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Research Article

# Effect of Hesperdin isolated from orange peels on Cisplatin-induced Nephrotoxicity

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**ABSTRACT**

Present study is designed to evaluate the effect of Hesperidin, a naturally occurring citrus flavanone isolated from orange peels, against cisplatin-induced nephrotoxicity. Nephroprotector activity was evaluated in male Albino rats. Nephrotoxicity induced by intraperitoneal administration (6 mg/kg,i.p., single dose) of cisplatin. Hesperidin was administered by gastric intubation. Nephroprotector activity of hesperidin was tested at two dose levels i.e., 200mg and 400mg/kg Body weight. Nephrotoxicity was assessed by estimating blood urea nitrogen, serum creatinine and estimating urinary parameters. Cisplatin nephrotoxicity was characterized by elevated levels of blood urea nitrogen, serum creatinine, and high protein excretion in urine and reduced levels of creatinine clearance. Hesperidin produced significant protection against cisplatin – induced nephrotoxicity in dose dependent manner.

**Key words**: Hesperidin, Cisplatin, nephrotoxicity.

## INTRODUCTION

Cisplatin is one of the most effective anticancer drugs administered to treat a variety of cancers such as ovarian cancer[1],[2],[3] , bladder and other genito– urinary tumors[4],[5] , head, neck cancers[6],[7],[8],[9] and lung cancer[10],[11],[12],[13]. High doses of cisplatin are more effective than low doses in Ovarian and colorectal cancer[14]. However high dose treatment with cisplatin-induce nephro and neurotoxicity. In spite of hydration, hypertonic saline and diuretics to protect against renal complications, a high percentage of treated patients develop from mild to severe renal imbalance. The mechanism of cisplatin nephrotoxicity still not fully understood, but this cause is the dose-limiting factor in clinical studies[15]. However the generation of free oxygen radials in tubular cells has been proposed as an important pathogenic process[16]. Various data indicate that the cisplatin induces Oxidative stress[17],[18] lipid peroxides[19] and DNA damage[20],[21].Histological results of earlier studies showed that cisplatin preferentially induced structural damage in the cortical zone of the kidney[7] several antioxidants such as L-histidinol[7] , thymoquinone[22] and lipoic acid[23] capsicin[24], Ascarbic acid[25], curcumin[26], have been tested for their ability to protect against cisplatin-induced nephrotoxicity in experimental animals and all of them exhibited significant protection against cisplatin-induced nephrotoxicity. Flavonoids, poly phenolic compounds are another group of interested compounds, which possess antioxidant properties, are found in fruits, vegetable, nuts and seeds as well as most types of tea and red wine[27]. Flavonoids have been reported to exhibit a wide range of biological effect. Flavonoids such as Quercetin[28], Naringenin [29] were showed good nephroprotection. Hesperidin is a citrus flavonone, which possess wide spectrum of biological activities including antioxidant activity. Literature survey reveals that substances with good antioxidant activity exhibited good nephroprotection against cisplatin-induced nephrotoxicity. Hence, present work designed to evaluate the nephroprotector activity of natural dietary anti oxidant, hesperidin against cisplatin-induced renal toxicity.

## MATERIALS AND METHODS

Chemicals: Cisplatin injection was purchased from market; and all other chemicals were purchased from either S.D. or Merck India. Biochemical parameters were estimated by using commercial kits.

Isolation and charcterisation of hesperdin- Orange peels were collected, dried and powdered isolated according to procedure mentioned in Harborne 1986. Characterized by IR, NMR and MASS.

