Alleviation Effect of Dimethylnitrosamine-Induced Liver Toxicity by Supplementation of Beta Vulgaris Leaves Powder in Rats

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Abstract

Hepatotoxicity is the damage to liver cells caused by exposure to toxic substances (drugs, chemicals, and environmental agents) which lead to functional impairment and liver disease. *Beta vulgaris* leaves are natural plants that have garnered attention due to its rich phytochemical composition and potential therapeutic effects. Therefore, the present study was carried out to investigate the effect of dried *Beta Vulgaris* leave*s* on the amelioration of hepatotoxicity induced by N-Dimethylnitrosamine (DMN) in rats. Forty adult male rats (Sprague Dawley Strain), weighing about 180±5g, were randomized into five groups, each with eight rats. Group 1, negative control group (healthy rats) and fed on a basal diet. Groups 2, 3, 4, and 5 were injected IP with DMN in a dose of 10 mg/kg body weight to induce liver toxicity. Group 2 kept as a positive control group fed on the basal diet. While the other three groups fed on the supplemented diet with *Beta vulgaris* leaves at a level of 2.5%, 5%, and 10% of the diet, respectively. The obtained results exhibit that positive control group had a significant ($P<0.05$) reduction in FI, FBW, BWG, and RBWG, serum concentration of TP, Alb, HDL-c, and the activity of antioxidant enzymes (CAT, SOD, GSH, and GPx), and an increase in the activities of liver enzymes (AST, ALT, and ALP), serum levels of TBL, DBL, IDBL, TL, TG, TC and LDL-c, UN, Cr and UA , MDA, compared to negative control group fed the basal diet. Histopathological examination of liver sections of the positive control group revealed portal fibroplasia associated with inflammatory cells infiltration, portal congestion and hepatic atrophy. As well the size of the hepatocytes and their nuclei was reduced, with nuclei appearing more closely spaced with no evidence of glycogen accumulation. However, rats with hepatotoxicity fed the complemented diet with the different levels (2.5, 5, and 10%) of *Beta Vulgaris* leaves have a significant ($P<0.05$) increase in FI, FBW, BWG, and RBWG, serum levels of TP, Alb, TBL, DBL, and IDBL, HDL- c and activities of the antioxidant enzymes, as well as significant reduction in the liver enzyme activities, serum levels of TL, TG, TC and LDL-c, UN, Cr, UA and MDA, compared to the positive control group. Microscopic examination of liver sections of hepatotoxic rats in the treated group with added 2.5 and 5% of *Beta Vulgaris* leaves to the diet showed moderate improvement and visible normal hepatocytes with 10% of *Beta Vulgaris* leaves. Finally, we concluded

the beneficial health effect of the *Beta Vulgaris* leaves on hepatotoxic rats, which exhibits the ability to improve body weight, liver, and kidney functions. In addition to their beneficial effects on controlling and treating dyslipidemia, oxidative stress, and increasing activity of antioxidant enzymes.

Keywords: Liver Toxicity; Antioxidant Enzymes; Beta Vulgaris; Liver Functions; Kidney Functions.

1. INTRODUCTION

The liver plays a pivotal role in numerous processes such as food and drug biotransformation, protein synthesis, detoxification, and the formation of enzymes essential for digestion (**Ozougwu, 2017**). Throughout these processes, both toxic chemicals and drug overdose may create drug-induced liver injury, and hepatocytes become the primary target (**Zillen *et al.,* 2021**). The conventional hepatotoxic substance includes alcohol, drugs, anti-inflammatory drugs, analgesics and heavy metals. Throughout the process of drug-induced liver injury, liver cell play various roles in inflammatory and fibrotic processes (**Edwards and Wanless, 2013**). The inflammatory response initiated by a damaged hepatocytes accelerates the injury process, leading to tissue damage (**Imaeda *et al*., 2009**).

Additionally, liver disorders generated by oxidative stress promote the pathogenesis of hepatic fibrosis, liver cirrhosis, and property hepatocellular carcinoma (**Yang *et al.,* 2017**). Oxidative stress is considered one of the pathological mechanisms that cause the initiation and progression of liver injury via inducing irreversible modification in lipid membranes, proteins, and DNA and, more importantly, through modulating pathways that control biological function (**Cichoż-Lach and Michalak, 2014**).

Recently, there is a growing concern in the use of natural products and curative plants as alternative therapies to discourage and treat liver diseases. These natural materials are often rich in bioactive components, which have powerful antioxidant and anti-inflammatory characteristics (**Gupta and Misra, 2006**). *Beta vulgaris*, known as beetroot, is among natural plants that have garnered attention due to its rich phytochemical composition and potential therapeutic effects.

Beta vulgaris leaves are an excellent source of vitamins (A, C, and K), minerals (iron, magnesium, potassium), dietary fiber, and bioactive

phytochemicals (betacyanins and polyphenols), which exhibit powerful antioxidant characteristics (**Georgiev *et al.,* 2010**). Although much research has been devoted to the beet roots, some latest studies have exhibit that the beetroot leaves also contain high levels of bioactive components, creation them a hopeful candidate for therapeutic utilization (**Clifford *et al.,* 2015)**.

The antioxidant activity of *Beta vulgaris* leaves is mainly due to the existence of betalains (a group of water-soluble pigments) that have been exhibited to free radicals scavenge, hinder lipid peroxidation, and diminish inflammation (**Clifford *et al.,* 2015**). Despite the promising properties of *Beta vulgaris* leaves, there is a shortage of profound studies investigating the protective role of it against chemically caused liver injury. Therefore, the present study was carried out to investigate the effect of dried *Beta Vulgaris* leaves powder on the amelioration of hepatotoxicity induced by N-Dimethylnitrosamine (DMN) in rats.

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Beta Vulgaris: Fresh whole Beta vulgaris (Photo 1) were obtained from the National Center for Agricultural Research, Cairo, Egypt.

Figure 1: Beta Vulgaris plant

2.1.2. Rats: Forty adult male rats (Sprague Dawley Strain), weighing about 180±5g, were obtained from the Laboratory Animal Colony, Helwan, Egypt.

