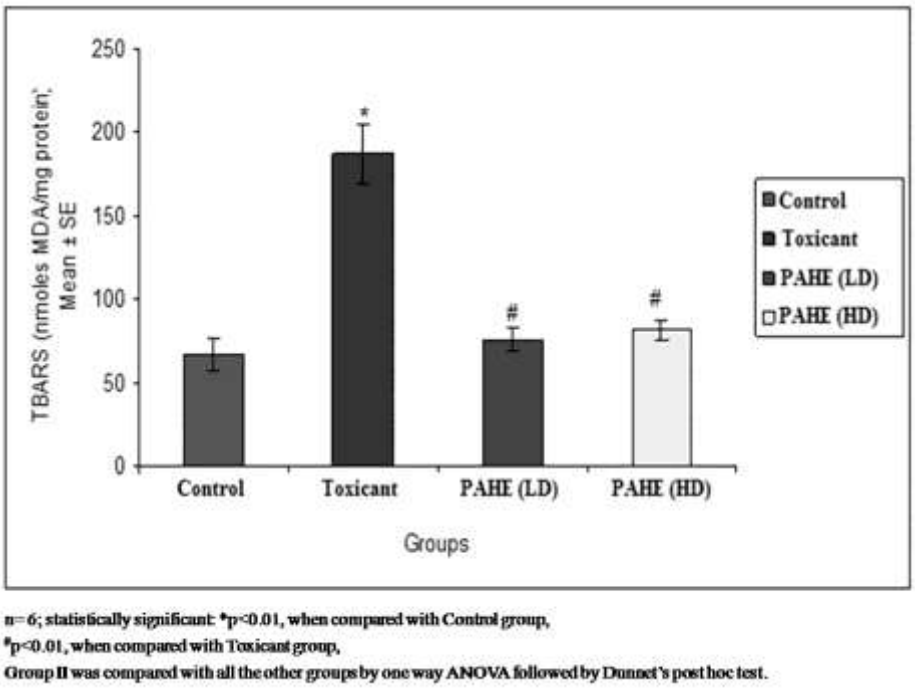
n= 6; statistically significant: *p<0.01, when compared with Control group,

$^{\#}$p<0.01, ^{ns}p>0.05, when compared with Toxicant group,

Group II was compared with all the other groups by one way ANOVA followed by Dunnet's post hoc test.

3.4 Effect of PAHE on TBARS in Cisplatin induced nephrotoxicity in rats

The results show significant inhibition of raised TBARS level in test drugs. Hydroalcoholic extract of *Kaknaj* (*Physalis alkekengi*) at a dose of 420 mg/kg significantly inhibited the rise of TBARS level. The mean score were 66.44 ± 9.702 in control group, 186.61 ± 17.38 in toxicant group, 75.86 ± 6.60in group III PAHE (420 mg/kg) and 81.60 ± 6.53 in group IV PAHE (980 mg/kg). The percentages of toxicity inhibition were 59.34, 56.27 in groups III and IV respectively. Kaknaj (*Physalis alkekengi*) inhibited the rise of BUN level by 59.64% at a dose of 420 mg/kg and 56.27% at a dose of 980 mg/kg. The results are shown in Table IV/Figure 4.



n= 6; statistically significant: *p<0.01, when compared with Control group,
#p<0.01, when compared with Toxicant group,
Group II was compared with all the other groups by one way ANOVA followed by Dunnet's post hoc test.

Fig. 4 Effect of PAHE on TBARS in Cisplatin induced nephrotoxicity in rats

Table 4: Effect of PAHE on TBARS in Cisplatin induced nephrotoxicity in rats

Groups	Treatment	Dose	TBARS (nmoles MDA/mg protein) Mean ± SE	% Inhibition
I (Control)	Vehicle	10ml/kg	66.45 ± 9.70	-
II (Toxicant)	Cisplatin	7 mg/kg *i.p.*	186.61 ± 17.38*	-
III	PAHE(LD)	420 mg/kg	75.87 ± 6.60#	59.34
IV	PAHE(HD)	980 mg/kg	81.60 ± 6.54#	56.27

n= 6; statistically significant: *p<0.01, when compared with Control group,

#p<0.01, when compared with Toxicant group,

Group II was compared with all the other groups by one way ANOVA followed by Dunnet's post hoc test.

