Protective Effect of Hesperidin (HDN) on Carbon Tetrachloride (CCl4)-Induced Hepatic Toxicity in Male Albino Rats

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Conflict of Interest - None

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Abstract:

An experimental study was conducted to evaluate the protective effects of an antioxidant (Hesperidin) on carbon tetrachloride-induced hepatic toxicity. This effect was evaluated through assessment of liver functions as well as histopathological changes in livers of rats exposed to Hesperidin prior to carbon tetrachloride. Thirty two male albino rats were distributed to four equal groups each of 8 rats. Group I (Negative control). Group II (Positive control group): received vehicle (Carboxymethyl Cellulose) for 10 days and were challenged with CCl4 2 ml/kg/SC (40% v/v in olive oil) on 8th day. Group III (HDN: 100 mg/kg): rats received HDN continuously for 8 days. On 8th day, they received CCl4 2ml/kg/SC in olive oil. HDN was further continued for 2 more days. Group IV (HDN: 200 mg/kg): rats received HDN continuously for 8 days. On 8th day, they received CCl4 2ml/kg/SC in olive oil. HDN was further continued for 2 more days. After ten days of treatment, liver enzymes, oxidant parameters as malondialdehyde and antioxidant parameters as glutathione and superoxide dismutase were assessed. Histopathological examination of the liver tissues was conducted. Hesperidin in the dose of 100 and 200 mg/kg produced a significant decrease in the levels of liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and oxidant parameters as malondialdehyde. Antioxidant parameters as glutathione and superoxide dismutase also have shown significant increase. These findings were confirmed by histopathology. Hesperidin in a dose of 100 and 200 mg/kg offers dose-dependent significant protection against hepatotoxicity produced by CCl4 in albino rats.

Keywords: Hesperidin, Antioxidants, Hepatic Toxicity, CCl4
Introduction:
The liver is the main organ involved in metabolism of biological toxins and medicinal agents. Such metabolism is associated with disturbance of hepatocyte biochemistry and generation of reactive oxygen species (ROS). [1] Oxidative stress, resulting from an imbalance in generation of free radicals and antioxidant defense molecules, affects biological macromolecules causing their structural alterations that lead to cell damage and death. [2] This phenomenon is considered a major factor in the pathogenesis of a variety of liver diseases. In this regard, reduction of oxidative stress may be a good target for prevention and treatment of hepatic and renal toxicity. [3] Considering hazards of treatment failure, drug resistance and high costs associated with current hepatic and renal therapy, there is an interest in study of natural compounds with free radicals scavenging capacity. [4]

Hepatotoxicity Implies chemical-driven liver damage induced by certain medicinal agents and other chemical agents.[5] Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failure.[5&6] There are increasing evidences that free radicals and reactive oxygen species play a crucial role in the various steps that initiate and regulate the progression of liver diseases independently of the agent in its origin.[7]

Oxidative stress in hepatotoxicity, resulting from increased generation of reactive oxygen species (ROS) and other reactive intermediates as well as by decreased efficiency of antioxidant defenses, actively contributes to excessive tissue remodeling.[8] In the present study, induction of acute hepatic toxicity in Wistar male albino rats was done by SC injection of CCl₄ 2 ml/kg (40% v/v in olive oil) characterized model for acute hepatic toxicity has been extensively performed and revealed microscopically in the liver as extensive damage, very severe vaculation, inflammatory cells infiltration, irregular architecture (damaged sinusoids, rows and disintegrated central vein) and degenerated nuclei.[9]

The mechanism of hepatotoxicity undergoes two phases. The first resulted from its metabolic conversion to trichloromethyl free radical (CCl₃⁺) by cytochrome P450 mainly (CYP2E1 and CYP2B1) which react very rapidly with oxygen to produce more reactive trichloro-methylperoxy (CCl₃O₂) free radical.[10] These free radicals attack microsomal lipids, DNA and proteins in the endoplasmic reticulum leading to initiating a chain of lipid peroxidation, cell necrosis and liver fibrosis.[11]

