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Effect of Nanoparticles of *Ocimum Basilicum* Methanolic Extract on Rat's Liver

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***Abstract*—** *Ocimum basilicum is a popular herb used in traditional medicine and as a typical ingredient of the healthy diet. The current investigation aimed to evaluate the nontoxic effect of methanolic basil extract. Two groups of twenty rats were used for 8 weeks: group G1 (control) and group G2 (Ocimum basilicum treated). Serum liver function and serum lipid profile were measured using Elisa Kits, and histological studies were affected. No significant changes in levels of alkaline phosphatase (ALP), Alanine Aminotransferase (ALT), and aspartate aminotransferase (AST) were detected. Furthermore, no significant changes are observed in the levels concentration of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL), comparing O. basilicum group with control croup. However, moderate decreasing is observed in the concentration of High-Density Lipoprotein (HDL). Other, no histological changes were observed in the liver of O. basilicum group comparing with the control group. Our study suggests that, the sweet basil methanolic extract appears to be nontoxic.*

***Keywords*—** *Ocimum basilicum, liver function, lipid profile, liver histology*.

I. INTRODUCTION

Sweet basil (*Ocimum basilicum Linn*.) is a well-known herb that belongs to the Lamiaceae family. It has originated in India, Africa, and southern Asia although it is cultivated in the world (Shirazi et al. 2014; Yacout, Elguindy, and El Azab 2012). It is an annual, broadly cultivated herb known for its medicinal value in traditional medicine and also in modern pharmacological investigations.

O. basilicum is added to a variety of foods to provide a specific aroma. In the traditional medicine, the seed is used as demulcent, diuretic and diaphoretic, and headache. The leaves and flowers are used as an aromatic repellant, antispasmodic, reduce fever, nausea, migraine, dysentery, chronic diarrhea, fatigue, tonic, and vermifuge. The roots are used to reduce intestinal diseases and cough. Whereas the oil is used for colds, spasms, and rhinitis (Ahmed et al. 2019; Patel et al. 2018).

O. basilicum contains essential oils such as chavicol, linalool and eugenol, which are extensively used in the food and pharmaceuticals industries (Stanojevic et al. 2017). The essential oils are able to reduce unpleasant odors and replace antioxidants (Ahmed et al. 2019). Besides essential oils, basil also contains phenol and flavonoid compounds which have antioxidant properties (Siti Mahirah, Rabeta, and Antora 2018; Skrypnik, Novikova, and Tokupova 2019). Furthermore, basil has been shown to have a variety of pharmacological effects in many disorders such as brain damage (Singh, Krishan, and Shri 2018), diabetes (Widjaja and Savira 2019), asthma (Eftekhar et al. 2019), anemia (Zangeneh et al. 2019) and hepatic fibrosis (Alomar and Al-Attar 2019).

The liver is an essential and complex organ that plays a central role in numerous metabolic processes, including protein synthesis, lipid and cholesterol homeostasis, blood volume regulation, immune system support, endocrine control of growth signaling pathways, and the breakdown of xenobiotic compounds, in particular many current drugs (Trefts, Gannon, and Wasserman 2017; Vaja and Rana 2020).

Therefore, the aim of this investigation was to assess the effects nanoparticles of *O. basilicum* in liver of Wistar rats.

II. MATERIALS AND METHODS

1. Preparation of Ocimum basilicum extract

Healthy and mature leaves of *O. basilicum* were purchased from The Qatar Alnada plantation, Al Madinah Al Munawara, Saudi Arabia. Fresh basil leaves (400gm) were washed with double distilled water and was dried at room temperature and ground in a grinder with a 2 mm diameter mesh. The dried and powdered plant materials were extracted using methanol 80% by using Soxhlet extractor for 24 hours at a temperature not exceeding the boiling point of the solvent (Vogel and Furniss 1978). The extracts were filtered using Whatman filter paper (Number 1) and then concentrated in vacuo using a Rotary evaporator (Buchi CH-9230). The residues obtained were stored in dark glass bottles at −20°C in airtight containers for further tests (Yacout, Elguindy, and El Azab 2012).

1.1. Synthesis of Herbal Nanoparticles

The methanolic extract was ground into a coarse powder then re-grinded into a fine powder. The obtained powder was milled again into nanoparticles ranging in size from (50- 100 nm) using a ball miller. After, the powder was given orally after being dissolved in 1 ml of distilled water**.** The prepared herbal nanoparticles were scannedin an Electron Microscope (SEM) and tested by the X-ray Diffraction (XRD) method (Salam, Sivaraj, and Venckatesh 2014; Pirtarighat, Ghannadnia, and Baghshahi 2019, Hummdi, and Qahl ., 2020).

1.2. Determination and Preparation of Plant Dried Leaves Extract Doses

The dose of basil dried leaves extract given in the experiment was determined and selected after many preliminary studies by the table below.

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TABLE 1. Determination and preparation of basil dried leaves extract doses

Ocimumbasilicum L. dried leaves	Weight	Methanolic extract	Nano extract	Percentage
		0.02 mg	ng /ml $*10^5$	%

According to the method of (Lalitha, Subbaiya, and Ponmurugan 2013, Hummdi, Qahl., 2020), the leaves extract was kept at a moderate temperature to prevent moisture and prepared daily by dissolving (ng/m b.w./day) of the dry powdered extract in (1ml) of distilled water, then giving it to the selected rats groups orally administered by gastric tube.

2. Animals

The animals were obtained from the animal house of the King Fahd Medical Research Center (KFMRC), King Abdul-Aziz University, Jeddah, Saudi Arabia. Forty adult healthy albino male *Wistar* rats weighing 190–200 g were used in the study. The rats were housed in polypropylene cages and were fed with industrial standard diet formulations that contain all essential nutrients and protein, salts, vitamins and fiber and water. All rats were exposed under standard environmental conditions (12 h light / 12 h dark cycles with controlled temperature 20– 25°C) throughout the study period.

