Effect of Sweet Violet (*Viola odorata* L.) Blossoms Powder on Liver and Kidney Functions as well as Serum Lipid Peroxidation of Rats Treated with Carbon Tetrachloride

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Abstract: This study was aimed to investigate the effect of sweet violet (*Viola odorata* L.) blossoms powder (SVBP) on liver and injuries of rats injected with carbon tetrachloride (CCl_4). Thirty six mature albino rats, weighting 130-150 g per each, were used and divided into two main groups, the first group (Group 1, 6 rats) fed on basal diet and the other main group (30 rats) was injected by CCl_4 for two weeks to induce liver impaired rats then classified into sex sub groups as follow: group (2), fed on standard diet only as a positive control; groups 3, 4, 5 and 6 fed on standard diet containing 0.2, 0.4, 0.8 and 1.6 % of SVBP respectively. At the end of the experiment, 28 days, liver and kidney functions as well as serum lipid peroxidation were determined. The results indicated that treatment of animals with CCl_4 caused a significant increased ($p \leq 0.05$) in liver functions (AST, ALT and ALP activities), kidney functions (urea and creatinine levels) and serum lipid peroxidation (malondialdehyde level, MDA) compared to normal controls. Supplementation of the animal diets with SVBP (0.2 to 1.6 g/100g) prevented significantly ($p \leq 0.05$) the rise of mean serum AST, ALT and ALP activities; urea, creatinine and MDA levels. The rate of preventative was increased with the increasing of the SVBP supplementation level. It could be concluded that SVBP was effective in protecting against CCl_4-induced liver and kidney injuries. Therefore, we recommended like of that plant part, sweet violet blossoms, by a concentrations ranged 0.2-1.6 % amount to be included in our daily diets, drinks and food supplementation.

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Key words: *Viola odorata*, blossoms, ALT, AST, ALP, urea, creatinine, malondialdehyde.

1. Introduction

Liver plays a critical role in all vertebrates (from human to fish). Reasons for this include the following: 1) this organ plays numerous important metabolic functions, such the regulation of carbohydrate metabolism, the production of plasma proteins, and the synthesis of bile, 2) nutrients derived from intestinal absorption are stored in hepatocytes and released for further catabolism by other tissues, 3) the yolk protein, vitellogenin, destined for incorporation into the oocyte, is synthesized entirely within the liver, 4) it is the major site of the cytochrome P450-mediated, mixed-function oxidase system, and, while this system inactivates or detoxifies some xenobiotics, it activates others to their toxic forms, and 5) bile synthesized by hepatocytes aids in the digestion of fatty acids and carries conjugated metabolites of toxicants into the intestine for excretion or intrahepatic recirculation (Voet and Voet, 1990 and Maton *et al*., 1993). It that means, the liver is responsible for many critical functions within the body and should it become diseased or injured, the loss of those functions can cause significant damage to the body.

The kidneys perform the essential function of removing waste products from the blood and regulating the water fluid levels. The kidneys receive blood through the renal artery. The blood is passed through the structure of the kidneys called nephrons, where waste products and excess water pass out of the blood stream. Kidney disease occurs when the nephrons inside the kidneys, which act as blood filters are damaged. This leads to the build up of waste and fluids inside the body (NKUDIC, 2012). Drug overdoses, accidental or from chemical overloads of drugs such as antibiotics or chemotherapy, may also cause the onset of acute kidney failure. Overuse of common drugs such as aspirin, ibuprofen, and acetaminophen (paracetamol) can also cause chronic kidney damage (Perneger *et al.,* 1994).