Pharmacological Screening: Animals- Healthy wistar adult male albino rats weighing 150-200g were used for study. They housed in poly propylene cages and fed standard rat pellet diet and water *ad libitium*

Experimental design- Animals were divided into 4 groups. Group I animals received vehicle (1%CMC). Group II animals received cisplatin on day 5. Group III and IV received 200mg/kg and 400 mg/kg Bd.Wt. hesperdin respectively from day 1 to day 10 and On day 5 these groups received cisplatin injection. GroupV received only hesperdin for 10days i.e., from day 1 to day 10. On day 9, urine was collected and estimated urinary functional parameters. Animals were sacrificed on day 10

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Table – 1 Effect of Hesperdin on normal rats

| S.No. | PARAMETER | GROUP-I | GROUP-V |
|---|---|---|---|
| | Treatment (mg/kg) | 1% carboxy methyl cellulose throughout the treatment | Hesperdin (400mg) given throughout the period. |
| 1. | BUN (mg/dl) | 23.56±0.15 | 24.06±0.19* |
| 2. | SC (mg/dl) | 0.69±0.8 | 0.67±0.01* |
| 3. | $U_{TP}$ (mg/24hrs) | 6.2±0.3 | 8.10±0.43* |
| 4. | $Cl_{Cr}$ (ml/hr/100g bd.wt) | 18.90±1.3 | 17.98±1.09* |
| 5. | LPO (n moles/gm) | 10.6±1.1 | 11.3±0.9* |

* Each value represents the mean ± SEM from 6 animals in each group

by cervical decapitation and blood samples were collected by cardiac puncture and were used for estimation of Blood urine nitrogen (BUN, Di acetylmonooxime method), Serum creatinine (SC, Alkaline Picrate method). In kidney tissue Malondialdehyde levels were estimated[30].

**STATISTICAL ANALYSIS**

The results are expressed as mean±SEM and the data analysed using one way analysis of variance followed by post hoc Student-Keuls test using SPSS computer software for *in vivo* studies. Statistical significance was set at $P \leq 0.05$.

**RESULTS**

Effect of Hesperdin extract on normal rat kidney. Effect on Serum markers- The BUN and SC levels were expressed in mg/dl. With the administration of hesperdin to group V animals there was no significant changes were observed in BUN and SC levels when compared with normal group I animals.

Effect on Urinary parameters-Urinary total proteins ($U_{TP}$) was expressed in mg/24hrs.Animals which received hesperdin showed almost similar values to that of normal control animals.

The levels of creatinine clearance ($Cl_{cr}$) were expressed in mg/hr/100g body weight. There were no significant changes in $Cl_{cr}$ levels in hesperdin given animals when compared to normal group I animals.

Effect on LPO activity-The animals on administration of only hesperidin to the animals no significant change was observed in LPO.

Effect of Hesperdin on cisplatin-induced nephrotoxicity

Effect on Serum markers-Animals which received cisplatin (6mg/kg) alone showed significant elevated levels of BUN when compared to group I animals. Animals which belongs to Gr IV (200mg/kg) and Gr V (400mg/kg) exhibited dose dependent protection.

The SC levels were increased in Gr III animals, which received only cisplatin when compared with normal animals. Animals treated with hesperdin at 200mg/kg dose (Gr IV) showed moderate protection and animals treated with hesperdin at 400mg/kg dose (Gr V) showed significant protection.

Effect on Urinary parameters-Animals administered with cisplatin excreted high amount of $U_{TP}$, when compared with normal group I animals, whereas Gr IV (200mg/kg) and Gr V (400mg/kg) reversed the effect caused by cisplatin in dose dependent manner.

The animals received cisplatin alone exhibited decreased levels of $Cl_{cr}$ when compared with normal. On oral administration of hesperidin showed significant increase in $Cl_{cr}$.

Effect on LPO activity:Animals which are received cisplatin alone increased levels of LPO when compared with normal group I. The animals received high dose of hesperidin (Group-V) showed decrease levels of LPO.

**DISCUSSION**

Cisplatin is an antitumour drug, it has been successful in the bladder, lung, head, neck, cervical especially testicular and ovarian cancers[31]. Although it is active against wide variety of tumors but serious and usually dose limiting toxicity of cisplatin is renal.