3.5 Histopathological Analysis

Normal kidney tissues of rat (Control) are shown in Fig. 5. Fig. 6 indicated cisplatin induced nephrotoxicity in rats. On the 11th day, when compared to normal sections the cisplatin treated rat kidney sections of group II showed marked congestion of the glomeruli with numerous tubular casts associated with epithelial desquamation. Marked peritubular congestion and edema were also observed.

The interstitium showed infiltration with inflammatory cells and congestion. These features suggest that cisplatin induces acute tubular necrosis. The hydroalcoholic extract of *Physalis alkekengi* (LD) (Fig. 7) showed complete normalization of kidney section. However, mild glomerular, peritubular congestion and inflammatory cells were apparent in hydroalcoholic extract of *Physalis alkekengi* (HD) (Fig. 8)

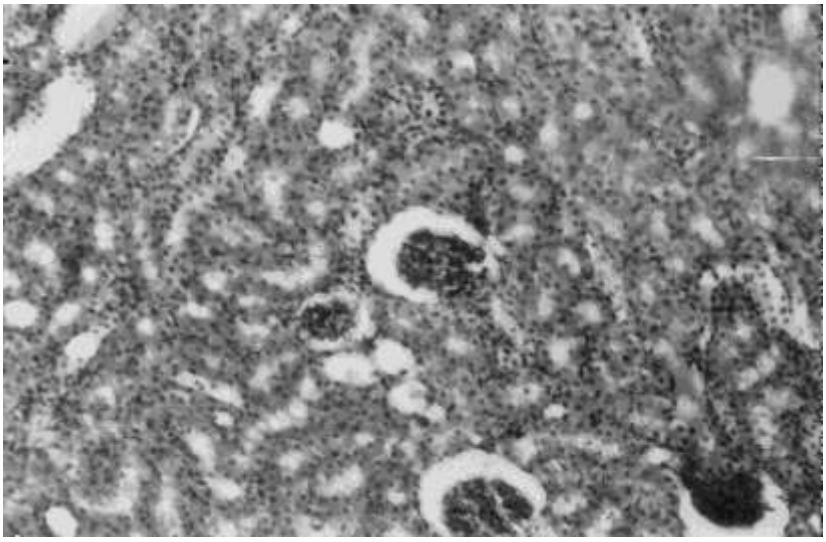


Fig. 5 Normal kidney tissues of rat (Control)