The modern pharmacological therapy is costly and associated with multiple side effects resulting in patient non-compliance. Thus there is a need to explore alternative therapies particularly from herbal sources as these are cost effective and possess minimal side effects. Plants produce an amazing amount of complex chemicals we can use as medicines to “curb and cure” disease. For example, *Viola*

odorata Linn. (*Violaceae*) is a popularly known as "Banafsaj" and sweet violet in Egypt . It is cultivated all over the world including Egypt as an ornamental plant and found wild in many regions of the world, e.g. South and eastern Africa, South America, France, Italy, Australia and New Zealand (Bown, 1995). It is a long trailing plant of a less than 6-in height. The plant has a thick and scaly underground stem, with rooting runners. It possesses heart-shaped leaves with scalloped or slightly serrated edges which are dark green, smooth, or sometimes downy underneath and grow in a rosette form at the base of the plant. Flowers are deep purple or blue to pinkish or even whitish-yellow in color (Warrier and Vaidya, 1995 and Fleming, 1998). Its history as a medicinal herb dates back as far as 500 BC, where it was known to be used to relieve pain due to cancer (Kapoor, 1990). In the traditional system, it has been used in anxiety, lower blood pressure, bronchitis, cough, fever, urinary infections, rheumatism, sneezing, and Kinney and liver disorders (Keville, 1991 and Duke *et al.,* 2002). *V. odorata* was reported by Lindholm *et al.,* (2002) as pharmacological tools and possibly as leads to antitumor agents. Furthermore, it is demonstrated that *V. odorata* showed a significant oral antipyretic activity, antihypertensive and antidyslipidemic (Khattak *et al.,* 1985 and Siddiqi *et al.,* 2012). In this study, we examined the influence of powder of blossoms of *Viola odorata* L. in the liver and kidney functions as well as serum lipid peroxidation of rats treated with Carbon Tetrachloride.

2. Materials and Methods

2.1 Materials

Sweet violet (*Viola odorata* L.) blossoms (SVB) were purchased from flower nurseries at Giza, Egypt. Carbon tetrachloride (CCl_4, 10% liquid solution), Dimethyl sulfoxide (DMSO) and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Casein was obtained from Morgan Chemical Co., Cairo, Egypt. Paraffin oil (10%) obtained from Eltampashawy Pharmacy, Shebin El-Kom City, Egypt. All organic solvents and other chemicals were of analytical grade were purchased from El-Ghomhorya for Drug and Chemical Trading Co. (Cairo, Egypt).

2.2 Preparation of powder from Sweet Violet Blossoms (SVB)

After arriving of the Sweet Violet Blossoms samples, they were prepared for drying process by manual sorting and washing. The drying process has been carried out using 55^{0}C under vacuum until arriving by the moisture in the final product to about 8%. The dried Sweet Violet Blossoms samples were put in polyethylene bags and kept in -20^{0}C until used in biological experiments.

2.3 Biological Experiments:

2.3.1 Animals

Animals used in this study, adult male albino rats (180-200 g per each) were obtained from Helwan Station, Ministry of Health and Population, Helwan, Cairo, Egypt.

2.3.2 Basal Diet:

The basic diet prepared according to the following formula as mentioned by (AIN, 1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride(0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin mixture component was that recommended by (Campbell, 1963) while the salt mixture used was formulated according to (Hegsted, 1941).

2.3.3 Experimental design

All biological experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n=36 rats), 130-150g per each, were housed individually in wire cages in a room maintained at 25 ± 2 ^{0}C and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1, 6 rats) still fed on basal diet and the other main group (30 rats) was injected by CCl_4 for two weeks to induce liver impaired rats then classified into sex sub groups as follow: group (2), fed on standard diet only as a positive control; group (3), fed on standard diet containing 0.2 % SVBP; group (4), fed on standard diet containing 0.4 % SVBP; group (5), fed on standard diet containing 0.8 % SVBP and group (6): fed on standard diet containing 1.6 % SVBP.

2.3.4 Blood sampling:

At the end of experiment period, 28 days, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to Drury and Wallington, (1980). Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20^{o}C until analysis.

