**THE EFFECTS OF ORAL ADMINISTRATION OF HESPERIDIN ON LIVER ENZYMES AND KIDNEY FUNCTIONS IN WHITE MICE**

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**Abstract**

Hesperidin (HDN) is a flavanone glycoside abundantly found in sweet orange and lemon and is an inexpensive by-product of citrus cultivation .

The study evaluates the effects of Hesperidin extract on blood glucose level, creatinine, urea and liver enzymes parameters in female white mice. Adult mice weighing between 26-30g.

The mice were grouped into four groups (each of six animals). Groups 2,3 and 4 were treated with 50,100 and 200 mg/kg body weight of Hesperidin extract respectively. while group 1 as a control group. The extracts were given to the animals orally for 14 days. At the end of the experimental period, mice were sacrificed using chloroformanesthesia. Blood samples were collected by cardiac puncture into EDTA capped bottles withthe aid of a 5ml syringe. The blood samples were then used for the experiments.

Results show that the liver enzyme was significantly (P<0.05) lower in treatment groups (2,3, and 4) than in control. Hb content, , creatinine, urea did not differ significantly among the groups, but blood glucose level was significantly higher in the fourth group (200mg/kg) compared with control group. In conclusion, the extract has little or no effect kidney function (creatinine, urea ), But the extract significantly decreased the The liver enzyme, probably due to its direct antioxidant effect.

**Introduction**

Citrus species have been studied for medicinal properties and reported to contain numerous bioactive compounds, such as flavanone glycosides (narirutin, naringin, hesperidin, and neohesperidin) and natural antioxidants. Particularly, the phenolic acids have attracted more attention for their antioxidant behaviour and beneficial health-promoting effects in chronic and degenerative diseases (Gil-Izquierdo, 2002).

Hesperidin (HDN) is a flavanone glycoside abundantly found in sweet orange and lemon and is an inexpensive by-product of citrus cultivation (Manach *et al.*, 2003).

HDN is effectively used as a supplemental agent in the treatment protocols of complementary settings. Its deficiency has been linked to abnormal capillary leakiness as well as pain in the extremities causing aches, weakness and night leg cramps. Supplemental hesperidin also helps in reducing oedema or excess swelling in the legs due to fluid accumulation (Akiyama *et al*., 2010). HDN was reported to possess anticancer (Alshatwi *et al*., 2013), health promoting effects (Li and Schluesener, 2015) and to protect against radiation-induced toxicity (Kuntić *et al*., 2014).

The present study was designed to investigate the role of HDN against γ-radiation and/or paraquat inducedbiochemical, hematological and histological changes.

Flavonoids are polyphenolic compounds present in all foods of plant origin. They have various effects on mammalian cellular systems and structures, and have been shown to protect biological membranes against free radical-induced oxidative damage (Jain *et al.*, 2011).

According to the results obtained in the current study, it is concluded that hesperidin could be a useful adjunct to attenuate biochemical, hematological and histological damage induced by the over exposure to free radicals.( **Abd El-Rahman ,2016).**

de Oliveira et al., (2013) have shown that hesperidin supplementation per se or in combination with swimming exercise protocols, continuous and interval, potentiates improvement of the biochemical profile and antioxidant biomarkersevidencing that the use of citrus flavonoids may be beneficial to reduce risk factors for metabolic and cardiovascular diseases. Moreover, hesperidin supplementation, in conjunction with continuous swimming, presented hypolipidemic effects and could be useful as an antioxidative compound to protect against oxidative damages during this type of exercise; on the other hand, hesperidin plus interval swimming exercise can help reduce increased levels of glucose in the bloodserum.

Previous studies in humans and animals, especially rodents, have demonstrated that hesperidin and its metabolites decrease blood serum glucose and lipids and neutralize markers of oxidative stress [. Kurowska ,2000 and Kim,2010]. Although a body of evidence has shown these benefits, most of the mechanisms are still being explored (Minato,2003, . Miyake ,2003; Cureton ,2009; . Di Giacomo ,2009; Aptekmann,2010).

Hesperidin is a flavanone glycoside (flavonoid) (found abundantly in citrus fruits. Its aglycone form is called hesperetin. It’s name is derived from the

Hesperides nymphs of Greek mythology. Hesperidin is believed to play a role in plant defense. It acts as an antioxidant according to *in vitro* studies (Hirata et al., 2005; Monforte et al., 1995). In human nutrition, it contributes to the integrity of the blood vessels. Various preliminary studies reveal novel pharmaceutical properties. Flavonoids are products of plant metabolism

and have different phenolic structures (Guzmán and Navarrete, 2009; Ohtusuki et al., 2003). They are effective antioxidants because of their free radical scavenging properties and because they are chelators of metal ions (Trivedi et al., 2001); thus, they may protect tissues against free oxygen radicals and lipid peroxidation. Flavonoids may also be activated by

mechanisms that apparently are not directly dependent on their antioxidative properties. Under certain conditions they may also behave as preoxidants (Garg et al., 2001).