CCl₄ not only initiates lipid peroxidation but also depletes tissue GSH and SOD.[12] Antioxidants such as vitamin E has been shown to be hepatoprotective molecules in animal models of acute toxicity where inflammation and fibrosis are primarily involved, but might not be efficient on early signs of toxicity.[13] Glutathione is an important intracellular antioxidant that also plays a role in the detoxification and elimination of potential carcinogens and toxins. Studies in animals have found that glutathione synthesis and tissue glutathione levels are significantly lower in aged animals than in younger animals, leading to decreased ability of aged animals to respond to oxidative stress or toxin exposure.[14] SOD catalyzes the destruction of the O₂⁻ free radical (2O₂⁻ + 2H⁺ → O₂ + H₂O₂). It protects oxygen-metabolizing cells against harmful effects of superoxide free-radicals. [15] CCl₄ challenge significantly decreased the levels of SOD and catalase in liver, by alteration in gene expression and depletion of SOD and catalase levels. [16] Antioxidants are agents that inhibit or neutralize potentially harmful elements known as free radicals. [17&18] Flavonoids are naturally occurring polyphenolic compounds in plants that are thought to have positive effects on human health. [19] HDN administration ameliorates the increased level of lipid peroxidation after CCl₄ treatment, able to show improvement in the levels of endogenous antioxidant enzymes SOD and improvement of hepatic GSH levels in HDN-treated rats in comparison to CCl₄ intoxicated rats, thereby, this demonstrates the antioxidant effect of HDN. [20] Flavonoids are known to operate via direct scavenging of ROS, chelation of redox active transition metal ions, inhibition of enzymes involved in ROS production, regeneration of endogenous antioxidants.[17&21] It was found that HDN has an important antioxidant activity in humans, it enhances the integrity of the blood.

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vessels and it is found in great quantity in citrus fruits (lemons and oranges).[22] HDN and Silymarin are polyphenolic compounds which play an important role as antioxidants; they can directly quench free radicals, inhibit the enzymes of oxygen reduction pathways and also prevent the sequestration of transient metal actions.[23&24]

The present study aims at the investigation of the ability of Hesperidin (HDN) as antioxidant to retard development of acute hepatic and renal toxicity induced by CCL₄ in rat. In addition to histopathological examination, the following will be evaluated: liver enzymes, markers of oxidative stress (oxidants and antioxidants) as malondialdehyde, glutathione and superoxide dismutase content in liver homogenate.

Materials and Methods:

Materials:

1. Experimental Animals: This study was conducted on 32 male albino rats. Their weight ranged between 160-200 gm. Rats were housed as 4 groups with 8 rats each in clean capacious macro lane cages under standard laboratory conditions.[25]

2. Drugs: CCL₄ (El-Nasser Pharmaceuticals chemical company, Egypt) and Hesperidin (HDN: Sigma, Aldrich)

3. Chemicals: Saline (El-Nasser Pharmaceuticals chemical company, Egypt), Phosphate buffered saline (Hi-media- Lab. Pvt. Inc., USA), SOD, Malondialdehyde and Glutathione reduced kits (Biochemical Enterprise, Italy) & ALT/AST kits (Centronic_Gmbh, Germany)

Experimental Design: Animals were divided into (4) groups, each consisted of (8) rats. Animals were fed on commercial pellet food, water was supplied freely.

Group-I (Control negative): rats received 5% carboxymethyl cellulose orally (as a vehicle) for 10 days and were injected by olive oil subcutaneously in the 8th day. [20]

Group-II (Control positive): These animals received 5% carboxymethyl cellulose orally for 10 days and were challenged with CCL₄, 2 ml/kg/SC (40 % v/v in olive oil) on 8th day. [9]

Group-III (HDN 100): These rats received HDN 100 mg/kg/PO daily for 10 days. On the 8th day they received CCL₄ 2ml/kg/SC in olive oil once. HDN was further continued for 2 more days. [20]

Group-IV (HDN 200): These rats received HDN 200 mg/kg/PO daily for 10 days. On the 8th day they received CCL₄ 2ml/kg/SC in olive oil once. HDN was further continued for 2 more days. [20]

Procedures:

Blood sampling:

At the end of the experiment, rats were sacrificed and blood samples were collected from the retro-orbital vein of each animal, under light anesthesia by diethyl ether, according to the method of Cocchetto and Bjornsson (1983). [26] Blood samples were then centrifuged and the serum from each animal was kept in epindorff tubes in the deep freezer at (-20°C) until analyzed for liver functions.