2.1.3. Basal Diet Components: Casein, cellulose, choline chloride, D-L methionine, vitamins, minerals, and other components were purchased from Al-Gomhoriya Pharmaceutical Company, Cairo, Egypt. While, starch, soybean oil, and sucrose were obtained from the local market.

2.1.4. Chemicals and Kits: N-Dimethylnitrosamine (DMN), reagent for biochemical analysis and the other chemicals were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

2.2. Methods:

2.2.1. Preparation of Dried Whole *Beta vulgaris* Leaves: Fresh whole Beta vulgaris leaves were cleaned from dust with flowing water to get free of any dirt or external bodies. As well, roots and all invalid parts were removed Photo 2. Then, the leaves were dried in a drying oven vacuum at 50ºC. Afterward, a grinder mill and sieves were used to obtain a powder particle size of less than 0.4mm for all plant. The final powder was packaged in a closed bag and stored in the refrigerator at 5ºC until use.

Figure 2: *Beta vulgaris* leaves.

2.2.2. Ingredients and Formulation of Purified Basal Diet (AIN-93M): All components of the basal diet were mixed together to fulfill the desirable adequate dietary intake for keeping the health state of rats as confirmed by **Reeves *et al*., (1993)** . Concisely, each 1 kg diet consists of 140g casein (85% protein), 465.70 g corn-starch, 155 g dextrinized corn-starch, 40g soybean oil, 100g sucrose, 50g fiber, 10g vitamin mixture, 35g mineral mixture, 2.5g choline chloride, 1.8 g L-cysteine and 0.008g Tert-butylhydroquinone.

2.2.3. Induction of Hepatotoxicity: Liver toxicity was induced in the rats by intraperitoneal injections of DMN in a dose of a 10 mg/kg body weight (diluted 1:10 with 0.15 M sterile NaCl) for 3 consecutive days a week for 4 weeks as described by **Choo *et al*., (2016)** .

2.2.4. Experimental Design and Grouping of Rats: All rats were housed in wire cages at the animal house of the Faculty of Home Economics, Helwan University under controlled environmental conditions of the light/dark cycle (12/12 hr), temperature (22±4°C) and relative humidity (45% to 50%). The supply of food and water was uninterrupted during the experimental period.

Prior to the trial study, rats were kept for a week to acclimatize. Subsequently, rats were randomized into five groups, each with eight rats as follows.

- **Group (1):** Rats were kept as a negative control group (healthy rats) and fed on a basal diet.
- **Group (2):** Rats were injected IP with DMN in a dose of 10 mg/kg body weight, fed on the basal diet, and maintained as a positive control group (untreated hepatotoxicity group).
- **Group (3):** Hepatotoxicity rats were fed the supplemented basal diet with Beta vulgaris leaves at a level of 2.5% of the diet.
- **Group (4):** Hepatotoxicity rats were fed the supplemented basal diet with Beta vulgaris leaves at a level of 5% of the diet.
- **Group (5):** Hepatotoxicity rats were fed the supplemented basal diet with Beta vulgaris leaves at a level of 10% of the diet.

2.2.5. Estimation of Feed Intake, Body Weight Gain and Percent Change in Body Weight Gain: Feed intake (FI) was calculated every day during the experimental period (6 weeks). The changes in body weight were determined by weighing the animals prior to the experiment (IBW) and at the end of the experimental period (FBW). The biological value of the diet was assessed by the determination of its effect on body weight gain (BWG) and the percent change of body weight gain was calculated using the following formula:

$$\mathbf{BWG = FBW - IBW}$$

$$\mathbf{\% \text{ Change of body weight gain} = BWG/IBW \times 100}$$

2.2.6. Blood Collection and Serum Separation: At the end of the experiment period (6 weeks), rats were fasted for 12-hr., except of water. Then rats were anaesthetized with diethyl ether and scarified. Blood samples were collected from the posterior vena cava into dry clean centrifuge tubes. Blood samples were left at room temperature to clot, and then centrifuged for 15 minutes at 4000 rpm for serum separation. Serum samples were carefully aspirated using a needle, transfers into dry, clean test tubes and frozen at -20°C for biochemical analysis.

2.2.7. Biochemical Assay:

2.2.7. 1. Estimation of Liver Functions: The activity of serum AST, ALT, and ALP enzymes was quantified using colorimetric (Diamond Co, Hanover, Germany) reagent packages in line with the guidance of **Young (2000)** . The

biometrics were spectrophotometer measurable (Hum star 200, automatic biochemistry analyzer, Germany) which adjusted at 505 nm for AST and ALT, and 510 nm for ALP.

Serum concentrations of total protein (TP), albumin (Alb), total bilirubin (TBL), and direct bilirubin (DBL) were quantified colorimetrically using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) as mentioned by **Tietz (1994) , Young (2000), Henry (1991),** and **Burtis and Ashwood (1999)**, respectively. The apparatus was adjusted at 545, 628, 520, and 548 nm, respectively, for measuring the color intensity that reflects the serum concentration of the tested parameters. While indirect bilirubin (IDBL) was calculated by computing the difference between total and direct bilirubin according to the mentioned formula (Indirect bilirubin (mg/dl) = Total bilirubin - Direct bilirubin).

2.2.7.2. Estimation of Kidney Functions: Quantitative ELISA-based colorimetric reagent assay was used for the measurements of serum urea nitrogen (UN), creatinine (Cr), and uric acid (UA) levels as described in procedures by **Friedman and Young (1997).** The absorbance of the colored solutions was recorded by using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) adjusted at 540,530 and 750 nm, respectively.

2.2.7. 3. Estimation of Lipid Profile: Serum lipid profile as indicated by levels of total lipid (TL), triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) was estimated using commercial reagent (Boomed diagnostic, Egypt) as outlined with **Hostmark *et al.,* (1991) , Vassault *et al*., (1986), Zöllner and kirsch (1962),** and **Young., (2001),** respectively**.**

2.2.7.4. Estimation of Malondialdehyde and Activities of Antioxidant Enzymes: The serum concentration of MDA and the activity of catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) enzymes were determined using commercial assaying kits (Cayman Practice ELISA Kits).