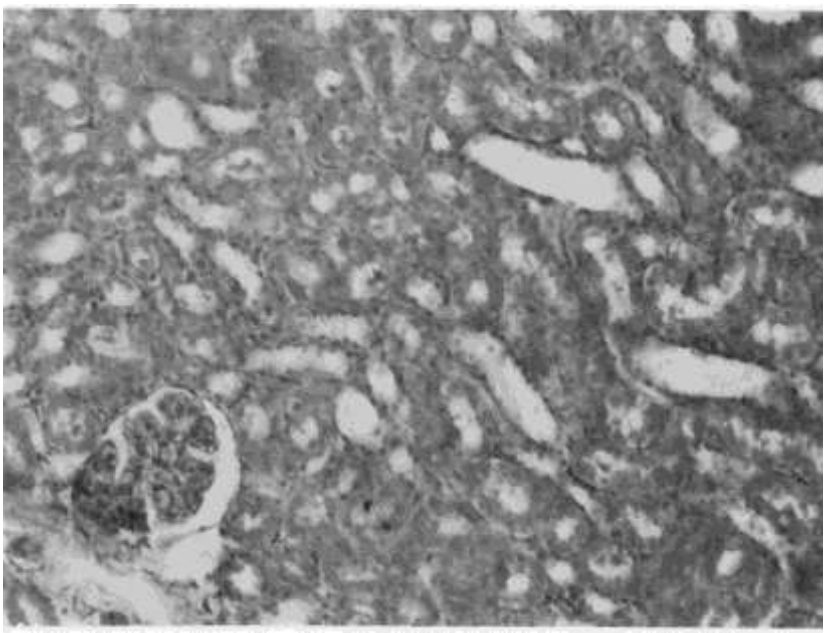


Fig. 6 Kidney tissues showing cisplatin-induced nephrotoxicity in rat

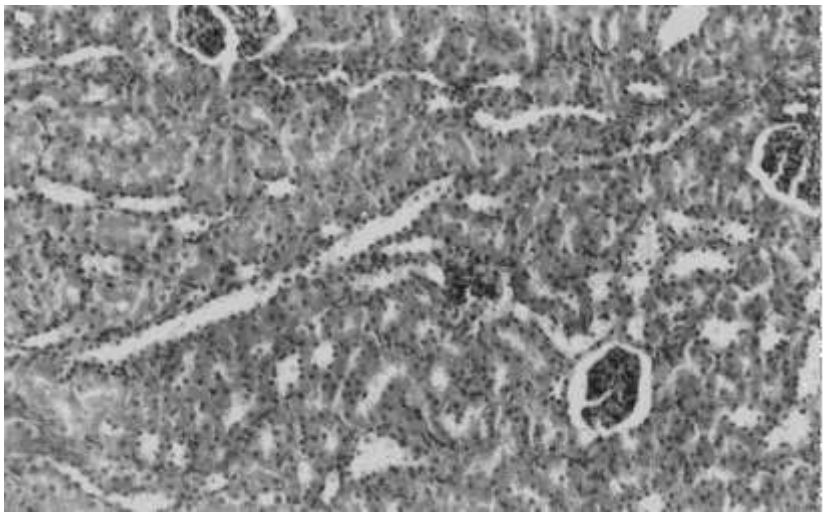


Fig. 7 PAHE (LD) of Kaknaj (Physalis alkekengi) treated rat showing normal kidney tissues in cisplatin-induced nephrotoxicity model

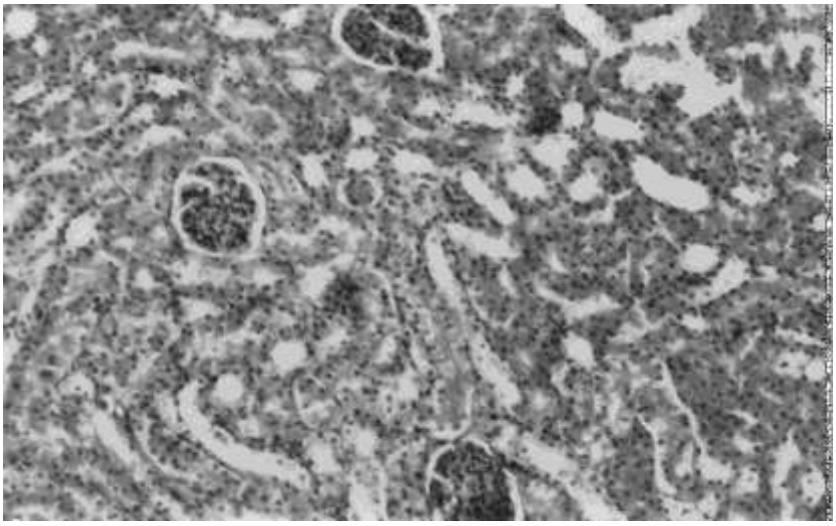


Fig. 8 PAHE (HD) of Kaknaj (Physalis alkekengi) treated rat showing tubular cell's swelling of kidney tissue in cisplatin-induced nephrotoxicity model

Lipid peroxidation is one of the mechanisms by which cisplatin induces nephrotoxicity (Francescato et al., 2001). Lipid Peroxidation is oxidative deterioration of polyunsaturated lipids and its major deleterious effect is intracellular free radical generation. Peroxidation of unsaturated lipids has great biochemical effects, especially when lipid peroxidation occurs in membrane lipids. Dramatic alterations in membrane structure and function can be the net result of this process due to disruptive effects of loops of membrane lipids, production of toxic lipid breakdown products and peroxidative damage to nearby membrane proteins (Freeman and Crapo., 1982).