Preparation of Liver homogenate:

Animals were sacrificed; livers were immediately excised, rinsed from blood in ice cold saline and blotted dry by filter papers. Small piece of each liver was fixed in 10% phosphate-buffered formalin for histological examination. About 0.5 gm of each liver was homogenized by ultrasonic homogenizer in 5 ml ice-cold phosphate buffered saline (PBS) to obtain ultimately10% (w/v) whole liver homogenate. [4] The homogenate was centrifuged at 3000 rpm for 15 min and the resultant supernatant was stored at (-20°C) until used for determination of reduced Glutathione (GSH), Malondialdehyde (MDA), Superoxide dismutase (SOD) and Hydroxyproline concentration.

- Determination of liver function: Commercial kit Purchased from (Centronic_Gmbh, Germany) based on the method described by Thomas, (1998) was used for determination of ALT & AST activity.[27]
- Determination of hepatic reduced glutathione (mg/g tissue): the method based on the reduction of 5, S’ dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm.[28]
- Determination of hepatic superoxide dismutase (U/g tissue): this assay relies on the ability of the enzyme to inhibit the
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- Phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.[29]
- Determination of hepatic lipid peroxide (Malondialdehyde) (nmol/g tissue): it was determined colorimetrically in serum according to Ohkawa et al., (1979).[30]

Figure (1): Showing Comparison of Results of Biochemical Tests Among Groups (I, II, III and IV)

Histopathological Results:

Group (I) Control negative: were fed on 5% Carboxymethyl cellulose (as a vehicle) only for 10 days & were injected by olive oil S.C in the 8th day Normal (liver tissue, architecture, rows, cellular appearance and apparent nuclei) No "Inflammatory cell infiltrate"

Figure (2): Liver tissue of group (I: Control negative)

Group (II) Control positive: fed on 5% Carboxymethyl cellulose (as a vehicle) only for 10 days & were injected by CCl4 in olive oil (2 ml/kg) SC in the 8th day. Extensive damage, very severe vaculation, inflammatory cell infiltration, disruption of the lattice

Figure (3): Liver tissue of Group (II: Control positive)

nature of hepatocytes and damaged hepatocyte cell membrane, irregular architecture (damaged sinusoids, rows and disintegrated central vein) & degenerated nuclei

Group (III): treated with HDN as 100 mg/kg in the vehicle for 10 days and were injected by CCl4 in olive oil (2 ml/kg) SC in the 8th day. Vaculation occurs but less than Control positive group, more eosinophilic infiltration than Control positive group.

Figure (4): Liver tissue of Group (III) treated with HDN (100 mg/kg)

Better viability and less damage than Control positive group, nuclei are healthier than PositiveControl group, less disruption of the lattice nature of hepatocytes and less damaged hepatocyte cell membrane, more regular architecture and rows than Control positive.
Group (IV): treated with HDN as 200 mg/kg in the vehicle for 10 days and were injected by CCl$_4$ in olive oil (2 ml/kg) SC in the 8$^{th}$ day. Faded vaculation (very mild), architecture and rows are so close to normal, normal viability, less infiltration by the inflammatory cells than treated groups by (HDN100), normal nuclei and cell membranes, normal central vein and sinusoids.

Figure (5): Liver tissue of Group (IV) treated with HDN (200 mg/kg)