A wide range of different biological activities, including antibacterial, antithrombotic, vasodilatory, antiinflammatory, and anticarcinogenic effects mediated by different mechanisms, are associated with flavonoid compounds

(Middleton et al., 2000). Hesperidin reduced cholesterol and blood pressure (Ohtsuki et al., 2003) in rats. In a mouse study, large doses of the glucoside hesperidin decreased bone density loss (Chiba et al., 2003).

Hesperidin has anti-inflammatory effects (Galati et al., 1994; Kawaguchi et al., 2004). Hesperidin is also a potential sedative, possibly acting through opioid or adenosine receptors (Loscalzo et al., 2008). Hesperidin also showed the ability to penetrate the blood-brain barrier in an *in vitro* model.

Flavonoids are non-nutritive dietary components that are widely distributed in plants (Mahmoud, 2012). Naringin (4´,5,7- trihydroxy flavonone 7-rhamnoglucoside) is the predominant flavonone found in grape fruit and related citrus species (Jagetia and Reddy, 2002). Among the naturally occurring flavonoids, naringin has been empirically proven to have no side effects, as humans have been ingesting grapes and citrus fruits for a long time (Choe et al., 2001). Hesperidin is an abundant and inexpensive byproduct of Citrus cultivation and isolated from the ordinary orange Citrus aurantium and other species of the genus Citrus (family: Rutaceae) (Kakadiya et al., 2010). Recently, we have reported the anti-diabetic potentials of both hesperidin and naringin in high fat diet/streptozotocin induced diabetic rats (Ahmed et al., 2012; Mahmoud et al., 2012, 2013).

It has been reported that, ingestion of medicinal plants or drugs can alter the normal hematological values (Ajagbonna et al., 1999). Therefore, hematological parameters could be an important tool in the assessment of deleterious effect of drugs, as well as medicinal plants (Yakubu et al., 2007).

Generally, There is limited information on hesperidin in Iraq. Thus, the intention of the present study was to scrutinize the influence of oral administration of hesperidin on the levels of hematological parameters in mice.

**MATERIAL AND METHODS**

### Experimental animals

White female mice weighing about 26 - 30 g were used. They were obtained from the animal house of the college of veterinary medicine, university of Diyala. They were kept under observation for about 15 days before the onset of the experiment to exclude any inter current infection. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25 ± 5 °C) and normal 12 hour light/dark cycle. Moreover, they had free access to water and were supplied daily with standard diet of known composition ad libitum. All animal procedures were in accordance with the recommendations of the Canadian Committee for Care and Use of Animals.

### Experimental design

The experimental animals were divided into four groups, each group comprising six mice as detailed follows.

* Group 1: Normal control mice
* Group 2: mice administered with hesperidin (50 mg/kg b.wt.) orally for 2 weeks
* Group 3: mice administered with hesperidin (100 mg/kg b.wt.) orally for 2 weeks
* Group 4: mice administered with hesperidin (200 mg/kg b.wt.) orally for 2 weeks

The dosage was adjusted every week according to any change in body weight to maintain similar dose per kg body weight of mice over the entire period of study for each group. By the end of the experiment, animals were sacrificed and blood samples were obtained. The blood samples were labeled and kept at room temperature until processing, which occurred within one hour of collection.

**Laboratory analysis**

**Liver enzymes determination**:

Liver enzymes tests performed by using the Reflotron apparatus according to the manufacturer instructions are:

1. Aspartate amino transferase (AST)
2. Alanine amino transferase (ALT)
3. Alkaline phosphatase (ALP)

Reflotron apparatus works on the principle of the reflectance photometry measurement that based on the color changes in the test strip.

Handling:

1. The samples were applied for liver enzymes tests whole blood be directly used. There is no need to prepare reagents or to calibrate the instrument.
2. Test Strip was inserted into the Reflotron measuring chamber, a test specific magnetic code recognizes the parameter of interest.
3. The result was recorded just 2 - 3 minutes later

All further processing was done automatically by the instrument within 2-3 minutes, 18 to 30 tests per hour can be performed. The results were shown on the display, documented via the integrated printer and were also stored to the internal memory for further processing.

**Statistical analysis**

Statistical analysis was applied by using Statistical program SPSS and Analysis of Variance (ANOVA) and used Least significant difference (L.S.D) for detect the significant differences between means of treatments (**Steel and Tarries,1980**). The significant difference statements were based on the possibility (P≤0.05)

**Results and Discussion**

**Liver enzymes evaluation:** the levels of AST, ALT and ALP significantly decreased with administration of higher doses of hesperidin (200 mg/kg). On the other hand, it seems that with administration of hesperidin, the level of total serum protein elevated, especially with the high­est dose. This effect was also shown in table (1).