The determination of oxidative stress method depends on colorimetric quantification by quantifying thiobarbituric acid (TBA) reactivity as malondialdehyde (MDA) in a spectrophotometer adjusted at 532 nm according to the described method by **De-Zwart *et al.,* (1999**). While, the procedure that

is used for the evaluation of CAT activity depends on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured spectrophotometrically at 540 nm as described by **(Wheeler *et al*., 1990).** As well the standard technique to assay the activity of SOD is that the kits used use an enzyme linked immunosorbent assaydouble antibody principle. The color change is measured spectrophotometrically at 450 nm as described by **(Wheeler *et al*., 1990)**. The serum activity of glutathione (GSH) and Glutathione peroxidase (GPx) was assayed according to the kit's instructions as described by **Ceballos-Picot *et al*., (1992)** using spectrophotometrically at 340nm.

2.2.8. Histopathological Examination: Liver of all the scarified rats were cleaned, dried and immersed in 10% formalin solution. Then, sections of liver were trimmed, washed and dehydrated in ascending grades of alcohol. Specimens was being then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns' thickness, and stained with Heamtoxylin and Eosin stain for examination as described by **Carleton, (1979)** .

2.2.9. Statistical Analysis: Data was evaluated statistically using computerized SPSS package program (SPSS 22.00 software for Windows) by one-way analysis of variance (ANOVA). The obtained data was expressed as Mean ± SD and the significant difference among means was estimated at $p<0.05$**.**

3. RESULTS

3.1. The Effect of *Beta Vulgaris* leaves on FI and BWG in Rats in Rats with Hepatotoxicity: The recorded results in **Table 1** interpret the effect of feeding hepatotoxic rats on the complemented diet with the different levels (2.5, 5, and 10%) of *Beta Vulgaris* leaves on feed intake (FI), final body weight (FBW), body weight gain (BWG), and relative body weight gain (RBWG %). Results exhibit that the hepatotoxic-rats (positive control group) fed the basal diet alone had a significant ($P<0.05$) reduction in FI, FBW, BWG, and RBWG, compared to healthy rats (negative control group) fed the basal diet. In comparison to the hepatotoxic rats fed the basal diet alone, hepatotoxic rats fed the complemented diet with the different levels (2.5, 5, and 10%) of *Beta Vulgaris* leaves have a significant ($P<0.05$) increase in FI, FBW, BWG, and RBWG. Whereas, the

increase in FI, FBW, BWG, and RBWG was gradually with increasing added levels of *Beta Vulgaris* leaves.

3.2. The Effect of *Beta Vulgaris* Leaves on Liver Functions in Rats with Hepatotoxicity: The obtained results in **Table 2** demonstrate the effect of feeding rats with hepatotoxicity on the complemented basal diet with *Beta Vulgaris* leaves on serum activity of AST, ALT, and ALP enzymes. Delimited outcome displays that the positive group fed on the basal diet alone had a significant ($P<0.05$) increment in the activities of liver enzymes (AST, ALT, and ALP), compared to a normal control group. Whilst feeding rats with hepatotoxicity on the combined basal diet with different levels of *Beta Vulgaris* leaves results in a significant ($p<0.05$) reduction in the liver enzyme activities compared with the positive control group.

As exhibited in **Table 3**, the serum concentration of TP, Alb was significantly reduced ($P<0.05$), while serum TBL, DBL, and IDBL levels increased in rats with hepatotoxicity, compared with healthy rats. On the other hand, feeding rats with hepatotoxicity on an added diet with *Beta Vulgaris* leaves at the three different levels (2.5, 5, and 10%) significantly ($P<0.05$) ameliorates serum levels of TP, Alb, TBL, DBL, and IDBL, compared with feeding rats with hepatotoxicity on a basal diet alone. It is obvious that there is better improvement in liver function as demonstrated by the enhancement in the tested parameters with increasing levels of Beta Vulgaris leaves added to the diet.

Table 1: The Effect of supplemented diet with *Beta Vulgaris* leaves on FI, FBW, BWG and RBWG in rats with hepatotoxicity.

Parameters / Groups		FI (g)	IBW (g)	FBW (g)	BWG (g)	RBWG (%)
Negative control group		13.50±1.20^{a}	203.50±1.20^{a}	260.63±1.80^{a}	56.98±2.59^{a}	27.95±1.38^{a}
Positive control group		11.50±1.20^{b}	203.50±1.20^{a}	229.00±1.30^{e}	25.50±1.20^{e}	12.53±0.62^{e}
Treated groups with BVL at levels of:	**2.5%**	13.50±1.20^{a}	203.25±1.04^{a}	242.25±1.80^{d}	39.00±1.31^{d}	19.19±0.63^{d}
	5%	13.50±1.20^{a}	203.25±1.04^{a}	251.13±1.80^{c}	47.88±1.81^{c}	23.56±0.93^{c}
	10%	13.50±1.20^{a}	203.25±1.04^{a}	256.13±1.90^{b}	52.88±2.10^{b}	26.02±1.09^{b}

Values are expressed as means ± SD; Values at the same column with different letters are significantly different at $P<0.05$; **BVL:** *Beta Vulgaris* leaves; **FI**: Feed Intake; **IBW:** Initial Body Weight; **FBW**: Final Body Weight; **BWG**: Body weight gain; **RBWG:** Relative Body Weight Gain.

Table 2: The Effect of *Beta Vulgaris* leaves on activity of serum AST, ALT and ALP enzymes in rats with hepatotoxicity.

Parameters / Groups		AST (U/L)	ALT (U/L)	ALP (U/L)
Negative control group		22.40±0.90^{d}	20.00±0.90^{d}	517.50±1.70^{e}
Positive control group		46.30±0.90^{a}	57.00±1.30^{a}	713.00±2.10^{a}
Treated groups with BVL at levels of:	**2.5%**	39.50±0.90^{b}	49.10±0.80^{b}	679.60±0.90^{b}
	5%	25.40±1.10^{c}	32.90±1.00^{c}	643.10±2.03^{c}
	10%	25.80±1.30^{c}	32.40±1.10^{c}	579.50±0.80^{d}

Values are expressed as means ± SD; Values at the same column with different letters are significantly different at P<0.05; **BVL**: *Beta Vulgaris* leaves **AST**: Aspartate Aminotransferase; **ALT**: Alanine aminotransferase; **ALP**: Alkaline Phosphatase.