2.3.5 Hematological analysis

Different tested parameters in serum were determination using specific methods as follow: glutamic oxaloacetic transaminas (AST/GOT), glutamic pyruvic transaminas (ALT/GPT), alkaline phsphatase (ALP), urea, creatinine and

malondialdehyde concentration according to Yound, (1975), Tietz, (1976), Moss (1992), Fawcett and Soctt, (1960), Bartles *et al.,* (1972) and Stroev and Makarova (1989), respectively.

2.4 Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

3. Results and Discussion

3.1 Liver Functions

Liver functions of rats injected CCl_4 and consumed sweet violet (*Viola odorata* L.) blossom powder were shown in Table (1). From such data it could be noticed that treatment of animals with CCl_4 caused a significant increased ($p \leq 0.05$) in AST (47.64%), ALT (93.58%) and ALP (194.20%) compared to normal controls. Supplementation of the rat diets with SVBP (0.2 to 1.6 g/100g) prevented the rise of mean serum AST, ALT and ALP activities. The rate of preventative was increased with the increasing of the SVBP concentration. Such as shown in figure (1), the rate of increasing in the liver enzymatic activities were recorded 53.70, 72.53, 49.08 and 38.27% (For AST); 39.72, 35.24, 23.64 and 19.68% (for ALT) and 146.26, 124.70, 97.31 and 64.84% (for ALP) with the rat diets supplemented by 0.2, 0.4, 0.8 and 1.6 g/100g of SVBP, respectively.

Table (1). Liver functions (Mean ± SD) of rats injected CCl_4 and consumed sweet violet (*Viola odorata* L.) blossom powder

Liver functions	Control (-)	Control (+)	Sweet violet blossom powder (%)			
			0.2	0.4	0.8	1.6
Aspartate aminotransferase (AST, IU/L)	50.95 ± 6.66^{d*}	75.22 ± 6.12^{a}	71.19 ± 5.36^{a,b}	68.91 ± 5.59^{b}	63.00 ± 5.80^{c}	60.98 ± 5.32^{c}
Alanine aminotransferase (ALT, IU/L)	29.74 ± 6.62^{e}	57.57 ± 9.08^{a}	45.71 ± 6.35^{c}	51.31 ± 10.08^{b}	44.33 ± 8.85^{c}	41.12 ± 7.72^{d}
Alkaline phosphatase (ALP, IU/L)	97.96 ± 17.31^{f}	288.20 ± 29.78^{a}	241.24 ± 32.03^{b}	220.12 ± 19.74^{c}	193.28 ± 13.68^{d}	161.48 ± 9.39^{e}

* Means in the same row with different litters are significantly different ($p \leq 0.05$)

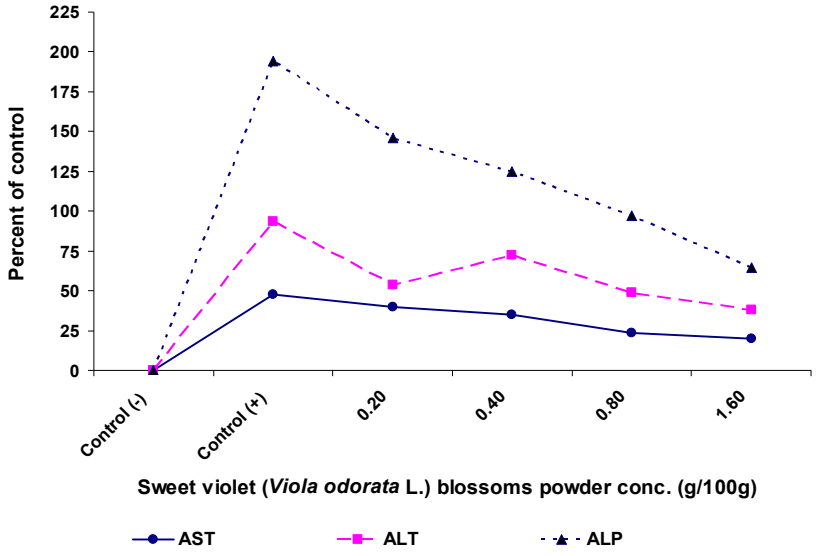

Figure (1). Effect of feeding sweet violet (*Viola odorata* L.) blossoms powder on Liver functions (% of control) of rats injected CCl_4

