

Protective Effects of *Jasonia montana* Against Lipid Peroxidation in Liver and Kidney of Iron-overloaded Rats

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Abstract: Massive iron deposition in parenchymal organs, particularly in the liver, causes organ dysfunction, fibrosis, cirrhosis, and also hepatocellular carcinoma. The aim of the present article is to investigate protective activity of *Jasonia* ethanolic extract against liver and kidney damage induced by iron-overloaded in adult rats in an attempt to understand its mechanism of action, which may pave the way for possible therapeutic applications. *In vivo* determination of antioxidant and chelating activities of the extract were employed. Orally administration of ferrous gluconate as a source of iron at 10 mg/kg b.w./day for 21 days to rats induced hepatocellular and renal cell damage. Oral administration of *Jasonia* ethanolic extract at a concentration of 150 mg/kg b.w. daily to rats treated with iron for 31 prior to iron overloaded-administration significantly prevented the increase in liver, kidney and serum iron, serum ferritin, serum transferrin levels, γ -GT, α -GST and γ -GT activities as well as serum NO and TNF- α level and hepatic MDA level as compare to iron-overloaded treated rats. The treatment also resulted in a significant increase in hepatic and kidney SOD, GPx, GR and CAT activities compare to iron-overloaded treated rats. High content of flavonoids and phenolic compounds was found in ethanolic extract, which may be responsible for free radical activity and as molecules of natural origin may serve as a template of new biologically active compounds possess capability of chelating iron. The results clearly suggest that the aerial parts of *J. montana* extract may effectively normalize the impaired antioxidant status in iron-overloaded rats model experiment. Thus the extract may have a therapeutic value in iron-overloaded-induced haemostasis.

Key words: *Jasonia montana*, iron-overloaded, ferritin, transferrin, γ -GT, α -GST, NO, TNF- α , SOD, GPx, GR, CAT, GPT, GOT, LDH, liver and kidney.

INTRODUCTION

Iron is indispensable for virtually all aspects of life. As an integral part of numerous proteins, iron serves as a reaction center for many metabolic processes. The chemical reactivity of iron, critical to its normal biochemical functions, is potentially cytotoxic. Ferrous iron is capable of the generation of reactive oxygen species (ROS) such as the hydroxyl radical and superoxide. Free radicals initiate lipid peroxidation of cell membranes and oxidative damage of proteins, which in turn cause changes in membrane fluidity, disruption of microsomes and lysosomes, and accumulation of peptide fragments and cross-linked protein aggregates (Pietrangelo, 2003). This ultimately leads to dysregulation of cellular processes, cell dysfunction, and eventually to apoptosis or necrosis.

Many biologically active compounds widely used as a chelating agent for iron such as deferoxamine, ascorbate (Young *et al.*, 1996) and kojic acid (Kotyzová *et al.*, 2004). The endogenous antioxidant enzymes (e.g SOD, CAT, GSH and GPx) are responsible for the detoxification of deleterious oxygen radicals (Jacob, 1995). Many plant extracts and plant products have been shown to have significant antioxidant activity (Scartezzini and Speroni, 2002), which may be an important property of medicinal plants associated with the treatment of several ill fated diseases including cholestasis (Hussein and Abdel-Gawad, 2010). Thus, herbal plants are considered a useful means to prevent and/or ameliorate certain disorders, such as diabetes, atherosclerosis and other complications (Scartezzini and Speroni, 2002). Plant-derived polyphenols minimize iron-overloaded-induced liver injury (Miller *et al.*, 1996). Therefore, new strategies to prevent haemostasis

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induced liver injury and fibrosis is needed. Accordingly, antioxidant therapy represents a potential strategy to prevent liver injury and fibrosis. Polyphenols exist in many plants and are especially abundant in *Jasonia montana* (Tawfeq *et al.*, 2005), whose dried leaves are used as antioxidant. *Jasonia montana* and polyphenol-enriched plant extracts have no known toxicity. Thus polyphenols from *Jasonia montana* and possibly other plant sources represent a promising potential species (Hussein, 2008). Not surprisingly, plants such as *Jasonia montana* contain high levels of polyphenols (Tawfeq *et al.*, 2005), which are excellent antidiabetic and antioxidant (Hussein, 2008), anticholestatic effects (Hussein and Abdel-Gawad, 2010) and represent a promising anti-haemostatic effect.

The plant *Jasonia montana* occurs in the Mediterranean and adjacent areas (Merxmuller *et al.*, 1977), including the *Sinai Peninsula* (Scartezzini and Speroni, 2002). The herb has a strong aromatic odor and is used in traditional medicine for diarrhea, stomachache, and chest diseases (Tackholm, 1974). A literature survey indicated that some mono- and sesquiterpenes (Tackholm, 1964; Ahmed and Jakupovic, 1990; Eid *et al.*, 1987; Ahmed *et al.*, 1988), flavonoids (Ahmed, 1991), and essential oils (Ahmed *et al.*, 1989) have been reported from the plant. The different extracts of the plant were also screened for hypoglycemic and antidiabetic (Tawfeq *et al.*, 2005) and antioxidant (Hussein, 2008). Recently, Hussein and Abdel-Gawad, 2010, studied the protective effect of ethanolic extract of *Jasonia montana* against ethinylestradiol-induced cholestasis in rats. These reports prompted us to investigate the other physiological and pharmacological functions of *Jasonia montana*. *In vivo* tests have been conducted with *Jasonia montana* to determine, for example, its, hypoglycemic, antioxidant (Hussein, 2008) and anticholestatic (Hussein and Abdel-Gawad, 2010) activities. But there are no reports of the effect of *Jasonia montana* on liver toxicity by iron-overloaded induced haemostasis.