Table 3: The Effect of *Beta Vulgaris* leaves on serum TP, Alb, TBL, DBL. And IDBL levels in rats with hepatotoxicity.

Parameters / Group		TP (gm/dl)	Alb (gm/dl)	TBL (gm/dl)	DBL (gm/dl)	IDBL (gm/dl)
Negative control group		10.50±0.19^{a}	4.63±0.25^{a}	0.65±0.02^{d}	0.18±0.01^{d}	0.47±0.02^{c}
Positive control group		7.45±0.22^{c}	3.69±0.12^{d}	0.95±0.02^{a}	0.36±0.06^{a}	0.59±0.06^{a}
Treated groups with BVL at levels of:	**2.5%**	7.47±0.26^{c}	4.04±0.37^{c}	0.89±0.01^{b}	0.36±0.05^{a}	0.53±0.01^{b}
	5%	8.56±0.25^{b}	4.30±0.03^{b}	0.75±0.01^{c}	0.28±0.01^{b}	0.47±0.02^{c}
	10%	8.57±0.23^{b}	4.50±0.08^{ab}	0.67±0.01^{d}	0.20±0.01^{c}	0.47±0.02^{c}

Values are expressed as means ± SD; Values at the same column with different letters are significantly different at P<0.05; **BVL:** *Beta Vulgaris* leaves; **TP**: Total Protein; **Alb**: Albumin; **TBL**: Total Bilirubin; **DBL**: Direct Bilirubin; **IDBL**: Indirect Bilirubin.

3.3. The Effect of *Beta Vulgaris* Leaves on Lipid Profile in Rats with Hepatotoxicity: The recorded results in **Table 4** represent the effect of supplemented diet with the three different levels (2.5, 5, and 10 %) of *Beta Vulgaris* leaves on serum concentration of TL, TG, TC, LDL-c and HDL-c in hepatotoxic rats. The results exhibited a significant ($p<0.05$) decrease in serum HDL-c and increase in serum TL, TG, TC and LDL-c concentrations in hepatotoxicity rats fed on the basal diet alone (positive control group), compared to normal rats. On the other hand, the results showed that there was a significant increase in serum HDL-c and decrease in serum TL, TG, TC and LDL-c concentrations of the hepatotoxicity rats of treated groups fed on the supplemented diet with the different levels of *Beta Vulgaris leaves*, compared to the hepatotoxicity rats fed on the basal diet alone. The best amelioration in

serum levels of TL, TG, TC, LDL-c and HDL-c was shown in the treated groups by 10% of *Beta Vulgaris* leaves

Table 4: The Effect of *Beta Vulgaris* leaves on serum TL, TG, TC, LDL-c and HDL-c levels in rats with hepatotoxicity.

Parameters / Groups		TL (mg/dl)	TG (mg/dl)	TC (mg/dl)	LDL-c (mg/dl)	HDL-c (mg/dl)
Negative control group		481.42±1.39^{e}	98.90±1.50^{d}	124.90±1.70^{e}	61.14±1.35^{e}	38.90±0.80^{a}
Positive control group		756.57±3.55^{a}	195.80±0.90^{a}	219.50±1.04^{a}	185.06±0.55^{a}	32.30±1.30^{b}
Treated groups with BVL at levels of:	**2.5%**	674.00±1.91^{c}	135.50±1.60^{b}	164.10±1.10^{b}	134.75±1.67^{b}	38.10±1.00^{a}
	5%	699.50±2.50^{b}	116.00±1.30^{c}	157.60±1.30^{c}	78.32±1.18^{c}	38.40±1.10^{a}
	10%	583.80±2.90^{d}	115.60±1.10^{c}	137.50±1.20^{d}	73.70±1.40^{d}	38.90±1.50^{a}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **BVL:** *Beta Vulgaris* leaves; **TL**: Total Lipid; **TC:** Total Cholesterol; **TG:** Triglycerides; **LDL**: Low Density Lipoprotein; **HDL**: High Density Lipoprotein;

3.4. The Effect of *Beta Vulgaris* Leaves on Kidney Functions in Rats with Hepatotoxicity: Results in **Table 5** exhibit the effect of a complemented diet with *Beta Vulgaris* leaves on serum concentration of BUN, Cr and UA in hepatotoxicity rats. The results exhibited a significant ($p<0.05$) increase in serum BUN, Cr and UA concentrations in rats with hepatotoxicity (positive control group), compared to normal rats. However, the results showed a significant decrease in serum BUN, Cr and UA concentrations of rats with hepatotoxicity-rats in fed groups on the supplemented diet with the different levels of *Beta Vulgaris* leaves, compared to the rats with hepatotoxicity fed on the basal diet alone. The most highly improvement in serum levels of BUN, Cr, and UA was shown in the treated hepatotoxicity-rats groups by *Beta Vulgaris* leaves at a level of 10%.

Table 5: The Effect of *Beta Vulgaris* leaves on serum BUN, Cr and BUA levels in rats with hepatotoxicity.

