



Research Article

HESPERIDIN (HDN) AN ANTIOXIDANT FLAVONOID PREVENTS CARBON TETRACHLORIDE (CCl₄) - INDUCED HEPATIC TOXICITY IN MALE ALBINO RATS

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ABSTRACT

Background: Through this research work, 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.

Materials and Methods: Thirty two male albino rats (160-200 gm) were chosen as an animal model for this study and distributed to four equal groups each of 8 rats. **Group I** (Negative control group, i.e., No CCl₄ or HDN). **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, the following were assessed: liver enzymes, tumour necrosis factor -alpha, oxidant parameters as malondialdehyde and antioxidant parameters as glutathione, superoxide dismutase, and total antioxidant capacity. Histopathological examination of the liver tissues was conducted.

Results: 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), tumour necrosis factor-alpha (TNF-α), and oxidant parameters as malondialdehyde (MDA). Antioxidant parameters as glutathione (GSH), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC), also have shown significant increase. These findings were confirmatory to histopathology.

Conclusion: Hesperidin in a dose of 100 and 200 mg/kg offers significant protection against hepatotoxicity produced by CCl₄ in albino rats, but this protection is dose-dependent.

KEYWORDS: Hesperidin (HDN), Antioxidants, Hepatic Toxicity and 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) (Fernandez-Checa, and Kaplowitz, 2005).

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 (Ryter, et al., 2007). 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 (Flora, 2007).

This 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, TNF-α, T-AOC in serum, markers of oxidative stress (oxidants and antioxidants) as MDA, GSH, and SOD in liver homogenate.

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MATERIALS AND METHODS**Materials:**

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 macrolane cages under standard laboratory conditions.

Drugs: CCl₄ (El-Nasser Pharmaceuticals chemical company, Egypt) and Hesperidin (HDN: Sigma, Aldrich).

Chemicals: Saline (El-Nasser Pharmaceuticals chemical company, Egypt), Phosphate buffered saline (Hi-media- Lab. Pvt. Inc., USA), SOD, MDA and GSH reduced kits (Biochemical Enterprise, Italy), ALT/AST kits (Centronic_GmbH, Germany) TNF-α, ELISA kit (Ray Biotech, Inc., USA), T-AOC assay kit, (Cayman Chemical Company, USA).

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 (Tirkey, et al., 2005).

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 (Mandal, et al., 2002).

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 (Tirkey, et al., 2005).

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 (Tirkey, et al., 2005).

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 anaesthesia by diethyl ether, according to the method of Cocchetto, and Bjornsson, (1983). Blood samples were then centrifuged and the serum from each animal was kept in epindorf tubes in the deep freezer at (-20°C) until analyzed for liver functions, TNF-α, and T-AOC.

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 ultimately 10% (w/v) whole liver homogenate (Ezz, et al., 2011). 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).

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.

Determination of serum tumor necrosis factor alpha (Pg /ml) by using rat specific ELISA kit (Cat#: ELR-TNFalpha-001)

Determination of serum total antioxidant capacity (μmol/l) by Koracevic, et al. (2001). The determination of T-AOC is performed by the action of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H₂O₂). The antioxidants in the

sample eliminate a certain amount of the provided H₂O₂. The residual H₂O₂ is determined calorimetrically by an enzymatic reaction with involves the conversion of 3,5-dichloro-2-hydroxy benzenesulphonate to a colored product. The absorbance were recorded spectrophotometrically immediately at 505 nm.

Determination of hepatic reduced glutathione (mg/g tissue): the method based on the reduction of 5, 5' 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 (Beutler, et al., 1963).

Determination of hepatic superoxide dismutase activity (U/g tissue): this assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye (Nishikimi, et al., 1972).

Determination of hepatic lipid peroxide (Malondialdehyde) (nmol/g tissue): it was determined colorimetrically according to Ohkawa, et al., (1979).

RESULTS

Serum levels of liver enzymes (AST and ALT) and TNF-α were significantly increased in group-II, but when treated with HDN (100 mg/kg/day) there was a significant decreased in activity of the two enzymes. The decrease in activities of the two enzymes was significant in rats treated with an increase HDN dose (200 mg/kg/day). Also hesperidin (100, 200 mg / kg/day) exerted a significant decrease TNF-α, Malondialdehyde level was increased significantly in rats treated with CCl₄ (group-II) and decreased significantly in rats treated with HDN (200 mg/kg/day). Glutathione, Superoxide dismutase, and total antioxidant capacity were decreased significantly in group-II and increased significantly in rats treated with HDN (100, 200 mg / kg / day) (table 1 and fig. 1).