As a continuation of our research program (Hussein, 2008; Hussein and Abdel-Gawad, 2010), the present study was undertaken to investigate the protective activity of *Jasonia montana* extract in the form of ethanolic formulation against iron haemostasis on liver and kidney toxicity.

MATERIALS AND METHODS

Chemicals:

Ferrous gluconate (EPICO, Egypt). It was given orally for three weeks in a daily dose at 10 mg/kg b.w. (Emmanuel *et al.*, 1996).

Plant Material:

Fresh aerial parts of *J. montana* were collected from the Sinai Peninsula.

Preparation of Ethanolic Extract:

Air-dried aerial parts of the plant (1.5 kg) was crushed to coarse powder and extracted exhaustively in a Soxhlet with 95% ethanol. The extract was concentrated under reduced pressure to yield viscous mass. The ethanolic extract was kept in airtight containers in a deep freeze maintained at 4°C until the time of further use.

Phytochemical Screening:

A phytochemical analysis of aerial parts of *J. montana* was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil, coumarins and triterpenes (Hammerschmidt *et al.*, 1993).

Animals:

Adult albino rats weighing around 190 ± 10 gms, at the age of 10 weeks were purchased from Faculty of Veterinary Medicine, Cairo University. The animals were kept under standard laboratory conditions with free access to standard pellet diet in a controlled-temperature room (23-25 °C) and 12 h light/dark cycle. Rats were kept under constant environmental condition and observed daily throughout the experimental work.

Experimental Set Up:

The animals were divided into five groups with six animals in each.

Group I: Normal control (was given similar volume of distilled water)

Group II: was treated with ethanolic extract (150mg/kg b.w.) suspended in distilled water orally for 31 days (Farnsworth, 1966).

Group III: was given ferrous gluconate (FG) (10 mg/kg b.w.) suspended in distilled water orally in a single

daily dose for the last 21 days of the experimental period (Emmanuel *et al.*, 1996).

Group IV: was pretreated with ethanolic extract (150 mg/kg b.w) alone for 10 days then received both ethanolic extract and ferrous gluconate (FG) (10 mg/kg b.w.) for other 21 days (prophylactic I) (Hussein and Abdel-Gawad, 2010).

Group V: was simultaneously given ethanolic extract (150mg/kg b.w) and ferrous gluconate (FG) (10 mg/kg b.w.) for 21 days followed by ethanolic extract alone for other 10 days (prophylactic II) (Hussein and Abdel-Gawad, 2010).

Sample Collection and Homogenate Preparation:

The experiment ended on the 31st day. Fasting blood samples were collected, 24 hours after the last treatments, from the retroorbital plexus (Schermer, 1967) under anaesthesia using diethylether. The blood samples were left to clot and the sera were separated using cooling centrifuge and stored at -20 °C until analysis. After blood collection, all animals were rapidly killed; liver and kidney tissues were dissected and immediately homogenized in phosphate buffer (pH 7.4) to give 20 % w/v homogenate (Lin *et al.*, 1998). The homogenate was centrifuged at 1700 r.p.m and 4°C for 10 min. and the supernatant (20%) was used for determination of lipid peroxides, glutathione reductase (GR) and catalase (CAT) activities, and it was further diluted with phosphate buffer solution to give 2% and 0.5 % dilutions for determination of glutathione peroxidase and superoxide dismutase, respectively.

Biochemical Assays:

Serum, liver and kidney iron according to Ceritti and Ceriotti 1980. Serum transferrin and ferritin were estimated according to Hellsing, 1973; Valberg, 1980, respectively. The activities GOT and GPT were estimated according to Reitman and Frankel, 1957. γ -GT and LDH in serum were estimated according to Szasz, 1969; Buhl and Jackson, 1978, respectively. Quantitative estimation of serum (α -GST) was carried out by enzyme linked immunosorbent assay (ELISA) according to Flendring *et al.*, 1999. Quantitative determination of tumor necrosis factor- α (TNF- α) was done by ELISA according to the method of Corti *et al.*, 1992. Serum nitrate concentration as a stable end product of nitric oxide was estimated by the Griess reaction after quantitative conversion of nitrate to nitrite by nitrate reductase according to the method of Moshage *et al.*, 1995. Lipid peroxidation was estimated in liver homogenates by thiobarbituric acid test according to Esterbauer *et al.*, 1991. The level of lipid peroxides was expressed as n mol malonaldehyde/mg protein. The protein content of liver tissue was measured by applying the method of Lowry *et al.*, 1951. CAT, GR, GPx and SOD activities/mg protien in liver homogenate were assayed according to Tukahara, 1960; Horn, 1965; Paglia and Valentine, 1967; Suttle, 1986, respectively.

Statistical Analysis:

All the grouped data were statistically evaluated with SPSS/7.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. *P* values of less than 0.01 were considered to indicate statistical significance. All the results were expressed as mean \pm SD for six separate determinations.