Parameters / Groups		BUN (mg/dl)	Cr (mg/dl)	UA (mg/dl)
Negative control group		26.50±1.5 0^{c}	0.72±0.08^{d}	3.20±0.10^{d}
Positive control group		31.10±1.1 0^{a}	0.93±0.07^{a}	4.00±0.10^{a}
Treated groups with BVL at levels of:	**2.5%**	29.00±1.1 0^{b}	0.83±0.09^{b}	3.70±0.10^{b}
	5%	26.10±1.0 0^{cd}	0.78±0.08^{c}	3.50±0.10^{c}
	10%	25.10±1.1 0^{d}	0.72±0.10^{d}	3.30±0.20^{d}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **BVL:** *Beta Vulgaris* leaves; **BUN:** Blood Urea Nitrogen; **Cr**: Creatinine; **UA:** Uric Acid**.**

3.5. The Effect of *Beta Vulgaris* leaves on Oxidative Stress and Activity of Antioxidant Enzymes in Rats with Hepatotoxicity: Table 6 represents lipid peroxidation as indicated by serum MDA level and serum activity of antioxidant enzymes (CAT, SOD, GSH and GPx) in normal rats and rats with hepatotoxicity. In comparison to the normal rats, rats with hepatotoxicity encourage a significant ($P<0.05$) increase in serum MDA level and decrease in the serum activity of CAT, SOD, GSH and GPx enzymes. In contrast, feeding the hepatotoxicity rats on the supplemented diet with the different levels of *Beta Vulgaris* leaves significantly ameliorate serum MDA levels and activities of antioxidant enzymes (CAT, SOD, GSH, and GPx), compared to the positive control group fed on the basal diet alone. The superior result in serum concentration of MDA and activity of antioxidant enzymes was shown in the treated group by the upper levels (10%) of *Beta Vulgaris* leaves.

Table 6: The Effect of *Beta Vulgaris* leaves on serum concentration of MDA and the activity of CAT, SOD, GSH and GPx enzymes in rats with hepatotoxicity.

Parameters / Groups		MDA mmol/ml	CAT (U/l)	SOD (U/l)	GSH (U/l)	GPx (U/l)
Negative control group		2.20± 0.10^{e}	97.16±2.67^{a}	913.50± 1.5^{b}	63.90±0.15^{a}	79.17±2.91^{a}
Positive control group		4.80±0.10^{a}	48.87±1.46^{d}	643.80± 2.1^{e}	52.30±0.16^{e}	37.16±1.34^{e}
Treated groups with BVL at levels of:	**2.5%**	3.80±0.10^{b}	79.20±1.63^{c}	794.30± 2.1^{d}	55.33±0.13^{d}	42.24±1.49^{d}
	5%	3.30±0.10^{c}	83.30±2.40^{b}	902.50±1.80^{c}	57.50±0.04^{c}	53.73±2.56^{c}
	10%	3.00±0.10^{d}	95.43±2.43^{a}	934.10± 1.00^{a}	59.10±0.06^{b}	74.43±2.57^{b}

Means with different letters in each column are significantly differs at $p< 0.05$; **BVL:** *Beta Vulgaris* leaves; **MDA:** Malondialdehyde; **CAT**: Catalase; **SOD**: Superoxide Dismutase; **GSH**: Reduced Glutathione; **GPX**: Glutathione Peroxidase.

3.6. Histopathological Examination of Liver: Histopathological examination of liver sections of health rats from the negative group showed normal histological in liver structure of hepatic parenchyma as shown in **Photo 3**. In comparison to the negative control group, examined liver sections of hepatotoxic-rats from the positive control group revealed portal fibroplasia associated with inflammatory cells infiltration (**Photo 4**), portal congestion (**Photo 5**) and hepatic atrophy (**Photo 6**). As well the size of the hepatocytes and their nuclei was reduced, with nuclei appearing more closely spaced with no evidence of glycogen accumulation. On the other hand examining liver sections of the hepatotoxic rats of treated group with added 2.5% of *Beta Vulgaris* leaves to the diet showed moderate fibroplasia associated with fewer mononuclear inflammatory cells infiltration in portal area and hyperplasia of

biliary epithelium as shown in **Photo 7**. Likewise, examined liver sections of the hepatotoxic-rats of treated group with added 5% of *Beta Vulgaris* leaves to the diet exhibited portal inflammation with thickening of the hepatic capsule with fibroplasia and inflammatory cells infiltration (**Photo 8**). While other examined sections were visible as normal hepatocytes. The examined liver sections of the hepatotoxic rats of treated group with added 10% of *Beta Vulgaris* leaves to the diet were visible as normal hepatocytes as shown in **Photo ٩**.

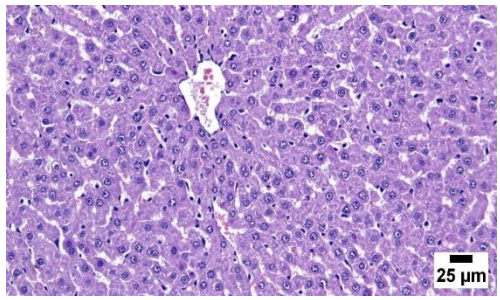

Photo ٣: Photomicrograph of liver sections from health rats (negative control group) showing normal histological in liver structure of hepatic parenchyma (H and E).

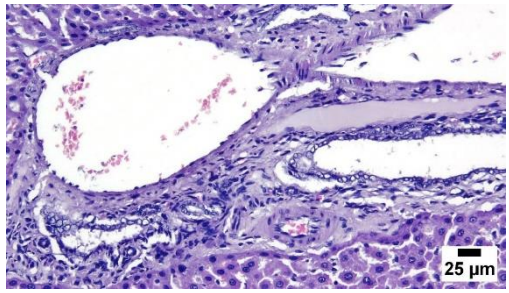

Photo 4: Photomicrograph of liver sections from the positive control group showing portal fibroplasia associated with inflammatory cells infiltration (H and E).

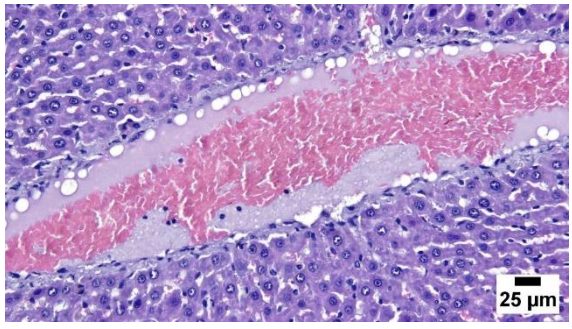

Photo 5: Photomicrograph of liver sections of hepatotoxic rats from the positive control group showing congestion (H and E).