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

Table No. 1: Showing Comparison of Results of Biochemical Tests Among Groups (I, II, III and IV)

Groups	Group (I) Control negative No CCl ₄ No HDN n = 8	Group (II) Control positive CCl ₄ NO HDN n = 8	Group (III) HDN (100 mg/kg) n = 8	Group (IV) HDN (200 mg/kg) n = 8
Parameters				
Malondialdehyde (nmol/gm tissue)	49.013 ± 1.03	82.763 ± 0.91	81.625 ± 0.68*	##50.2 ± 0.38
Glutathione (mg/gm tissue)	5.088 ± 0.06	2.88 ± 0.048	3.09 ± 0.067*	##5.025 ± 0.072
Superoxide dismutase (U/gm tissue)	107.888 ± 0.56	89.688 ± 0.45	90.863 ± 0.26*	##107.013 ± 1.77
AST (Aspartate aminotransferase) (IU/L)	48.725 ± 0.47	163.875 ± 2.99	#111.375 ± 1.78**	##71.375 ± 1.71*
ALT (Alanine aminotransferase) (IU/L)	38.5 ± 0.76	87.875 ± 1.46	#57.375 ± 1.28**	##46.5 ± 0.94*
TNF-α (Tumor necrosis factor-α) Pg /ml	35.75 ± 1.816	131.417 ± 3.150	#81.766 ± 2.994**	##73.666 ± 2.689**
T-AOC (Total antioxidant capacity) μmol/l	116.050 ± 2.024	35.400 ± 1.864	#56.566 ± 5.587**	##70.166 ± 2.734**

* 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)

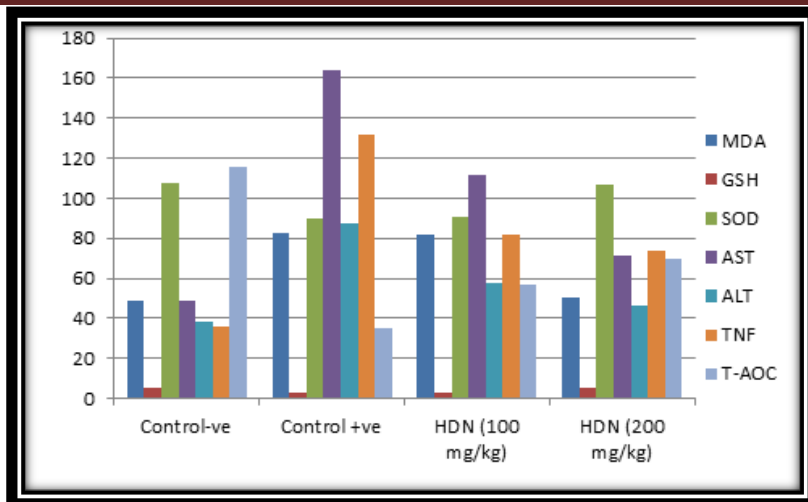


Fig. 1: Showing Comparison of Results of Biochemical Tests Among Groups (I, II, III and IV)

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" (Fig. 2).

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 vacuolation, 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 (Fig. 3).

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. Vacuolation occurs but less than Control positive group, more eosinophilic infiltration than Control positive group (Fig. 4).

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): 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 vacuolation (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 (Fig.5).

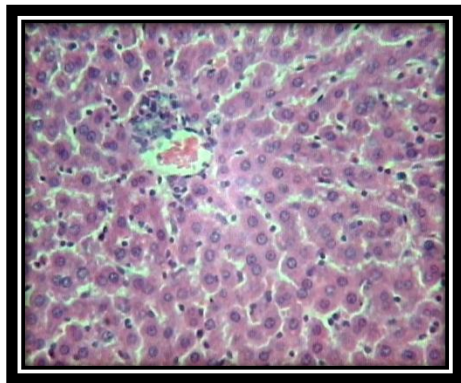


Fig. 2: Liver tissue of group (I: Control negative)