Aminotransferases are normally intracellular enzymes. Thus, the presence of elevated levels of aminotransferase in the plasma indicates damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting release of intracellular enzymes into the blood. Two amino transferases were found in plasma are of particular diagnostic value AST and ALT. AST enzyme is one of the enzymes tested in the cardiac enzyme series. This enzyme is found in very high

concentration within the heart muscles, skeletal muscle cells, and to a leaser degree in the kidney and panaceas (Pagana and Pagana, 1997). ALT is found predominately in the liver leaser quantities are found in the kidneys, heart and skeletal muscles (Pagana and Pagana, 1997). These enzymes are elevated in nearly all liver diseases, but are particularly high in conditions that the causes extensive cell necrosis, such as severe viral hepatitis and prolongated circulatory collapse. Serial enzyme measurments are often useful in determining the course of liver damage (Abd El-Aziz, 1990; Pagana and Pagana, 1997 and Hong *et al.,* 2002). Also, aminotransferases may be elevated in nonhepatic disease, such as myocardial infraction and muscle disorders; however, these disorders can usually be distingushed clinically from liver disease (Champe and Harvey, 1994). Alkaline phosphatatse (AP) is an enzyme which catalyzes the hydrolysis of phophate esters at an alkaline pH to give pi and the corresponding alcohol, phenol or sugar. Although ALP is found in many tissues, the highest concentrations are found in the liver, biliary tract , epithelium and bone. The intestinal mucosa and placenta also contain ALP (Pagana and Pagana, 1997). However, practically every body tissue contains at least a small amount of AP. Because of this wide distribution limited information can be obtain from a total AP assay. Elevated serum and leukocytic ALP leaves in patients with Hodgkin`s and non-Hodgkin`s lymbhoma were reported by several investigators (Thyss *et al*., 1985). Also, Aiba *et al*., (1980) found that the elevated leukocyte AP in patients who have hairy cell leukemia was inversely correlated to absolute number of neutrophils in the peripheral blood, *i.e*. the patients who had high leukocyte ALP scores has low or normal peripheral blood neutrophil counts. Abnormal leukocyte ALP scores are characteristic of certain myloprolifrative and lymphoproliferative disorders. Gobbi *et al*., (1982) found that among liver function tests that have been investigated in Hodgkin's disease, serum ALP activity was elevated in 20 out of 133 patients while it was elevated in 10 out of 20 patients with initial bone disease. The liver inflammation and functions-improving effects were evaluated according to ALT, AST (serum biochemical indicators for liver inflammation), albumin, total protein (liver cell regeneration indicators).

Such as reviewed in many studies plant parts including sweet violet are a rich source of different classes of phytochemicals such alkaloids, carotenoids, phytosterols, phenols and organosulfurs (Harborne, 1998; Harborne and Mabry, 1982, Beattic *et al.,* 2005 and Ssddiqi *et al.,* 2012). Many studies reported that the effect of many plant parts on decreasing the serum liver function enzymes activity could be attributed to their high level content of that phytochemicals. For example, El-Nashar, (2007) found that different doses of cinnamon extract showed slight-decreased in serum GOT, GPT and ALP after 12 week of feeding when compared with control group. The same observation was reported in rats injected with nitrosamine and treated with apricot kernel extracts (Hassan, 2011). Also, El-Sayed *et al.,*(2012) found that the addition of tested plant parts such Henada *(Jasonia montana)*, lemon balm leaves (*Melissa officinalis*), hawthorn leaves *(Crataegus azorolus),* rose of jericho *(Anastatica hierochuntica)* and corn cob silk *(zea mayz)* by 5 and 10% of the diet intake in the presence of CCl_4 induced significant improvements in all liver functions including the serum AST, ALT and ALP activities.