The precise mechanism of *cisplatin*-induced nephrotoxicity has not been elucidated, but it has been suggested that the oxygen free radicals play an important role[32],[33],[34]. Cisplatin-induced nephrotoxocity is related to increase in lipid peroxide levels in kidney[35]. Reports also suggest that there is an involvement of nitric oxide which induces the nephrotoxicity by cisplatin[36],[37]. Tsulsumishitha and coworkers reported the involvement of $H_2O_2$ in ciplatin-induced nephrotoxicity in outer medullary cortical tubule (OCMT) cells[38]. Additionally, more than half of patients developed hypomagnesemia and persisted for months after cisplatin treatment [39] and clinical reports demonstrated that patients who received cisplatin suffered reversible azotemia, 1-2 weeks following treatment[40]. Numbers of studies have shown that reduction of the glomerular filtration rate is common in patients receiving cisplatin treatment. On the other hand, there are well-known evidences that oxidative and nitrosative stress is involved in the kidney damage after cisplatin administration.

However the generation of oxygen free radicals in tubular cells has been proposed as an important pathogenic process[16]. Various data indicate that cisplatin induces oxidative stress[41],[17],[42], lipid peroxides[19] and DNA damage[20],[21] .

Natural antioxidants such as Ascorbic acid[25] , Spirulina, Quercetin[28], Rutin[43], have been tested for their ability to protect against cisplatin-induced nephrotoxicity in experimental animals and reported that they exhibited good nephroprotection. Flavonoids are the polyphenolic compounds are found in fruits and vegetables, nuts and

Table-2 Effect of isolated compound of *hesperdin* on cisplatin induced nephrotoxicity

| **Gr** | Treatment (mg/kg) | BUN (mg/dl) | SC (mg/dl) | **$U_{TP}$ (mg/24hrs)** | **$Cl_{cr}$ (ml/hr/100g bd.wt)** | **LPO (n moles/g)** |
|---|---|---|---|---|---|---|
| **I** | Normal (1%carboxy methyl cellulose ) | 24.5±0.15 | 0.69±0.8 | 6.2±0.3 | 18.90±1.3 | 10.6±2.1 |
| **II** | Cisplatin (6mg/kg) | 62.2±3.2[a] | 1.9±0.2 [a] | 17.9±0.9 [a] | 7.10±0.8 [a] | 15.7±0.3 [a] |
| **III** | Low dose (200mg/kg) | 31.0±.0.8[b] | 1.2±0.3 [b] | 12.3±0.1[b] | 10.07±1.4[b] | 12.6±0.6 [b] |
| **IV** | High dose (400mg/kg) | 27.2±0.7[c] | 2.0±0.5 [c] | 9.6±1.7 [c] | 16.06±0.9 [c] | 11.3±1.8 [c] |

Each value represents the mean ± SEM from 6 animals in each group
a: P<0.05 when compared with normal group.
b: P<0.05 when compared with group II (cisplatin)
c: P<0.01 when compared with group II (cisplatin)

seeds[27], activity and they may play a dietary role in protection against chronic diseases such as cancer and cardiovascular disease[44]. They have antioxidant and antiproliferative properties[45].

In present study, the effect of cisplatin is similar to those previously described[46],[47],[7],[22],[23] i.e., elevated serum urea, serum creatinine levels, reduced creatinine clearance, increased urinary protein excretion.

Oral administration of hesperidin at a dose of 200 and 400mg/kg/day for 10 days starting 5 days before cisplatin single *i.p.* injection produced significant protection of renal function. hesperidin reduced the extent of cisplatin-induced nephrotoxicity as evidenced by significant reduction in serum urea and creatinine levels, decreased urinary functional proteins and increased creatinine clearance.

Previous reports also evidenced that cisplatin exerted its nephrotoxic effects through LPO [48], [28]. In the present study, cisplatin induced accumulation of lipid peroxides Cisplatin-induced alterations in lipid peroxides is markedly improved by hesperidin.

In conclusion, the results provide hesperidin, is attenuates the nephrotoxicity of cisplatin in rats. The results provide further insight into the mechanisms of cisplatin-induced nephrotoxicity and confirm the antioxidant potential of hesperidin.

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