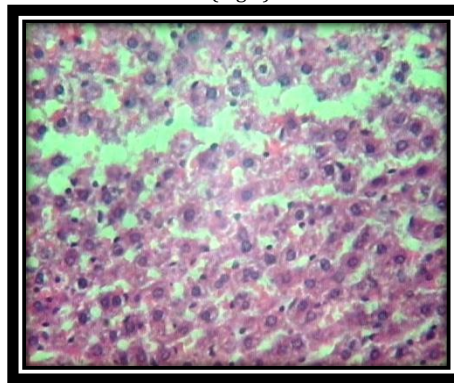


Fig. 3: Liver tissue of Group (II: Control positive)

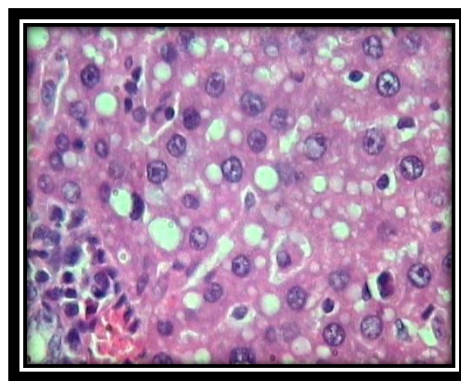


Fig. 4: Liver tissue of Group (III) treated with HDN (100 mg/kg)

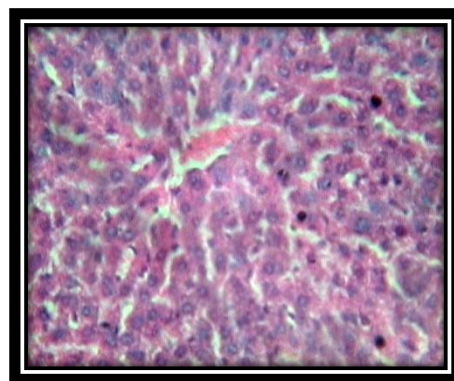


Fig. 5: Liver tissue of Group (IV) treated with HDN(200 mg/kg)

DISCUSSION

Hepatotoxicity Implies chemical-driven liver damage induced by certain medicinal agents and other chemical agents (Ostapowicz, *et al.*, 2002). Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failure (Ostapowicz, *et al.*, 2002 & McNally and Peter, 2006).

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 (Vitaglione, *et al.*, 2004).

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 defences, actively contributes to excessive tissue remodeling (Ismail, and Pinzani, 2009).

In the present study, induction of acute hepatic toxicity in Wister 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 vacuolation, inflammatory cells infiltration, irregular architecture (damaged sinusoids, rows and disintegrated central vein) and degenerated nuclei (Mandal, *et al.*, 2002).

These results are in agreement with the results obtained by Al-Qarawi, *et al.*, (2004), who reported the histopathological changes in acute hepatic toxicity by Montilla, *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. Kodama, *et al.*, (1990) and Prakash, *et al.*, (2008), have reported similar results to present study on the effect of CCl₄ on hepatic architecture. CCl₄, an industrial solvent, is a well-established hepatotoxin, (Abraham, *et al.*, 1999 & Gulmez, *et al.*, 2003) through free radical generation (Gonzalez, *et al.*, 1987).

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 trichloromethylperoxy (CCl₃OO) free radical (Vulimiri, *et al.*, 2011). 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 (Fang, *et al.*, 2008). CCl₄ not only initiates lipid peroxidation but also depletes tissue GSH and SOD (Augustyniak, *et al.*, 2005).

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 (Galicia-Moreno, 2009), but might not be efficient on early signs of toxicity.

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 Alam, *et al.*, (2000); Mousa, *et al.*, (2004) and Prakash, *et al.*, (2008) 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). This was associated with massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver (Shankar, *et al.*, 2012).

According to Abdel-sttar, *et al.*, (2017) a significant increase in serum tumor necrosis factor alpha (TNF-α) level. That is in harmony with these results, in the present study, injection of the rats with CCl₄ decreased the antioxidant capacity of liver as evidenced by the decreased GSH level and activities of SOD, and T-AOC, which is harmony with the results obtained by Abdalla, *et al.*, (2013)

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

The results of the present study are in contrast to (Stryjecka-Zimmer, *et al.*, 2003) reported no changes in SOD and glutathione peroxidase (GPX) activities were observed in the liver after CCl₄

administration and explained that by the ability of the liver to cope with oxidative stress.