Results:
Serum levels of liver enzymes (AST and ALT) were significantly increased in group-II, but when treated with HDN (100 mg/kg/day) there was a significant decreased in levels of the two enzymes. The decrease in values of the two enzymes was significant in rats treated with an increase HDN dose (200 mg/kg/day). Malondialdehyde level was increased significantly in rats treated with CCl$_4$ (group-II) and decreased significantly in rats treated with HDN (200 mg/kg/day). Glutathione and Superoxide dismutase levels were decreased significantly in group-II and increased significantly in rats treated with HDN (200 mg/ kg/ day) (table: 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Group (I)</th>
<th>Group (II)</th>
<th>Group (III)</th>
<th>Group (IV)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control negative</td>
<td>No CCL$_4$</td>
<td>Control positive</td>
<td>HDN (100 mg/kg)</td>
<td>HDN (200 mg/kg)</td>
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<tr>
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<td>(100 mg/kg)</td>
<td>(200 mg/kg)</td>
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<td></td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
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<tr>
<td>Malondialdehyde</td>
<td>(nmol/gm tissue)</td>
<td>49.013 ± 1.03</td>
<td>82.763 ± 0.91</td>
<td>81.625 ± 0.68$^*$</td>
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<tr>
<td>Glutathione</td>
<td>(mg/gm tissue)</td>
<td>5.088 ± 0.06</td>
<td>2.88 ± 0.048</td>
<td>3.09 ± 0.067$^*$</td>
<td>#5.025 ± 0.072</td>
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<tr>
<td>Superoxide dismutase</td>
<td>(U/gm tissue)</td>
<td>107.888 ± 0.56</td>
<td>89.688 ± 0.45</td>
<td>90.863 ± 0.26$^*$</td>
<td>#107.013 ± 1.77</td>
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<tr>
<td>AST (Aspartate aminotransferase)</td>
<td>(IU/L)</td>
<td>48.725 ± 0.47</td>
<td>163.875 ± 2.99</td>
<td>#111.375 ± 1.78$^{**}$</td>
<td>#71.375 ± 1.71$^{**}$</td>
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<tr>
<td>ALT (Alanine aminotransferase)</td>
<td>(IU/L)</td>
<td>38.5 ± 0.76</td>
<td>87.875 ± 1.46</td>
<td>#57.375 ± 1.28$^{**}$</td>
<td>#46.5 ± 0.94$^{*}$</td>
</tr>
</tbody>
</table>

Table (1): Showing Comparison of Results of Biochemical Tests Among Groups (I, II, III and IV)

* means statistical significance at P < 0.05 as compared to group (I); n = number of rats
# means statistical significance at P < 0.05 as compared to group (II)
** means P < 0.001 which indicates high significance as compared to group (I)
## means P < 0.001 which indicates high significance as compared to group (II)

Both doses of HDN have prominent prevention of hepatic damage which was assessed microscopically, but this prevention is dose dependent (figures: 4 and 5).
Histopathological Results:

Group (I) Control negative: was fed on 5% Carboxymethyl cellulose (as a vehicle) only for 10 days & were injected by olive oil S.C in the 8th day. Normal (liver tissue, architecture, rows, cellular appearance and apparent nuclei) No "Inflammatory cell infiltrate"

Group (II) Control positive was fed on 5% Carboxymethyl cellulose (as a vehicle) only for 10 days & were injected by CCl4 in olive oil (2 mL/kg) SC in the 8th day. Extensive damage, very severe vaculation, inflammatory cell infiltration, disruption of the lattice nature of hepatocytes and damaged hepatocyte cell membrane, irregular architecture (damaged sinusoids, rows and disintegrated central vein) & degenerated nuclei.

Group (III) was treated with HDN as 100 mg/kg in the vehicle for 10 days and were injected by CCl4 in olive oil (2 mL/kg) SC in the 8th day. Vaculation occurs but less than Control positive group, more eosinophilic infiltration than Control positive group. Better viability and less damage than Control positive group, nuclei are healthier than Positive Control group, less disruption of the lattice nature of hepatocytes and less damaged hepatocyte cell membrane, more regular architecture and rows than Control positive.

Group (IV) was treated with HDN as 200 mg/kg in the vehicle for 10 days and were injected by CCl4 in olive oil (2 mL/kg) SC in the 8th day. Faded vaculation (very mild), architecture and rows are so close to normal, normal viability, less infiltration by the inflammatory cells than treated groups by (HDN100), normal nuclei and cell membranes, normal central vein and sinusoids.