The possible mode of action of liver serum enzymes-lowering activity of the sweet violet plant could be explained by one or more of the following process. Dawson, (1998) reported that flavonoid is known to block the hepatocellular uptake of bile acids. Beattic *et al.,* (2005) reviewed that flavonoids pretreatment improved the antioxidant capacity of the liver, diminished the bilirubin concentration compared with the groups without treatment. Also, flavonol glycosides reduced the elevated levels of the following serum enzymes, AST, ALT and ALP. El-Nashar, (2007) reported that pre-treatment with flavonoids were not only able to suppress the elevation of GOT and GPT but also reduce the damage of hepatocytes *in vitro*. Also, they found that flavonoids have exhibited strong antioxidant activity against reactive oxygen species (ROS) *in vitro*. The hepatoprotective activity of flavonoids was possibly due to its antioxidant properties, acting as scavengers of reactive oxygen species (ROS). Finally, Hassan (2011) found that pre-treatment with apricot kernel extract rich in phytochemicals were able to reduce the damage of liver i.e. suppress the elevation of AST, ALT and ALP through the improvement of antioxidant defense system in red blood cells.

3.2 Kidney Functions

Kidney functions of rats injected CCl_4 and consumed sweet violet (*Viola odorata* L.) blossom powder were summarized in Table (2). Treatment of animals with CCl_4 caused a significant increased ($p \leq 0.05$) in serum urea (29.52%) and creatinine (50.98%) compared to normal controls. Supplementation of the rat diets with SVBP (0.2 to 1.6 g/100g) prevented partially the rise of mean serum urea and creatinine levels. The rate of preventative in particular for the creatinine level was increased with the increasing of the SVBP concentration. Such as shown in Figure (2), the rate

of increasing in the kidney functions parameters were recorded 16.71, 10.72, 10.48 and 10.47% (For urea) and 39.22, 35.29, 32.94 and 29.02% (for creatinine) with the rat diets supplemented by 0.2, 0.4, 0.8 and 1.6 g/100g of SVBP, respectively.

Urea is formed in the liver as the end product of protein metabolism. During ingestion, protein is broke down into amino acids. In the liver, these amino acids are catbolized and free ammonia is formed. The ammonia is combined to form urea (Pagana and Pagana, 1997). Urea, the major product of protein catabolism measuring urea is the most popular laboratory procedure for assessing renal function. (Bennett *et al*., 1995 and Pagana and Pagana, 1997). Creatinine is a catabolic product of creatine phosphate, which is used in skeletal muscle concentration (Pagana and Pagana, 1997). In the skeletal muscle serum creatinine levels are elevated by renal disease and dehydration.

The decreasing in serum uric acid and creatinine as the result of feeding plant parts including sweet violet could be attributed to their higher content of phytochemicals. For example, El-Nashar, (2007) found that different doses of cinnamon extract showed slight-decreased in serum creatinine after 12 week of feeding when compared with control group. Bedawy, (2008) suggested that the decreasing in serum uric acid and creatinine as the result of feeding with onion, garlic and cabbage could be attributed to their higher content of phenolic compounds. Also, El-Sayed *et al.,* (2012) found that the addition of tested plant parts such Henada *(Jasonia montana)*, lemon balm leaves (*Melissa officinalis*), hawthorn leaves *(Crataegus azorolus),* rose of jericho *(Anastatica hierochuntica)* and corn cob silk *(zea mayz)* by 5 and 10% of the diet intake in the presence of CCl_4 induced significant improvements in all kidney functions including the serum urea and creatinine levels. The possible mode of action of kidney serum parameters-lowering level of the sweet violet plant could be explained by one or more of the following process. El-Nashar (2007) reviewed that polyphenols improved the kidney weight and serum levels of urea nitrogen, creatinine and creatinine clearance as well as increased the activity of superoxide dismutase in the kidney. While, many authors such *Badary et al., (2005) and Mohamed et al., (2005)* found that flavanone produced significant protection of renal function by significant reduction in serum urea and creatinine concentrations, decreased polyuria and reduction in body weight loss, marked reduction in urinary fractional sodium excretion as well as protected kidney tissues. Finally, *Van Hoorn et al., (2006)* noticed that flavonoids lowered plasma creatinine and urea concentration, both indicating a better postoperative kidney functions.