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| **Table 1.** Effects of Various Doses of hesperidin on Liver enzymes in Studied mice |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **G1 Control** | **G2 hesperidin, 50 mg/kg** | **G3 hesperidin, 100 mg/kg** | **G4 hesperidin, 200 mg/kg** |
| **AST, IU/L** | 92 ± 2.35 | 86.75 ± 3.98 | 84 ± 2.12 | 75.18 ± 1.09 |
| **ALT, IU/L** | 46.86 ± 1.26 | 44.57 ± 1.04 | 42.56 ± 0.66 | 38.87 ± 0.89 |
| **ALP, IU/L** | 483.11 ± 19.68 | 430.76 ±19.25 | 413.37 ± 15.34 | 341.75 ± 20.33 |
| **Total protein, g/dL** | 6.42 ± 0.08 | 6.55 ± 0.10 | 6.2 ± 0.09 | 7.66 ± 0.06 |

|  |
| --- |
| Abbreviations: ALT, Alanine Transferase; ALP, Alkaline phosphatase; AST, Aspartate Transferase; g/dL, gram per deciliter; IU/L, International Unit Per Liter; . |

Liver enzymes such as ALT, AST, GGT and ALP are enzymes that significantly reflect liver hepatocytes necrosis and cholestasis and can be used in detection of sever liver diseases such as liver inflammation (Romeo et al.,2010 and Kramer et. al. ,1997).

Administration with hesperidin extract could decrease the level of liver function enzymes including AST, ALT and ALP. In liver dam­age, the level of these enzymes would be increased (Giannini et al.,2005). This effect may be due to the presence of anti-oxidant material in this plant. Polyphenols and flavonoid components are important anti-oxidants in this plant (Chen et al.,2002). These components have protective effect on the liver against toxins and free radicals (Areias et al.,2001 and . Perez-Carreon et al.,2002). This protective effect of Hesperidin can be correlated to its direct antioxidant effect.

In addition, AST has been recommended to identify liver abnormalities (Rukkumani et al.,2004). ALP is a membrane-bound glycoprotein enzyme found in different tissues such as liver, bone and in kidney, intestine and placenta in less amounts. Also, it is a valuable biochemical index used in detection of hepatobiliary disorders, osteoporosis, and fatty liver disease (Donmez et al.,2008 and Dvorska et al.,2008). In this research, the acivity of ALT enzyme in group recieving 200mg/kg hesperidin showed significant decrease compared to control group and injection of 100 and 50 mg/kg doses of hesperidin extract decreased significantly enzyme ALP compared to control group. On the other hand change in the activity of liver aminotransferases encourages the renovation of glucose from amino acids and changes activity of plasma of AST and ALT. Activity of these two enzymes can be changed with a variety of chemical and biological substances and physiological factors or disorder in Krebs cycle. Decreased activity of the Krebs cycle causes decrease in some compounds and finally decreases in AST and ALT (Sethuraman et al.,2003). It seems that decrease in activity of ALT and ALP enzymes in treatment groups is due to presence of hesperidin compound that prevents activity of the ATP precursors and aminotransferases. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney ( Aptekmann,2010).

**Table 2:** Effect of hesperidin extract on biochemical parameters of the mice blood

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **G1 Control** | **G2 hesperidin, 50 mg/kg** | **G3 hesperidin, 100 mg/kg** | **G4 hesperidin, 200 mg/kg** |
| Urea  (Mg/dl) | 43±11.91 | 42.6±11.12 | 41.33±9.33 | 40.78 ± 8.66 |
| Creatinine (Mg/dl) | 0.39 ± 0.08 | 0.37 ± 0.05 | 0.38± 0.04 | 0.40± 0.1 |
| Glucose  (Mg/dl) | 105.00+5.21 | 108.00±8.99 | 109.90±8.10 | 120.00±8.31\* |

The number of mice in each group was 6, the data are as Mean ± SD,

\*P<0.05 compared to control group.

There was no statistically significant (P>0.05) difference in urea and creatinine in all the groups compared to the control (**Table 2).**

Urea and creatinine are considered as a suitable prognostic indicator of renal dysfunction and kidney failure for any toxic compounds(Gnanamani et al.,2008). In this study, the absence of significant differences in these parameters after the Day 14 that hesperidin extract has no harmful effect on the kidney.

blood glucose concentration was significantly increased when the animals were treated with hesperidin (**200 mg/kg)**,( de Oliveira et al., 2013). On other hand hesperidin supplementation has important hypoglycemic effects by modulation of gene expression of hepatic enzymes such as glucokinase and glucose-6-fosfatase which are involved in the final step of catalyzing the gluconeogenesis and glycogenolysis, thus playing a role in regulating the homeostatic plasma glucose (Liu et al., 2008).

One of the signs of progression of chronic liver disease is decreased level of total protein. The level of this deduction is proportionate with the severity of liver damage (Sethuraman et al.,2003 and Jayasekhar et al.,1997). In this study, we showed that the level of total protein could be elevated by hesperidin. This elevation is significant with higher doses of hesperidin. Therefore, we can say that this extract can have protective effect for liver. Polyphenols and flavonoids in this plant can protect cells against diminution of reduced glutathione. This action is performed by elevation of antioxidative enzymes such as glutathione, glutathione reductase, glutathione peroxidase and catalase (Chu et al., 2002). In conclusion, hesperidin may have a protective effect on liver in animal models and more studies are needed to evaluate its effect in human.

**RECOMMENDATIONS:**

1. We recommend most students specially group of Veterinary Medicine College / department of public health to important for this project and make deep study on hesperidin.
2. In Future studies with a prospective design and larger sample size should be able to ascertain the results of our findings and develops this study to master thesis.

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