Discussion:

The results of the present study are in agreement with the results obtained by Al-Qarawi A.A. et al., 2004, who reported the histopathological changes in acute hepatic toxicity by Montilla M.P. et al., 1990, who proved CCl4 hepatotoxicity by 2 mL/kg/SC (LD50) of CCl4, the modification of Nembutal-induced sleep, the action on bile flow, serum transaminase and hepatic fatty acids levels and a histopathological study of liver tissue.[31&54] Kodama K. et al., 1990 and Prakash T. et al., 2008, have reported similar results to our study on the effect of CCl4 on hepatic architecture.[32&33] CCl4, an industrial solvent, is a well-established hepatotoxin, through free radical generation.[34&35&36]

In the present study, CCl4 induces a severe hepatic damage as represented by markedly elevated levels of ALT and AST. These results are in agreement with the studies of Mousa H.M. et al., 2004, Prakash T. et al., 2008; and Alam K. et al., 2000) that reported administration of CCl4 caused hepatotoxicity detected by increased levels of ALT and AST. Usually, the extent of hepatic damage is assessed by increased level of cytoplasmic enzymes (ALT and AST).[31&33&37] This was associated with massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver.[38]

The results of the present study have been showed that; subcutaneous injection of CCl4 lead to decreased levels of hepatic reduced glutathione (GSH), superoxide dismutase (SOD) and increased Malondialdehyde (MDA) level. These results are in agreement with the studies done by Manjrekar A. et al., 2008 who found that CCl4 causes decreased hepatic GSH level and increased MDA level.

Oxidative stress, presumably by favoring mitochondrial permeability transition, is able to promote hepatocyte death (necrotic and/or apoptotic). In some of clinically relevant conditions, generation of ROS within hepatocytes may represent a consequence of an altered metabolic state (like in NAFLD and NASH), with ROS being generated mainly by mitochondrial electron transport chain or through the involvement of selected cytochrome P450 isoforms like (CYP2E1). [39&53]

The results of the present study showed that oral administration of HDN (100 mg/kg) and (200 mg/kg) significantly decrease the ALT and AST in CCl4-treated rat and in the group of the dose (200 mg/kg) produces more decrease in ALT and AST. The results of the present study are similar to the study done by Ahmad S.T. et al. (2012) who proved that HDN ameliorates the hepatotoxicity-induced by acetaminophen and this was detected by decrease in ALT and AST not only that but also he noticed that the acuity of toxicity is decreased gradually by increasing the dose of HDN similar to our results. [40]