In the present study cisplatin treatment (7 mg/kg IP on 4th day) caused nephrotoxicity as evidenced by mark elevation in blood urea nitrogen, serum creatinine, serum uric acid and TBARS compared to saline treated (p.o) animals. Ten days treatment (three days prior to administration of cisplatin) with PAHE, at two different doses (420 mg/kg, 980 mg/kg) inhibited the rise in blood urea nitrogen, serum creatinine, uric acid and TBARS. However, the effect of lower dose (420 mg/kg) was more pronounced. Histopathological examination of the kidney tissues of the rats, treated with PAHE, indicate that it has significant protective effect against cisplatin-induced nephrotoxicity.

4. CONCLUSION

The PAHE significantly inhibited glomerular congestion, tubular casts, peritubular congestion, epithelial desquamation, blood vessel congestion, interstitial edema and inflammatory cells produced by the cisplatin-induced nephrotoxicity. On the basis of these findings it may be concluded that PAHE showed significant nephroprotective action.

REFERENCES

1. Ali, M.N., 1301. Mufradat-e-Nasiri Mai Takmla-e-Nasiri. Dar Mataba Qaisry, CITY, 78.
2. Arzani, H.M.A., 1952. Meezan-ut-Tib. Taj Press, Hyderabad, 183: 193.
3. Daunay, M.C., Laterrot, H., Janick, J., 2007. Iconography of the Solanacae from Antiquity to the XVIIth century: A rich source of information on genetic diversity and uses. *Acta. Hort.*, 745: 59-88.
4. Franciscato, H.D., Costa, R.S., Rodrigues Camargo, S.M. et al., 2001. Effect of selenium administration on cisplatin-induced nephrotoxicity in rats. *Pharmacol. Res*., 43: 77-82.
5. Freeman, B.A., Crapo, J.D., 1982. Biology of disease: Free radicals and tissue injury. *Lab. Invest*., 47: 412.
6. Ghani, M.N., 1920. Khazain-ul-Advia. Sheikh Basheer Ahmad & Sons, Lahore, 263-264.
7. Godkar, P.B., 1994. Clinical Biochemistry: principles and practice. Bhalani publishing house, Bombay, 118-132: 233-251.
8. Hakim, M.A., 1311. Bustanul Mufradat. Idara Taraqqi Urdu wa Publications, Lucknow, 240.
9. Huang, H., Zhul., Rheid, B.R., Drobny, G.P., Hopkins, P.B., 1995. Solution structure of a cisplatin induced DNA interstrand cross-link. *Science*., 270: 1842-1845.
10. Kabiruddin, H., 1951. Makhzanul Mufradat. Sheikh Mohammad Bashir & Sons, Lahore, 426-427.

11. Nadkarni, K.M., 1989. Indian Materia Medica. Bombay Popular Prakashan, Bombay, 49: 950.
12. Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95: 351-358.
13. Razi, A.M.Z., 2002. Kitabul Hawi. Central Council for Research in Unani Medicine(CCRUM), Ministry of Health and Family Welfare, Govt. of India, New Delhi, 22: 166.
14. Sina, I., 1927. Alqanoon Fit Tibb. Sheikh Mohammad Bashiir & sons, Lahore, 170-175: 304-308.
15. Veda, N., Kaushal, G.P., Shah, S.V., 2000. Apoptotic mechanisms in acute renal failure. *Am. J. Med*., 108: 403-415.