Results:

Table 1 shows the specific haemostatic parameters among the different groups. Ferrous gluconate (FG) (10 mg/kg b.w.) administration for 21 days resulted in significant increase ($p < 0.01$) in liver and kidney iron, serum iron, serum ferritin and serum transferrin levels by 774.9%, 50.05%, 65.1%, 101.43% and 39.02%, respectively as compare to control group. These data indicate that under the influence of ethanolic extract of *Jasonia montana* a statistically significant decrease in liver iron ($p < 0.01$), kidney iron ($p < 0.01$), serum iron ($p < 0.01$), serum ferritin ($p < 0.01$) and serum transferrin ($p < 0.05$) levels by 45.37%, 25.3%, 30.11%, 45.57% and 22.34%, respectively in the group of rats continuously treated with ethanolic extract before FG injection as compare to FG-treated group. A significant decrease in liver iron ($p < 0.01$), kidney iron ($p < 0.01$) serum iron ($p < 0.01$), serum ferritin ($p < 0.01$) and serum transferrin ($p < 0.01$) levels by 24.11%, 16.86%, 14.26%, 33.28% and 14.16%, respectively were observed in the group of rats continuously treated with ethanolic extract after FG injection as compared to FG treated group. The protective effect of extract was more obvious in case of simultaneous administration of ethanolic extract and FG (group 4).

Table 2 shows the effect of ethanolic extract of *Jasonia montana* on serum tumor necrosis factor alpha (TNF- α), serum nitric oxide (NO), serum lipid peroxides (TBARS) levels and alpha glutathione-s-transferase (α -GST) activity. Serum of TNF- α , NO and TBARS increased significantly ($p < 0.01$) in FG injected group by

Table 1: The effect of ethanolic extract of *Jasonia montana* on liver iron, kidney iron, serum iron, serum ferritin and serum transferrin of iron-loaded and control rats.

Groups	Liver iron ($\mu\text{g/g}$ of tissue wet.)	Kidney iron ($\mu\text{g/g}$ of tissue wet.)	S. iron ($\mu\text{g/dl}$)	S.ferritin (ng/ml)	S. transferrin (mg/dl)
Normal Control	134.68 \pm 10.41	57.82 \pm 4.93	129.36 \pm 12.72	35.48 \pm 5.98	190.77 \pm 18.09
Ethanolic extract 150 mg/kg.b.w.	125.67 \pm 8.60*	43.59 \pm 3.61*	118.40 \pm 10.79*	29.83 \pm 4.17*	177.52 \pm 13.76*
Ferrous gluconate (FG)(10 mg/kg b.w.)	1178.36 \pm 90.56 [@]	86.77 \pm 6.42 [@]	213.63 \pm 19.27 [@]	71.47 \pm 6.22 [@]	265.20 \pm 20.48 [@]
Ethanolic extract + (FG)(Prophylactic I)	643.67 \pm 73.40 [@]	64.80 \pm 5.14 [@]	149.30 \pm 15.72 [@]	39.11 \pm 4.96 [@]	205.93 \pm 15.26*
(FG) + Ethanolic extract (Prophylactic II)	894.26 \pm 66.48 [@]	72.11 \pm 4.83 [@]	183.16 \pm 13.77 [@]	47.68 \pm 7.31 [@]	227.64 \pm 24.07

[@]Values are given as mean \pm SD for groups of six animals each. Values are statistically significant at [@] $P < 0.01$, Values are statistically significant at * $P < 0.05$. Ethanolic extract and Ferrous gluconate (FG) treated rats were compared with normal control rats. Experimental groups (4 & 5) were compared with Ferrous gluconate (FG) treated rats.

Table 2: The effect of ethanolic extract of *Jasonia montana* on serum tumor necrosis factor alpha (TNF- α), serum nitric oxide (NO), serum lipid peroxides (TBARS) levels and alpha glutathione-s-transferase (α -GST) activity of iron-loaded and control rats.

Groups	(Serum TNF- α) (pg/ml)	(Serum NO) ($\mu\text{mol/L}$)	TBARS nmol/ml	(α -GST) ($\mu\text{g/L}$)
Normal Control	42.46 \pm 4.7	53.55 \pm 7.02	5.09 \pm 0.11	225.67 \pm 5.8
Ethanolic extract 150 mg/kg.b.w.	37.22 \pm 5.1*	41.44 \pm 6.19*	3.70 \pm 0.08*	214.2 \pm 7.1*
Ferrous gluconate (FG) (10 mg/kg b.w.)	85.92 \pm 7.36 [@]	114.08 \pm 8.3 [@]	10.56 \pm 2.31 [@]	154.7 \pm 10.4 [@]
Ethanolic extract + (FG) (Prophylactic I)	64.38 \pm 5.71 [@]	77.14 \pm 7.66 [@]	6.75 \pm 1.27 [@]	195.32 \pm 11.48 [@]
(FG) + Ethanolic extract (Prophylactic II)	75.10 \pm 6.08*	94.25 \pm 8.05*	8.68 \pm 2.1*	174.35 \pm 14.90*

Values are given as mean \pm SD for groups of six animals each. Values are statistically significant at [@] $P < 0.01$, Values are statistically significant at * $P < 0.05$. Ethanolic extract and Ferrous gluconate (FG) treated rats were compared with normal control rats. Experimental groups (4 & 5) were compared with Ferrous gluconate (FG) treated rats.