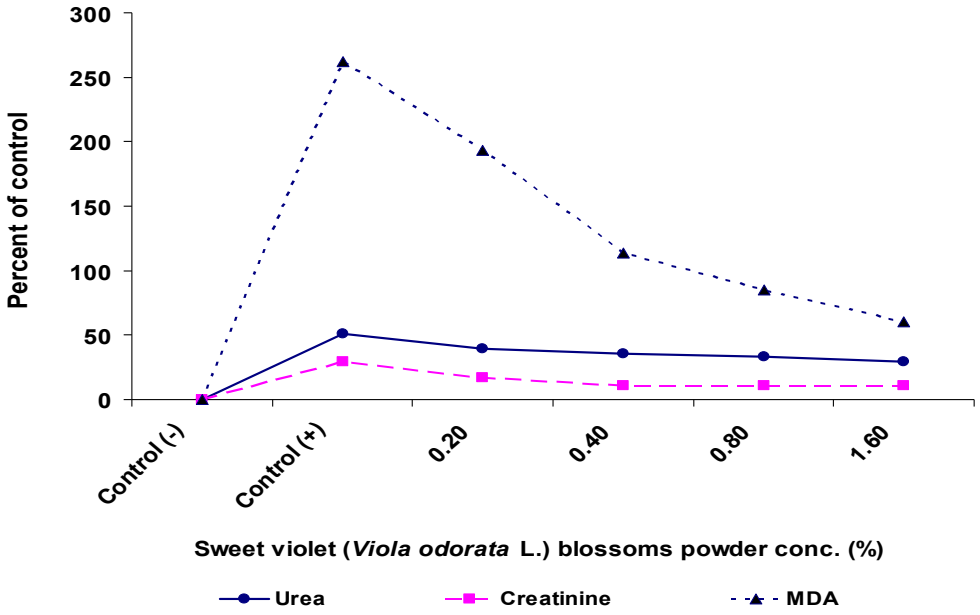

Figure (2). Effect of feeding sweet violet (*Viola odorata* L.) blossoms powder on kidney functions and serum lipid peroxidation (% of control) of rats injected CCl_4

Table (2). Kidney functions (Mean ± SD) of rats injected CCl_4 and consumed sweet violet (*Viola odorata* L.) blossom powder

Kidney functions	Control (-)	Control (+)	Sweet violet blossom powder (%)			
			0.2	0.4	0.8	1.6
Serum urea (mg/dl)	53.18 ± 3.85^{d*}	68.88 ± 7.96^{a}	62.07 ± 3.93^{b}	58.88 ± 3.53^{c}	58.75 ± 2.29^{c}	58.10 ± 1.14^{c}
Serum creatinine (mg/dl)	0.51 ± 0.05^{c}	0.77 ± 0.11^{a}	0.71 ± 0.10^{b}	0.69 ± 0.10^{a}	0.68 ± 0.12^{b}	0.66 ± 0.08^{b}

* Means in the same row with different litters are significantly different ($p \leq 0.05$)

Table (3). Serum lipid peroxidation (Mean ± SD) of rats injected CCl_4 and consumed sweet violet (*Viola odorata* L.) blossom powder

Serum lipid peroxidation	Control (-)	Control (+)	Sweet violet blossom powder (%)			
			0.2	0.4	0.8	1.6
Serum malondialdehyde (MDA, nmol/mg tissue protein)	3.82 ± 0.93^{d*}	13.84 ± 4.84^{a}	11.21 ± 2.70^{a}	8.17 ± 1.44^{b}	7.07 ± 1.76^{b,c}	6.12 ± 2.45^{c}