Balakrishnan A. and Menon V.P. (2007) reported that administration of HDN to nicotine-treated rats...
at different doses affects these enzymes significantly but in dose-dependent manner. Anandan R. and Ramaswamy P. (2012) reported protective effects of HDN (100 mg/kg) for 14 days against Gentamycin-induced hepatotoxicity (GEN 100 mg/kg) for 8 days detected by decrease in ALT and AST. Park S.H. et al. (2012) reported that protective effects of "HDN + Curdlan (CDN 100 mg/kg)" for 7 days against γ-irradiation-induced hepatotoxicity.[43] CCl₄ induced a severe hepatic damage as represented by markedly elevated levels of ALT and AST coupled with a marked hepatic oxidative stress.[20] Oxidative stress in hepatotoxicity, resulting from increased generation of reactive oxygen species (ROS) and other reactive intermediates as well as by decreased efficiency of antioxidant defenses, actively contributes to excessive tissue remodeling.[8] HDN in combination with diosmin shows a marked protective effect against inflammatory disorders, both in vivo and in vitro, possibly through a mechanism involving an inhibition of eicosanoid synthesis and/or antioxidant free radical scavenger activity. [44] The results of the present study showed that oral administration of HDN (100 mg/kg) causes insignificant decrease in MDA and insignificant increase in hepatic GSH and SOD levels. The results of the present study are similar to the study done by Tirkey N. et al., (2005) who proved that; oral administration of HDN (100 mg/kg) causes insignificant decrease in MDA and insignificant increased hepatic GSH and SOD levels.[20] The results of the present study are in contrast to the study done by Park et al. (2012) who observed protective effects of "HDN + CDN 100 mg/kg" for 7 days against γ-radiation-induced hepatotoxicity, through significant decrease in MDA and significant increased hepatic GSH and SOD levels. The results of the present study showed that oral administration of HDN (200 mg/kg) causes significant decrease in MDA and significant increased hepatic GSH and SOD levels. [43] The present study disagrees with Stryjecka-Zimmer. et al., 2003. The results suggest that change in antioxidant enzyme activities may be relevant to the ability of the liver and other investigated organs to cope with oxidative stress during CCl₄ poisoning No statistically significant changes in SOD and glutathione peroxidase (GPX) activities were observed in the liver after CCl₄ administration. [16] The radical scavenging power of flavonoids is thought to be related to their structure. Flavonoids in general, scavenge oxidizing radicals preferentially via their B-ring catechol; in particular the ortho-dihydroxy structure in the B-ring gives a higher stability during the formation of aroxyl radicals and participation in electron dislocation. The presence of the 3’ and 5’ OH functions together give a maximum radical scavenging potential; this property is found in both Silymarin and Hesperidin. [45&46&47] The results of the present study are in agreement with the study done by Xiao-min Y. et al., (2011) who reported significant decrease in MDA and significant increased hepatic GSH and SOD levels by studying the protective effect of HDN on hepatotoxicity-induced by Cisplatin. [48] Wei L. and Jun L. (2010) reported that HDN had protective effects on CCl₄-induced chemical liver injury. It was possibly related to removal of free radicals and inhibition of lipid peroxidation. HDN (250 and 500 mg/kg) could reduce the levels of MDA and significant increased hepatic SOD level. Wei L. and Jun L. (2010) also observed certain cytokines as (IL-1 and TNF) are inhibited by HDN (250 and 500 mg/kg) through decreasing mRNA expression.[32] Xiao-min Y. et al. (2011) reported that, administration of HDN (300 mg/kg P.O.) for 7 consecutive days had a remarkable protective effect on hepatotoxicity-induced by Cisplatin (5 mg/kg, intraperitoneally for 5 consecutive days from the third day of HDN administration).[48] The protective effect of HDN was possibly related to removal of free radicals and inhibition of lipid peroxidation produced by Cisplatin intoxication. HDN (300 mg/kg) could reduce the levels of MDA, significant increased hepatic SOD level and significant increased GSH. Also, Tirkey N. et al., (2005) and Pradeep K. et al. (2008) obtained similar results to our study on the effect of Hesperidin on oxidants and antioxidants parameters. [20&49] Kodama K.M. et al. (1995) reported that certain natural extracts containing antioxidants protect against the CCl₄-induced increased lipid peroxide levels and impairment in hepatic GSH status. [51] Hepatic MDA levels were also highly significantly increased in CCl₄ treated group.
showing an increased oxidative stress compared to control group. The increased MDA level suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals as described by (Pereira-Filho G. et al., 2008) and this is confirmed by (Kim H.Y. et al., 2010).[50&52]

The results of the present study showed that; oral administration of HDN (100 and 200 mg/kg) significantly improves hepatic architecture microscopically in a dose-dependent manner as the group of HDN administration (100 mg/kg) shows slight improvement while the group of HDN administration (200 mg/kg) show no difference with control normal group.

**Conclusion:**

The present study suggested that the antioxidant properties of HDN might be the main factor responsible for its strong protective action on CCl4-induced hepatotoxicity. **Hesperidin** in a dose of 100 and 200 mg/kg produced a significant decrease in the levels of liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and oxidant parameters as malondialdehyde. Antioxidant parameters as glutathione and superoxide dismutase also have shown significant increase. These findings were confirmed by histopathology. **Hesperidin** in a dose of 100 and 200 mg/kg offers significant protection against hepatotoxicity produced by CCl4 in albino rats, and this protection is dose-dependent.

**Conflict of Interest:**

The authors confirmed that this article and its content have no conflicting interest.

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**References:**


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24. Berker K., Gucu K., Tor I. and Apak R. Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP) and ferricyanide reagents. Talanta 2007; 72:1157–1165.


48. Xiao-min Y., Jun-ge Q., Qing-zhi L., Hong-wei L., Guo-kang W. and Quan-yi X. Comparative Effects of Baicalin and

International Journal of Contemporary Research and Review, Vol. 8, Issue. 11, Page no: MS 20328-20338
Hesperidin on Hepatotoxicity Induced by Cisplatin in Mice. Journal of Liaoning University of Traditional Chinese Medicine, 2011; 06.