102.35%, 113.03% and 31.44%, respectively as compared to control group with concomitant decrease in the activity of α -GST ($p < 0.01$) by 31.45% as compare to control group. Serum levels of TNF- α ($p < 0.05$), NO ($p < 0.01$) and TBARS ($p < 0.01$) were significantly decreased by 25.06%, 32.38% and 36.26%, respectively in rats treated with ethanolic extract of *Jasonia montana* before FG injection with concomitant increase in the activity of α -GST ($p < 0.01$) by 26.26% as compared to FG treated group. A significant decrease in serum activity of Pi TNF- α ($p < 0.05$), NO ($p < 0.05$) and TBARS ($p < 0.05$) were significantly decreased by 12.59%, 17.38% and 18.03%, respectively in rats treated with ethanolic extract of *Jasonia montana* after FG injection with concomitant increase in the activity of α -GST ($p < 0.05$) by 12.70% as compared to FG treated group. The protective effect of extract was more obvious in case of simultaneous administration of ethanolic extract and FG (group 4).

Table 3 shows that significantly increased in serum gamma glutamyl transferase (γ -GT) ($p < 0.01$) with non-significantly increased in serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) activity in FG-treated group by 23.18%, 4.5% , 9.37% and 3.73%, respectively as compared to control group. A significant decrease in serum γ -GT($p < 0.05$) and GPT($p < 0.05$)activity with non-significantly decreased in serum GOT and LDH activity were observed in the group of rats treated with ethanolic extract of *Jasonia montana* before FG injection by 15.83%, 12.39% , 2.94% and 7.79%, respectively as compared to FG-treated group. In the group of rats continuously treated with ethanolic extract after FG injection both γ -GT, GOT, GPT) and LDH activity decreased non-significantly by 2.6%, 1.01% , 4.06% and 1.46%, respectively as compared to FG-treated group. The effect was more pronounced in case of simultaneous administration of extract before FG injection (group 4) compared to administration of extract after FG injection (group 5).

Table 4 showed significant decreased in hepatic superoxide dismutase (SOD) ($p < 0.01$), glutathione peroxidase (GPx) ($p < 0.05$), glutathione reductase (GR) ($p < 0.01$) and catalase (CAT) ($p < 0.01$) activities upon FG injection by 37.20%, 14.95%, 28.31% and 28.35%, respectively as compared to the control group. A significant increase ($p < 0.01$) in hepatic SOD, GPx, GR and CAT activities were observed in the group of rats treated with ethanolic extract of *Jasonia montana* before FG injection by 50.89%, 31.03%, 46.49% and 29.94%, respectively as compared to FG-treated group. In the group of rats continuously treated with ethanolic

Table 3: The effect of ethanolic extract of *Jasonia montana* on serum gamma glutamyl transferase (γ -GT), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) activity of iron-loaded and control rats.

Groups	(γ -GT) (U/l)	(GOT) (U/l)	(GPT) (U/l)	(LDH) (U/l)
Normal Control	11.43 \pm 2.05	185.68 \pm 7.45	66.35 \pm 5.88	225.09 \pm 13.74
Ethanolic extract 150 mg/kg.b.w.	10.74 \pm 1.78	178.62 \pm 16.93	60.56 \pm 5.98	219.78 \pm 14.16
Ferrous gluconate (FG) (10 mg/kg b.w.)	14.08 \pm 3.85*	194.11 \pm 10.76	72.57 \pm 8.25	233.50 \pm 21.48*
Ethanolic extract + (FG) (Prophylactic I)	11.85 \pm 2.34	188.40 \pm 19.44	63.18 \pm 5.02	215.29 \pm 16.83*
(FG) + Ethanolic extract (Prophylactic II)	13.71 \pm 3.19	196.07 \pm 14.80	69.62 \pm 6.36	230.08 \pm 17.56

Values are given as mean \pm SD for groups of six animals each. Values are statistically significant at * P <0.05. Ethanolic extract and Ferrous gluconate (FG) treated rats were compared with normal control rats. Experimental groups (4 & 5) were compared with Ferrous gluconate (FG) treated rats.

Table 4: The effect of ethanolic extract of *Jasonia montana* on liver superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) activity of iron-loaded and control rats.

Groups	(SOD) (U/g prot.)	(GPx) (mU/mg prot.)	(GR) (U/mg prot.)	(CAT) (mU/mg prot.)
Normal Control	134.52 \pm 12.43	3.41 \pm 0.18	2.19 \pm 0.09	105.33 \pm 5.67
Ethanolic extract 150 mg/kg.b.w.	142.61 \pm 15.37*	4.06 \pm 0.13*	2.59 \pm 0.11*	121.70 \pm 13.57*
Ferrous gluconate (FG) (10 mg/kg b.w.)	84.47 \pm 6.83 [@]	2.90 \pm 0.08 [@]	1.57 \pm 0.07 [@]	75.46 \pm 11.26 [@]
Ethanolic extract + (FG) (Prophylactic I)	127.46 \pm 17.62 [@]	3.80 \pm 0.15 [@]	2.3 \pm 0.15 [@]	98.06 \pm 6.25 [@]
(FG) + Ethanolic extract (Prophylactic II)	113.93 \pm 11.82 [@]	3.30 \pm 0.17*	1.8 \pm 0.09*	87.45 \pm 5.34*

Values are given as mean \pm SD for groups of six animals each. Values are statistically significant at [@] P <0.01, Values are statistically significant at * P <0.05. Ethanolic extract and Ferrous gluconate (FG) treated rats were compared with normal control rats. Experimental groups (4 & 5) were compared with Ferrous gluconate (FG) treated rats. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the protein concentration.

extract after FG injection both hepatic SOD (p <0.01), GPx (p <0.05), GR (p <0.05) and CAT (p <0.05) activities increased significantly by 34.87%, 13.79%, 14.64% and 15.88%, respectively as compared to FG-treated group. The protective effect of extract was more obvious in case of simultaneous administration of ethanolic extract and FG (group 4).