* Means in the same row with different litters are significantly different ($p \leq 0.05$)

3.3 Serum lipid peroxidation

Table (3) showed the effect of SVBP on the Serum lipid peroxidation (malondialdehyde, MDA) concentration of rats injected by CCl_4 and CCl_4 plus sweet violet blossoms powder. Treatment of animals with CCl_4 caused a significant increased ($p \leq 0.05$) in serum malondialdehyde (262.30%) compared to normal controls. The control positive recorded 13.84 nmol/mg tissue protein which decreased after treated with 0.2, 0.4, 0.8 and 1.6 g/100g SVBP to 11.21, 8.17, 7.07 and 6.12 nmol/mg tissue protein, respectively. Therefore, supplementation of the rat diets with SVBP prevented significantly ($p \leq 0.05$) the rise of mean serum malondialdehyde levels.

In different organisms, endogenous and exogenous free radicals can damage lipids, proteins, carbohydrates and nucleic acids which themselves end up as new free radicals. Among all biomolecules, lipids are the most sensitive molecules to free radical attacks (Frodovrich, 1993). Double bonds in fatty acids form peroxide products by reacting with free radicals, and lipid radicals can be formed subsequently upon removal of electrons *(Cheeseman, 1993 and Girotti, 1998).* As a result of lipid peroxidation, harmful degradative products [namely malondialdehyde (MDA)] can be formed in cell membranes. Malondialdehyde shows both mutagenic and carcinogenic effects by changing membrane properties (*Nielsen et al., 1997 and Gerbhart, 2002).* Lipid peroxidation usually results in decreasing membrane fluidity, cell injury and may cause the formation of atherosclerotic plaques *(Kris-Etherton, 1999).* Cross linking with the membrane components, MDA causes inactivation of enzymes and receptors in membranes and thus changes membrane properties. Malondialdehyde also causes mutations by reacting with guanine nucleotide in DNA *(Girotti, 1998; Cline et al., 2004).* The estimation of free radical activity was done through the determination of malondialdehyde (MDA) which is a by-product of lipid peroxidation *(Chen et al., 1995).* The decreasing in serum MDA levels as the result of feeding plant parts including sweet violet could be attributed to their higher content of phytochemicals. For example, El-Nashar, (2007) found that cinnamon extract showed highly decreased in rats serum MDA when compared with control group. Mohammed, (2008) reported that oral administration of both the aerial parts extracts of Henada *(J. montana)* at a concentration of 150 mg/kg b.w daily for 30 days leads to effectively normalize the impaired antioxidant status in Streptozotocin induced diabetes than the glibenclamide-treated groups. The extract exerted rapid protective effects against lipid peroxidation by scavenging of free radicals by reducing the risk of diabetic complications. Also, Hassan (2011) reported that the administration of apricot kernel extracts specially oil extract prevented the diethylnitrosamine induced elevation of MDA and resulted in a significantly ($p \leq 0.05$) decrease in its content of liver homogenates. High levels of MDA, lipid peroxidation products, were noted in the present study represents an important finding to support our hypothesis, i.e., depression of the antioxidant defence potential in plasma of rats as the result of CCl_4 injection. Based on the current findings, highly significant decreasing rate on the formation of MDA in serum as the result of sweet violet blossoms powder treatment could be represented an important mode of action of the antioxidant activity of the plant.

Conclusion,

Sweet violet blossoms powder (SVBP) was effective in protecting against CCl_4-induced liver injuries. These results supported our hypothesis that SVBP contains several classes of phytochemicals with other compounds that are able to prevent or inhibit CCl_4 hepatotoxicity through liver serum enzymes-lowering activity and decreasing rate on the formation of MDA in serum. Therefore, we recommended like of that plant part (SVBP) by a concentrations ranged 0.2-1.6 % amount to be included in our daily diets, drinks and food supplementation.

4. Acknowledgment

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