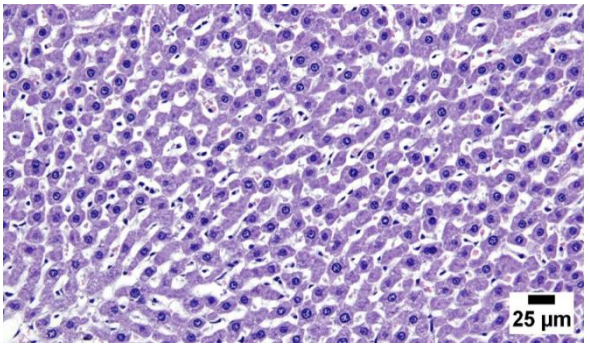

Photo 6: Photomicrograph of liver sections of hepatotoxic rats from the positive control group showing atrophy of hepatocyte with sinusoidal dilation (H&E).

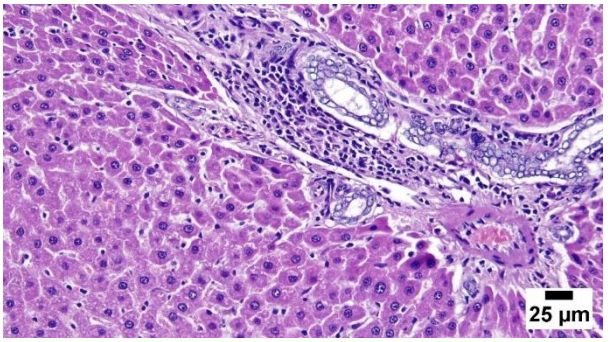

Photo 7: Photomicrograph of liver sections of hepatotoxic-rats from the treated group with 2.5% of *Beta Vulgaris* leaves showing moderate fibroplasia associated with fewer mononuclear inflammatory cells infiltration in portal area and hyperplasia of biliary epithelium (H&E).

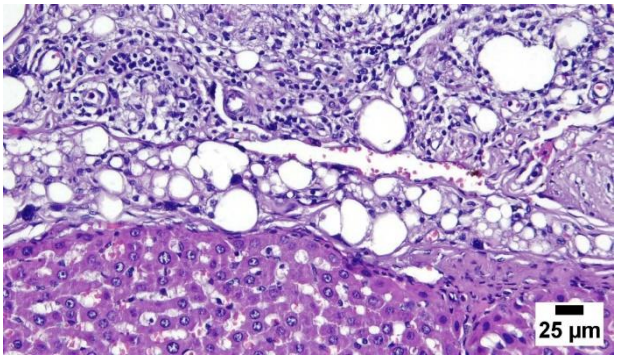

Photo 8: Photomicrograph of liver sections of hepatotoxic rats from the treated group with 5% of *Beta Vulgaris* leaves showing thickening of hepatic capsule with fibroplasia and inflammatory cells infiltration (H&E).

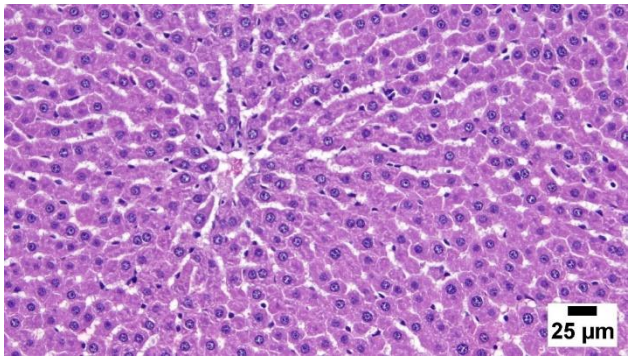

Photo 9: Photomicrograph of liver sections of hepatotoxic-rats from the treated group with 10% of *Beta Vulgaris* leaves showing normal hepatocytes (H&E).

4. DISCUSSION

The hepatoprotective effect of *Beta vulgaris* leaves (BVLs) against hepatotoxicity induced by N-Dimethylnitrosamine (DMN) in rats. This effect was verified by investigating its influence on numerous biological and biochemical parameters, inclusive alteration in feed intake, body weight, liver and kidney functions, lipid profile, lipid peroxidation, and the activities of antioxidant enzymes. Furthermore, histopathological examination of liver tissues was carried out to assist with the biochemical outcome and corroborate the effect of *Beta vulgaris* leaves on liver toxicity.

The acquired results discovered that rats with hepatotoxicity presented a significant reduction in FI, FBW, BWG, and % change of BWG, accompanied by pronounced elevation in liver enzyme activities (AST, ALT, and ALP), serum bilirubin fractions (TBL, DBL, IDBL), BUN, Cr, UA, TL, TG, TC, LDL-c, and MDA levels. Also, there was a significant decline in serum TP, Alb, and HDL-c concentrations, and the activity of antioxidant enzymes (CAT, SOD, GSH, and GPx). In addition, histopathological examination of liver sections of hepatotoxic rats revealed portal fibroplasia associated with inflammatory cell infiltration, congestion, and hepatic atrophy. As well, the size of the hepatocytes and their nuclei was reduced, with nuclei appearing more closely spaced. These results may be attributable to the hepatocellular injury induced by DMN, which diminishes metabolic and digestive efficiency, suppresses appetite, and disrupts protein and energy metabolism. Therefore,

hepatic disorder reduces the ability of hepatocytes to synthesize essential proteins and enzymes required for nutrient utilization, thereby leading to growth retardation **(Hou et *al*., 2020).** Comparable remarks were informed by **Al-Harbi *et al*., (2021)**, who mentioned that hepatotoxicity often leads to significant body weight loss due to lipid peroxidation and oxidative stress that accommodation liver metabolism and energy production. Similar, **Kamal *et al*., (2022) and Niu *et al*. (2023)** showed that hepatotoxic agents such as dimethylnitrosamine (DMN) and carbon tetrachloride (CCl4) cause significant increases in serum AST, ALT, and ALP activities as a result of oxidative stress–induced damage to hepatocyte membranes. Also, result was in the line with the obtained findings by **Akinmoladun *et al*., (2007)** who highlighted that hepatic damage often leads to dyslipidemia due to impaired lipid clearance. Additionally, the obtained results was in agreement with the obtained results by **Aly *et al*., (2016)** , who observed that chemically induced hepatotoxicity in rats was associated with impaired renal markers due to oxidative stress and metabolic overload.