Table 5 showed significant decreased in renal superoxide dismutase (SOD) (p <0.01), with non-significant decreased of glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) activities upon FG injection by 35.21%, 3.92%, 2.86% and 3.34%, respectively as compared to the control group. A significant increase in renal SOD (p <0.01) and CAT (p <0.05) with non-significant increased GPx and GR activities were observed in the group of rats treated with ethanolic extract of *Jasonia montana* before FG injection by 53.33%, 12.84%, 1.96% and 5.90%, respectively as compared to FG-treated group. In the group of rats continuously treated with ethanolic extract after FG injection, renal SOD (p <0.01) activity increased significantly by 42.09% with non-significant increased in renal GPx, GR and CAT activity by 3.20%, 2.53% and 8.24%, respectively as compared to FG-treated group. The protective effect of extract was more obvious in case of simultaneous administration of ethanolic extract and FG (group 4).

Table 5: The effect of ethanolic extract of *Jasonia montana* on kidney superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) activity of iron-loaded and control rats.

Groups	(SOD) (U/g prot.)	(GPx) (mU/mg prot.)	(GR) (U/mg prot.)	(CAT) (mU/mg prot.)
Normal Control	120.27 \pm 16.44	3.57 \pm 0.15	2.44 \pm 0.06	86.62 \pm 7.33
Ethanolic extract 150 mg/kg.b.w.	143.61 \pm 10.52*	3.72 \pm 0.09	2.55 \pm 0.08	96.15 \pm 8.48
Ferrous gluconate (FG) (10 mg/kg b.w.)	77.92 \pm 8.11 [@]	3.43 \pm 0.07	2.37 \pm 0.11	83.72 \pm 6.95
Ethanolic extract + (FG) (Prophylactic I)	119.48 \pm 13.82 [@]	3.64 \pm 0.11	2.51 \pm 0.10	94.47 \pm 5.81 *
(FG) + Ethanolic extract (Prophylactic II)	110.72 \pm 8.47 [@]	3.54 \pm 0.09	2.43 \pm 0.07	90.62 \pm 7.57

Values are given as mean \pm SD for groups of six animals each. Values are statistically significant at [@] P <0.01, Values are statistically significant at * P <0.05. Ethanolic extract and Ferrous gluconate (FG) treated rats were compared with normal control rats. Experimental groups (4 & 5) were compared with Ferrous gluconate (FG) treated rats. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the protein concentration.

Discussion:

Iron is essential for maintaining proper cell functions (Boldt, 1999). However, iron, especially ferrous iron, may cause deleterious reactions. Ferrous iron is a catalyst in the Haber-Weiss reaction, hydroxyl radical formation, and is involved in initiation and propagation of lipid peroxidation. Therefore, the reactivity of iron is usually tightly controlled by binding to transport and storage proteins. Haemostasis occurs when the body absorbs too much iron. This disease causes extra iron to gradually build up in the body's tissues and organs, a term called iron overload. If this iron buildup is untreated, it can, over many years, damage the body's organs.

The aim of our study was to evaluate the effect of *Jasonia montana* extract in the form of ethanolic formulation on iron accumulation and oxidative damage in the in vivo model of iron-overload in rat liver and kidney tissue. In the present study, we used a dietary iron ferrous gluconate (FG) (10 mg/kg b.w.) to induce oxidative stress in liver and kidney. The data in the present study indicate that, oral administration of ferrous gluconate (FG) (10 mg/kg b.w.) for 21 days resulted in significant increase in liver and kidney iron, serum iron, serum ferritin and serum transferrin levels as compare to control group (table 1). Many authors prove that, iron-overloaded induce liver cell damage and the iron and ferritin saturation in serum was significantly increased, probably indicating the release of intracellular iron-rich ferritin into the blood (Peter *et al.*, 2000). These data indicate that under the influence of ethanolic extract of *Jasonia montana* a statistically significant decrease in liver and kidney iron, serum iron, serum ferritin and serum transferrin levels in the group of rats continuously treated with ethanolic extract before and/or after FG injection as compare to FG-treated group. The effect of ethanolic extract of *Jasonia montana* more pronounced in group 4, Ethanolic extract + (FG).

Not surprisingly, plants such as *Jasonia montana* contain high levels of polyphenols (Tawfeq *et al.*, 2005), some mono- and sesquiterpenes (Tackholm, 1964; Ahmed and Jakupovic, 1990; Eid *et al.*, 1987; Ahmed *et al.*, 1988), flavonoids (Ahmed, 1991), and essential oils (Ahmed, *et al.*, 1989) which are excellent antioxidant (Hussein, 2008) and chelating agent for iron. *Jasonia montana* flavonoids scavenge reactive oxygen species and free radicals by several proposed mechanism, including delocalization of electrons, formation of intramolecular hydrogen bonds (Van Acker *et al.*, 1996(a)) and rearrangement of their molecular structure (Van Acker *et al.*, 1996(b)). Chelation of free iron is another property of *Jasonia montana* flavonoids that contributes to preventing oxidative reactions, as these metal is known to catalyze formation of reactive oxygen species in vivo (Morel *et al.*, 1993). My results hypothesize that, *Jasonia montana*, which chelates iron in a form that does not support the Haber-Weiss reaction, on the other hand, is a well-known inhibitor of iron absorption (Ito *et al.*, 2001).