Indeed, oxidative stress, presumably by favouring 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) (Tilg, and Hotamisligil, 2006).

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. Also decrease in serum (TNF-α) level is in agreement with the results of Abdel-sttar, *et al.*, (2017), and of Abdel-Monium *et al.* (2011) who discussed that decreased in elevated TNF-α by hesperidin and naringin in diabetic rats along with their blood glucose lowering effect suggests that the immunomodulatory properties of both flavonoids.

Feng *et al.* (2011) and Wang *et al.* (2010) who found that the different flavonoid-rich extracts have the ability to decrease oxidative stress by promoting T-AOC, that is agreement with this result, which indicate the antioxidant properties of HDN.

The results of the present study are similar to the study done by Ahmad, *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 this results.

Balakrishnan, and Menon, (2007) reported that administration of HDN to nicotine-treated rats at different doses decreases these enzymes significantly but in dose-dependent manner.

Anandan, and Ramaswamy, (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, *et al.* (2012) who reported that protective effects of "HDN + Curdlan (CDN 100 mg/kg)" for 7 days against γ-radiation-induced hepatotoxicity.

CCl₄ induced a severe hepatic damage as represented by markedly elevated levels of ALT and AST coupled with a marked hepatic oxidative stress (Tirkey, *et al.*, 2005). 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 (Ismail, and Pinzani, 2009).

Hesperidin 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 (Jean, and Bodinier, 1994).

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, *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.

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.

The results of the present study are in agreement with the study done by Xiao-min, *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.

Wei, and Jun, (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, and Jun, (2010)**, also observed certain cytokines as (IL-1 and TNF) are inhibited by HDN (250 and 500 mg/kg) through decreasing mRNA expression.

Xiao-min, 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).

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, et al. (2005)** & **Pradeep, et al., (2008)** obtained similar results to this study on the effect of Hesperidin on oxidants and antioxidants parameters.

Ko, 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.

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, et al., 2008)** and this is confirmed by **(Kim, et al., 2010)**.

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 **(Hagen, et al., 2000)**.

SOD catalyzes the destruction of the O₂⁻ free radical ($2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$). It protects oxygen-metabolizing cells against harmful effects of superoxide free-radicals **(Petkau, et al., 1975)**

CCl₄ challenge significantly decreased the levels of SOD and catalase in liver, by alteration in gene expression and depletion of SOD and catalase levels **(Stryjecka-Zimmer, et al., 2003)**.

Antioxidants are agents that inhibit or neutralize potentially harmful elements known as free radicals **(Zielinska, et al., 2001 & Galati, and O'brien, 2004)**.

Flavonoids are naturally occurring polyphenolic compounds in plants that are thought to have positive effects on human health **(Wahsha, and Al-Jassabi, 2009)**.

Hesperidin 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 **(Tirkey, et al., 2005)**.

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 **(Fitzgeorge, et al., 1994 & Zielinska, et al., 2001)**. It was found that HDN has an important antioxidant activity in humans, it enhances the integrity of the blood vessels and it is found in great quantity in citrus fruits (lemons and oranges) **(Tripoli, et al., 2007)**.

Hesperidin 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 **(Chatterjee, et al., 1999 & Berker, et al., 2007)**.

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 aroxy 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 **(Markham, 1982; Joshi, et al., 2005 & Andersen, and Markham, 2006)**.

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.

This result is in agreement with the study done by **(Balakrishnan, and Menon, 2007)** who observed that administration of HDN to nicotine-treated rats at different doses improves hepatic architecture significantly in dose-dependent manner even in high doses, he doesn't observe any morphological changes compared to normal.

Ahmad, et al., (2012) observed that HDN alleviates acetaminophen-induced toxicity in a dose-dependent manner and in high doses he doesn't observe any morphological changes compared to normal.

Also **Bentli, et al., (2013)** obtained similar results to present study on the effect of HDN on hepatic architecture.

CONCLUSION

The present study suggested that the imbalance between production of oxygen free radicals and the endogenous antioxidant defense system, as a result of the effect of CCl₄, so antioxidant properties of HDN might be the main factor responsible for its strong protective action on CCl₄-induced hepatotoxicity, through its ability to inhibit the lipid peroxidation and increase the activity of cellular antioxidant enzymes. Based on this study HDN at these doses are safe and effective antioxidant also has cytoprotective property in dose depended manner.

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