The significant amendment in all the tested biochemical parameters and histopathological inquiry was shown in the hepatotoxic rats fed the complemented diet with the different levels (2.5%, 5%, and 10%) of *Beta vulgaris* leaves. Where the effect exhibits augmentation in body weight, betterment in renovated liver and kidney functions, lipid profile, reduced oxidative stress, and increased antioxidant enzyme activities in a dose-dependent manner. The most pronounced amelioration was respected in rats fed an added diet with 10% *Beta vulgaris* leaves, indicating a strong protective and restorative effect. Histopathological observation of liver sections confirmed this biochemical progress, showing reduced hepatic degeneration, less inflammatory infiltration, and nearly normal liver and kidney architecture compared to the hepatotoxic control group. The discovered improvement in all the tested biological and biochemical parameters may be clarified by the progress in hepatic detoxification and antioxidant ability. Where the phenolic compounds and flavonoids in *Beta vulgaris* scavenge free radicals and prevent lipid peroxidation in hepatocytes, thereby preserving cell integrity and enzyme activity. This recovery of liver function furnish to better appetite regulation, nutrient absorption, and metabolic turnover, which together improve growth parameters. **Al-Harbi *et al.,* (2021)** and **Yousefi *et al.,* (2022)** inveterate that

Beta vulgaris increases hepatic lipid metabolism by activating the PPARα pathway and down-regulating lipogenic genes such as SREBP-1c and FAS, consequent in enhanced energy employment and body weight. These findings are in line with the results of **Georgiev *et al*., (2022)** and **Madić *et al*., (2023)** , who proved that *Beta vulgaris* extract is a rich in betalains, betaine, and polyphenols which augmented antioxidant enzyme activity (SOD, CAT, GPx) and reduced oxidative damage, leading to preferable feed efficiency and growth performance in hepatotoxic rats.

As well, **Eklund *et al*., (2023)** stated the nutritional and physiological importance of betaine in restoring the activity of liver metabolic and improved growth performance under oxidative stress conditions. They reported that the betaine in *Beta vulgaris* acts as a methyl contributor in the methionine–homocysteine cycle, upgrading protein synthesis and osmoregulation in hepatocytes. This mechanism supports cellular hydration, stabilizes enzymes, and discourage apoptosis in affected liver tissue, all of which promote body weight recovery.

Additionally, the attained outcome agreed with **Clifford *et al*., (2020)** , who stated that beetroot leaves improved antioxidant status, diminished inflammation, and supported energy metabolism in rats exposed to hepatotoxic agents. Therefore, the hepatoprotective influence of *Beta vulgaris* may be due to its bioactive component, which has strong antioxidant properties, thereby protecting hepatocytes from oxidative damage. These results were consistent with the outcome of **Georgiev *et al.,* (2022)** and **Madić *et al*., (2023)** , who discovered that *Beta vulgaris* leaves extracts have the ability to restore normal activity of the hepatic enzyme and enhance antioxidant protecting mechanisms in chemically-induced liver injury in animal models. Furthermore, the discovered improvement in serum TP and Alb concentrations in hepatotoxic rats fed the supplemented diet with *Beta vulgaris* leaves indicates partial restoration of hepatic biosynthetic activity. These consequences were in agreement with the outcome of **Yousefi *et al*., (2022)** , who proved that *Beta vulgaris* extract increases hepatic protein synthesis via its antioxidant action and for activation of hepatocellular repair pathways. As well as the simultaneous reduction in the serum levels of total, direct, and indirect bilirubin in the treated groups with *Beta vulgaris* leaves suggests that its ability to improve hepatobiliary function and facilitate bilirubin conjugation and excretion. These

outcomes may be a consequence of the ability of *Beta vulgaris* bioactive to retain hepatocyte integrity and normalize bile flow, as previously described by **Al-Harbi *et al*., (2021).** Likewise, **Kasabri *et al*., (2017)** noted that *Beta vulgaris* supplementation improved serum TP and bilirubin levels in rats with metabolic disorders, attributing these outcomes to its higher content of beta-carotene and polyphenolic components. Moreover, **Kujala *et al*., (2000)** pointed out that betalains in *Beta vulgaris* possess powerful free radical-scavenging action, which protects hepatocytes from oxidative injury and maintains their synthetic capacity, therefore explaining the better TP and Alb levels in treated groups.

As showed, *Beta Vulgaris* leaves ameliorated serum lipid profile (TL, TG, TC, LDL-c and HDL -c). This aligns with **Abd-El-Ghffar *et al*., (2023)** who proven that beet (*Beta vulgaris*) leaf extract significantly diminished serum lipid profile parameters in alloxan-induced diabetic rats and enhanced antioxidant markers. As well as **Al-Etly *et al.,* (2023)** observed that *Beta vulgaris* treatment significantly lower serum cholesterol, triglycerides, and LDL levels in hyperuricemic rats, additional supporting its lipid-lowering efficacy. These effects are potential due to the antioxidant and anti-inflammatory characteristics of phytochemicals like flavonoids and polyphenols identified in the leaves, which help protect toward the harmful effects of a high-fat diet and diminish lipid peroxidation (**El shahat *et al*., 2021**).

Moreover, the improvement effect of Beta Vulgaris leaves on kidney functions, the obtained results was in agreement with the obtained results by **Kapadia *et al*., (2011**) who reported that Beta vulgaris extract protected renal tissues in experimental models of toxicity, which supports the present outcome. Similarly, **Vulić *et al*., (2014)** Confirmed that phenolics and betalains utilize nephroprotective and hepatoprotective action by modulating oxidative and inflammatory pathways.