Serum of TNF- α , NO and TBARS increased significantly in FG injected group as compared to control group with concomitant decrease in the activity of α -GST as compare to control group. As shown in table 2, iron treatment induced inflammation and lipid peroxidation expressed in terms of serum of TNF- α , NO and MDA formation (Patrick *et al.*, 2008). Many authors (Kadiiska *et al.*, 2005; Walter *et al.*, 2006) reported that, the amount of lipid peroxide in the serum was increased dependent on the iron contents of diets. It is well known that NF κ B is activated by a wide range of agents and cytokines including TNF- α and IL-1 α secreted from the injured hepatic macrophages (Jaeschke *et al.*, 2002). TNF- α was reported to induce NO formation (Strater and Moller, 1998). The increased NO production is recognized as an important mediator of physiological and pathological processes (Fox *et al.*, 1997). As a result of these inflammatory and destructive processes, the oxidative stress on the hepatic cells was increased leading to the depletion of the antioxidant enzymes that scavenge the toxic superoxide and hydrogen peroxide radicals which promote lipid peroxidation (Dunger *et al.*, 1996). The present data demonstrated that *Jasonia montana* extract as a powerful iron chelator induced a significant reduction of inflammation and lipid peroxides due to its antioxidant and free radical scavenger properties (Hussein, 2008).

The present work revealed that, non-significantly increased in serum GOT, GPT and LDH activity in FG-treated group as compared to control group (table 3). The enzymes activities were not affected, suggesting that liver cell permeability was not affected by necrosis.

In the present study, the significant increase in serum γ -GT with the concomitant decrease in serum α -GST, hepatic and renal SOD, GPx, GR and CAT activity (table 4&5) were detected after FG-administration. Treatment with iron-overloaded produced a significant decrease in hepatic SOD, GPx, GR and CAT activities as compare to control group, these activities were not affected in kidney as compared to control values, except for SOD activity that was decreased.

These findings revealed the role of iron-overloaded in inducing oxidative stress on the liver and renal tissue. This is because the main function of SOD is represented in the removing of the superoxide radical in order to prevent formation of hydroxyl radical and the function of GPx is represented in its ability to utilize

and metabolize hydrogen peroxide in both cytosolic and mitochondrial compartments (Gross and Wolin, 1995). Catalase (CAT) is a heme protein which catalyses the reduction of liver and renal hydroperoxides and protects the tissues from highly reactive hydroxyl radicals (Hussein, 2008).

The hepatoprotective effect of *Jasonia montana* extract was demonstrated through correcting the value of serum α -GST, hepatic SOD, GPx, GR and CAT that were significant decreased by FG administration. This result indicated that ethanolic extract of *Jasonia montana* has a membrane stabilizing effect (Asai and Miyazawa, 2002). Additionally, there is growing evidence that the hepatoprotective effect of extract takes place directly at the level of hepatocytes by lowering serum and liver levels of iron (Ito *et al.*, 2001). Moreover, it has been reported that the ethanolic extract of *Jasonia montana* exert a cytoprotective effect by inducing liver glutathione-s-transferase (Hussein, 2008). This antioxidant action of extract is attributed to its ability to suppress many types of transcription factors including hydrogen peroxide, tumor necrosis factor- α .

Finally, this result proved that, dietary *Jasonia montana* canceled the effect of iron overloading on lipid peroxidation in rat liver and kidney by chelates iron in a form that does not support the Haber-Weiss reaction, on the other hand, is a well-known inhibitor of iron absorption. *Jasonia montana* flavonoids protective effect may be due to its high and specific affinity for Fe^{+3} and form a stable complex. Iron within the complex failed to be reduced in a form that would catalyze Fenton's reaction. So *Jasonia montana* accelerates $Fe^{+2} \rightarrow Fe^{+3}$ autoxidation and blocked the cytotoxic effects of iron through the formation of such complex.

The preliminary phytochemical screening of ethanolic extract of *Jasonia montana*, revealed the presence of flavonoids. Flavonoids (or bioflavonoids) are natural products, they are capable of modulating the activity of enzymes and affecting the behavior of many cell systems and they possess significant antihepatotoxic, antiallergic, anti-inflammatory, antiosteoporotic, and even antitumor (Carlo *et al.*, 1991; Rathee *et al.*, 2006), antioxidant (Hussein, 2008) and anticholestatic activities (Hussein and Abdel-Gawad, 2010). High content of flavonoids and phenolic compounds was found in ethanolic extract, which may be responsible for free radical activity.

Protective effects of ethanolic extract of *Jasonia montana* against liver and renal toxicity induced by iron-overloaded have not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind.

In conclusion, the present study showed that ethanolic extract of *Jasonia montana* has a powerful iron chelator activity against liver and renal toxicity induced by iron-overloaded. In addition, to its hepatoprotective, antioxidant action and free radicals scavenging activity.