In addition, **Ohta *et al*., (2000)** noted that dietary supplementation with dehydrated beet stalks and leaves in mice fed HFD significantly mitigating hepatic oxidative damage, reducing MDA levels and enhancing activities of antioxidant enzymes (SOD, GPx, and GSH). Also, **Al-Etly *et al*., (2023)** demonstrated that *Beta vulgaris* leaf treatment greatly reduced serum oxidative stress (as indicated by lower MDA) and restored activities of antioxidant enzymes in hyperuricemic rats.

Conclusion:

The obtained results concluded that the beneficial health effect of the *Beta Vulgaris* leaves on hepatotoxic rats, which exhibits the ability to improve body weight, liver, and kidney functions. In addition to their beneficial effects on controlling and treating dyslipidemia, oxidative stress, and increasing activity of antioxidant enzymes.

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تأثير إضافة مسحوق أوراق البنجر علي تخفيف سمية الكبد المُحدثه بواسطة ثنائي ميثيل نيتروسامين في الفئران

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المستخلص العربي:

تعرف السمية الكبدية بانها تلف يحدث في خلايا الكبد الناتج عن التعرض للمواد سامة (مثل الأدوية والمواد الكيميائية والعوامل البيئية) مما يؤدي إلى ضعف وظائف الكبد وأمراضه. تُعد أوراق بيتا فولغاريس (البنجر) من بين النباتات الطبيعية التي حظيت بالاهتمام نظرًا لتركيبها الكيميائي وتأثيراتها العلاجية المحتملة. لذلك، أجريت هذه الدراسة للتحقيق في تأثير أوراق بيتا فولغاريس المجففة على تحسين السمية الكبدية التي يسببها ثنائي ميثيل نيتروسامين في الفئران. تم تقسيم أربعين فأرًا ذكرًا (سلالة سبراغ داولي)، يزن حوالي ١٨٠ ± ٥ جرام، بشكل عشوائي. و تم تقسيمهم على خمس مجموعات، كل منها تضم ثمانية فئران. المجموعة الأولى، مجموعة ضابطة سالبة وتغذت على نظام غذائي أساسي. حُقنت المجموعات ٢، ٣، ٤، و٥ داخل الغشاء البريتوني بـثنائي ميثيل نيتروزامين بجرعة ١٠ ملجم/كجم من وزن الجسم لأحداث السُمية في الكبد. وُضعت المجموعة ٢ كمجموعة ضابطة إيجابية، حيث تغذت على النظام الغذائي الأساسي. بينما تغذت المجموعات الثلاث الأخرى على النظام الغذائي المضاف إليه أوراق نبات بيتا فولغاريس بنسبة ٢.٥٪، ٥٪، و١٠٪ من النظام الغذائي، على التوالي. تظهر النتائج التي تم الحصول عليها أن فئران مجموعة ضابطة الإيجابية التي تغذت على النظام الغذائي الأساسي وحده كان لديها انخفاض معنوي كبير في كميه الغذاء المتناوله و نسبة كفاءة التغذية و وزن الفئران و نسبه الزياده فى الوزن وتركيز البروتين الكلي و الالبومين و البروتين الدهنى منخفض الكثافة جدا ونشاط إنزيمات مضادات الأكسدة (الكتاليز و سوبروكسيد ديسموتاز و الجلوتاثيون و الجلوتاثيون بيروكسيديز) وزيادة في أنشطة إنزيمات الكبد (الاسبارتات ترانساميناز و الانين ترانس اميناز و الالكلين فوسفاتيز) ومستويات البيليروبين الكلي و المباشر و الغير مباشر و الدهون الكليه و الدهون الثلاثية و الكوليسترول الكلي و البروتين الدهني منخفض الكثافة و اليوريا و الكرياتينين و حمض اليوريك و المالونديالديهيد في السيرم، مقارنة بالمجموعة ضابطة السالبة التي تغذت على النظام الغذائي الأساسي. كشف الفحص الهستوبثولوجي للكبد في المجموعة الضابطة الموجبة عن تليف بابي مرتبط بتسلل الخلايا الالتهابية واحتقان البابي وضمور الكبد. كما انخفض حجم الخلايا الكبدية ونواها، حيث ظهرت النوى متقاربة أكثر دون وجود دليل على تراكم الجليكوجين. ومع ذلك، أظهرت الفئران المصابة بتسمم الكبد، والتي تغذت على نظام غذائي مُكمل بمستويات مختلفة (٢,٥ و٥ و١٠٪) من أوراق بيتا فولغاريس، زيادة ملحوظة في كميه الغذاء المتناوله و نسبة كفاءة التغذية و وزن الفئران و نسبه الزياده فى الوزن ، ومستويات البروتين الكلي و الالبومين و البيليروبين الكلي و المباشر و الغير مباشر في المصل، و البروتين الدهنى منخفض الكثافة جدا ونشاط إنزيمات مضادات الأكسدة، بالإضافة إلى انخفاض ملحوظ في أنشطة إنزيمات الكبد، ومستويات و الدهون الكليه و الدهون الثلاثية و الكوليسترول الكلي و البروتين الدهني منخفض الكثافة في المصل، اليوريا و الكرياتينين و حمض اليوريك و المالونديالديهيد في السيرم، مقارنةً بالمجموعة الضابطة الموجبة. أظهر الفحص الهستوبثولوجي لكبد للفئران المصابة بتسمم الكبد في المجموعة المعالجة بإضافة ٢,٥ و٥٪ من أوراق بيتا فولجاريس إلى النظام الغذائي تحسنًا معتدلًا وخلايا كبدية طبيعية مرئية مع إضافة ١٠٪ من أوراق بيتا فولغاريس. أخيرًا، استنتجنا التأثير الصحي المفيد لأوراق نبات بيتا فولغاريس على الفئران المصابة بتسمم الكبد، حيث أظهرت قدرتها على تحسين وزن الجسم ووظائف الكبد والكلى. بالإضافة إلى آثارها المفيدة في التحكم باضطراب دهون الدم والإجهاد التأكسدي وعلاجهما، وزيادة نشاط إنزيمات مضادات الأكسدة.

الكلمات المفتاحية: سمية الكبد؛ إنزيمات مضادات الأكسدة؛ بيتا فولغاريس؛ وظائف الكبد؛ وظائف الكلى.