REFERENCES

- Ahmed, A.A., 1991. Two geraniol derivatives from *Jasonia montana*, Pharmazie., 46: 362-63.
- Ahmed, AA., A. Ali, T.J. Mabry, 1989. Flavonoid aglycones from *Jasonia montana*, Phytochemistry, 28: 665-667.
- Ahmed, A.A., J.F. Jakupovic, 1990. Sesqui and monoterpenes from *Jasonia montana*. J. Phytochemistry, 29: 3656-61.
- Ahmed, A.A., J.F. Jakupovic, A. Ali, 1998. 11- Hydrojasione; A new sesquiterpene type from *Jasonia montana*, Phytochemistry, 27: 3875-77.
- Asai, A., T. Miyazawa, 2001. Dietary curcuminoids prevent high fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. J.Nutr., 131: 2932-2935.
- Boldt, D.H., 1999. New perspectives on iron: an introduction. Am. J. Med. Sci., 318: 207-212.
- Buhl, S.N., K.Y. Jackson, 1978. Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate to pyruvate to lactate reactions in human serum at 25, 30 and 37 °C. Clin. Chem., 2415: 828-836.
- Carlo, G., N. Mascolo, A. Izzo, F. Capasso, 1991. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. Life Sci., 65: 337-353.
- Ceritti, F., C. Ceriotti, 1980. Improved direct specific determination of serum iron and TIBC. Clin.Chem., 26: 327-331.
- Corti, A., J. Fassino, F. Marcucci, E. Barbenti, G. Cassani, 1992. Oligometric tumor necrosis factor- α slowly converts into the reactive forms at bioactive levels. Biochem. J., 284: 905-910.
- Dunger, A., J. Cunningham, C. Delaney, J. Lowe, M. Green, A. Bone, I. Green, 1996. Tumor necrosis factor α and interferone- γ inhibit insulin secretion and cause DNA damage in unweanted-rat islets. Diabetes, 45: 183-189.
- Eid, F., S. El-Dahmy, P.K. Gupta, 1987. Constituents from *Jasonia montana* and *Allagopappus dicotoma* Pharmazie, 42: 423-24.

- Emmanuel, B., R. Florian, S. Submann, A. Scholmerich, H. Wieland, 1996. Hair iron content: possible marker to complement monitoring therapy of iron deficiency in patients with chronic in-inflammatory bowel disease. *Cli.Chem.*, 42: 1270-1274.
- Esterbauer, H., R. Schaure, H. Zollner, 1991. Chemistry and biochemistry of 4-hydroxynonenal. Malonaldehyde and related aldehydes. *Free Rad. Biol. Med.*, 11: 81-128.
- Farnsworth, N.R., 1966. Biological and phytochemical screening of plants. *J. Pharm. Sci.*, 55: 225- 232.
- Flendrig, L., R. Chamuleau, M. Maas, J. Daalhuisen, B. Hasset, C. Kilty, S. Doyle, N. Ladiges, G. Jorning, J. La Soe, D. Sommeijer, A. Velde, 1999. Evaluation of a novel bioartificial liver in rats with complete liver ischemia: treatment efficacy and species specific α - GST detection to monitor hepatocyte viability. *J. Hepatol.*, 30: 129-134.
- Fox, E., J. Kim, T. Tracy, 1997. NF-Kappa β activation and modulation in hepatic macrophages during cholestatic injury. *J. Surg. Res.*, 72: 129-134.
- Gross, S., M. Wolin, 1995. Nitric oxide: pathophysiological mechanism. *Annu. Rev. Physiol.*, 57: 737-769.
- Hammerschmidt, F.J., A.M. Clark, F.M. Soliman, E.S. El-Kashoury, M.M Abd El-Kawy, A.M. El-Fishawy, 1993. Chemical composition and antimicrobial activity of essential oils of *Jasonia candicans* and *Jasonia montana*, *Planta Medica.*, 59: 68-70.
- Hellsing, K., 1973. In protides of the biological fluids. *J.Immuno.*, 123: 579-583.
- Horn, D., 1965. In: *Methods in enzymatic analysis*, edited by: Bergmayer, H; Academic Press, London, 75-879.
- Hussein, M.A., 2008. Antidiabetic and antioxidant activity of *Jasonia montana* extract in streptozotocin-induced diabetic rats. *Saudi Pharmaceutical Journal*, 16: 214-221.
- Hussein, M.A., S.M. Abdel-Gawad, 2010. Protective effect of *Jasonia montana* against ethinylestradiol-induced cholestasis in rats. *Saudi Pharm. J.*, 18: 35-45.
- Ito, H., K. Sawada, Y. Ohishi, W. Yoshida, T. Yokoi, T. Watanabe, T. Yokokura, 2001. Suppressive effects of bifidobacteria on lipid peroxidation in the colonic mucosa of iron-overloaded mice. *J. Dairy Sci.*, 84: 1583-1589.
- Jacob, R.A., 1995. The integrated antioxidant system. *Nutr. Res.*, 15: 755-67.
- Scartezzini, P., E. Speroni, 2002. Review on some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol.*, 71: 23-43.
- Jaeschke, H., G. Gores, A. Cederbaum, J. Hinson, D. Pessayre, J. Lemasters, 2002. Mechanism of hepatotoxicity. *Toxicol. Sci.*, 65: 166-176.
- Kadiiska, M., B. Gladen, D. Baird, D. Germolec, L. Graham, C. Parker, 2005. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl₄ poisoning. *Free Radic. Biol. Med.*, 38: 698-710.
- Kotyzová, D., V. Eybl, J. Koutenský, J. Brtko, E. Glattre, 2004. Effects of kojic acid on oxidative damage and on iron and trace element level in iron-overloaded mice and rats. *Cent. Eur. J. Publ. Health*, 12: S41-S44.
- Lin, C., Y. Hus, T. Lin, F. Hus, H. Hus, 1998. Antioxidant and hepatoprotective activity of punicalagin and punicalin on carbon tetrachloride-induced liver damage in rats. *J. Pharm.*, 50: 789-794.
- Hellsing, K., 1973. In protides of the biological fluids. *J.Immuno.*, 123: 579-583.
- Lowry, O., N. Rosebrough, A. Farr, R. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Merxmuller, H., P. Liens, H. Roessler, 1977. In: J.B. Heywood J.B, Harborne B and Turner L. *The Biology and Chemistry of Compositae*, p. 590. Academic Press, New York.
- Miller, N.J., L. Castelluccio, C. Tijburg, L. Rice-Evans, 1996. The antioxidant properties of theaflavins and their gallate esters-radical scavengers or metal chelators. *FEBS Lett.*, 392: 40-44.
- Morel, I., G. Lascoat, P. Cogere, O. Sergent, N. Padeloup, P. Brissot, P. Cillard, J. Cillard, 1993. Antioxidant and iron-chelating activity of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochem. Pharmacol.*, 45: 13-19.
- Moshage, H., B. Rok, R. Huizenga, P. Jansen, 1995. Nitrite and nitrate determination in plasma: A critical evaluation. *Clin. Chem.*, 41: 892-896.
- Pietrangelo, A. 2003. Iron-induced oxidant stress in alcoholic liver fibrogenesis. *Alcohol*, 30: 121-129.
- Paglia, D., W. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, 70: 158-169.

- Patrick, B., A. Walter, J. Macklin, E. Patricia, L. Janet, J. Kwiatkowski, T. Neufeld, J. Patricia, E. Giardina, O. Nancy, A. Daniele, H. Jaymes, H. Paul, 2008. Inflammation and oxidant-stress in b-thalassemia patients treated with iron chelators deferasirox (ICL670) or deferoxamine: an ancillary study of the Novartis. *Haematologica.*, 93: 817-825.
- Peter, N., G. Ulrike, D. Matthias, F. Roland, D. Jochen, 2000. Serum ferritin iron in iron overload and liver damage: Correlation to body iron stores and diagnostic relevance. *J Lab Clin Med.*, 135: 413-418.
- Rathee, J.S., S.A. Hassarajani, S. Chattopadhyay, 2006. Antioxidant activity of *Mammea longifolia* bud extract. *Food Chem.*, 99: 436-443.
- Reitman, S., A.A. Frankel, 1957. Colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. *Am J Clin Pathol.*, 28: 56-63.
- Scartezzini, P., E. Speroni, 2002. Review on some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol.*, 71: 23-43.
- Schermer, S., 1967. In: *The blood morphology of Laboratory Animals*. 3rd ed, Philadelphia F.A. Davi Co., pp: 42.
- Strater, J., P. Moller, 1998. Pathogenesis of primary biliary cirrhosis: CD 95-induced apoptosis at last. *Eur. J. Gastroenterol Hepatol.*, 10: 553-557.
- Suttle, N. 1986. Copper deficiency in ruminants; recent developments. *The Veterinary Record*. 119: 519-522.
- Szasz, G., 1969. A kinetic photometric method for serum gamma glutamyl transferase. *Clin.Chem.*, 15: 124-136.
- Tackholm, V. *Students' Flora of Egypt*, 1974; p. 562. Cairo University, Beirut.
- Tackholm, V. *Alfred Kaiser's Sinai-Herbarium*, 1969; p. 562. Cairo University Herbarium, Cairo.
- Tawfeq, A., J. Adnan, A. AL-Rehailyb, R. Joanna, J. Polsc, S. Porter, A. Bahar, 2005. Three new diterpenes and the biological activity of different extracts of *Jasonia montana*. *Natural Product Research*, 19: 253-265.
- Tukahara, S., B.H., Hamilton, J.V. Nell, Y. Ogura, E.T. Nishmura, 1960. Hypocatalasemia, a new genetic carrier state. *J Clin Invest*, 29: 610-16.
- Valberg, L., 1980. Plasma ferritin concentration, their clinical significance and relevance to patient care. *J.Canda.Medicine Association*, 122: 1240-1247.
- Van Acker, S., M. Van den Berg, G. Tromp, W. Griffioen, W. Bennekom, V. Van der, A. Bast, A. 1996(a). Structure aspects of antioxidant activity of flavonoids. *Free Rad. Biol. Med.*, 20: 331-342.
- Van Acker, S., D. Groot, M. Van den Berg, G. Tromp, W. Donne-Op de Kelder, V. Van der, A. Bast, 1996(b). A quantum chemical explanation of the antioxidant activity of flavonoids. *Chem. Res. Toxicol.*, 9: 1305-1312.
- Walter, P., E. Fung, D. Killilea, Q. Jiang, M. Hudes, J. Madden, 2006. Oxidative stress and inflammation in iron-overloaded Patients with b-thalassemia or sickle cell disease. *Br J Haematol.*, 135: 254-263.
- Young, I., S. Tate, H. Jant, M. Dorothy, R. Trimble, 1996. The effect of deferoxamine and ascorbate on oxidative stress in streptozotocin diabetic rats. *Free Radi. Biol. Med.*, 18: 833-840.