2.1. Experimental design

Forty (40) adult rats type Wister "Rutus norvegigus" were divided into two equal groups each containing twenty animals. Animals are treated as follows for 8 weeks:

Group (G1), control-group, included 20 rats, received distilled water for 8 weeks.

Group (G2), included 20 rats were orally administrated with (0.02 ng /1ml. b.w./day/week) of nano extract of *Ocimum basilicum* L. leaves for 8 weeks.

Body weights of the rats were weekly measured. All animals were treated and daily observed for signs of toxicity up to 48 hours after each treatment.

3. Blood and Tissue Sample Collection

At the end of the experiment, 3.5ml blood samples from the abdominal aorta were collected immediately and deposited into a plastic tube containing anticoagulant solution, followed by plasma separation at 3000 rpm for 15 min and at 4°C. Samples were then kept at −20°C until the analysis of liver function parameters. After that, rats were anaesthetized with diethyl ether and sacrificed by cervical decapitation to avoid stress. The liver tissue was collected for histological analyses.

4. Biochemical Analysis

All plasma parameters, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), high-density lipoprotein (HDL) cholesterol, Low-density lipoprotein (LDL) cholesterol, and triglyceride(TG) were assayed by using the rate Elisa Kitsobtained from My Biosource, U.S.A. the liver ALT, AST, ALP, GGT enzymes were assayed according to the methods described (Lala *et al.*, 2021; Odiegwu *et al.*, 2021) and the serum of lipid profile of the TG, TC, HDL, and LDL has been estimated according to the methods described by (Justice *et al.*, 2019, Aisha *et al.*,2022).

5. Histological study

Microscopic examination was conducted in the current research to study structural changes in liver tissue at the end of experimental period (8 weeks) for each experimental group. Small sections with thickness of 0.5 cm from liver of animals was placed in a solution of neutral buffered formalin 10% cooled at 4°C for 24 hours and then wash sections using running water. Then samples are washed again in buffer phosphate. The specimens are dehydrated in ascending series of ethyl alcohol (from 30 to 100%), and are clearing using Xylene, and were embedded in paraffin wax. Semithin sections (1-3µm) were prepared using rotary microtome and were subsequently stained with hematoxylin/eosin. The sections were viewed and photographed (Zhanmu et al., 2020 Aisha *et al.*,2022).

6. Statistical Analysis

According to the (Aisha *et al.*,2022) methods data analysis was performed using SPSS version 23 (SPSS for Windows, version 23). Data obtained were analyzed using Student's *t*-test and one-way ANOVA. *p* values less than 0.05 were considered as statistically significant. Our results are expressed as mean ± SEM

III. RESULTS

1. Serum marker enzymes

The levels of serum marker enzymes, including alkaline phosphatase (ALP), Alanine Aminotransferase (ALT), and aspartate aminotransferase (AST) serve as markers of liver damage and oxidative stress, which favor the release of aminotransferases from hepatocytes into the blood stream. The serum levels of (ALP), (ALT), and (AST) are shown in Table 2. No significant changes in levels of serum liver function are observed comparing O. *basilicum* group with control croup.

2. Serum lipid profile

The effect of O. *basilicum* on levels of serum lipid profile is shown in table 3. Administration of O. *basilicum* for 8 weeks to Wistar rates significantly ($p < 0.05$) decreased the concentration of High-Density Lipoprotein (HDL) level compared with the control group. After 8 weeks, no significant changes are observed in the levels concentration of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL), comparing O. *basilicum* group with control croup.

3. Histopathological Study

3.1. Control Groups

Examination of liver sections of control group were given distilled water and stained by H&E showed that the liver in rats consists of 5 lobes, each lobe consists of hepatic lobules intertwined with each other and not separated by barriers of stroma which characteristic of other mammals. The hepatocytes are arranged in strands of interconnected plates, its thickness is usually a single cell that is radially arranged around the central vein (Cv) in the center of the hepatic lobule. Normal hepatocytes are polygonal in shape and contain a spheroid, central nucleus (N). Some hepatocytes are

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mononuclear and others are diploidy (Bi-nuclear). The nuclei of hepatocytes are characterized by their large size, and smooth, regular surfaces. Likewise, hepatocytes contain anacidophilic cytoplasm that includes fine granules that aggregate into basophilic masses that can be easily distinguished in sections stained with the H&E (Fig A). The hepatocytes strands are separated by blood sinusoids (Bs), which penetrate, contain endothelial cells (En) with along nucleus, and Kupffer cells (Kc) witha polygonal and dark nucleus, which are considered as fixed macrophages cells in the hepatic tissue (Fig A-1). Hepatic tissue in rats is also characterized by the presence of portal areas (Pa) that contain a portal vein (Pv), hepatic artery (Ha), and one bile ductules (Bd) or more. The bile duct is lined with an epithelial layer consisting of cuboidal cells containing large vesicular nuclei and coated with a thin layer of collagen fibers. Some myofibroblasts and histiocytesare also present in the portal areas (Fig A-1,2,3), which are frequently increased in disease cases.

3.2. Ocimum basilicum Group

There was no fundamental difference observed in the histological structure of the liver tissue of *Ocimum basilicum* group when compared to the control group during the experiment period (8 weeks)**.** In addition to, the most of hepatocytes appeared bi-nucleated. Hypertrophy, proliferation of Kupffer cells, and elongation of endothelial cells lining the blood sinusoids were observed in the hepatic tissue, and around central veins and portal areas of rats treated with the nano extract of *Ocimum basilicum* L. leaves (Fig B).

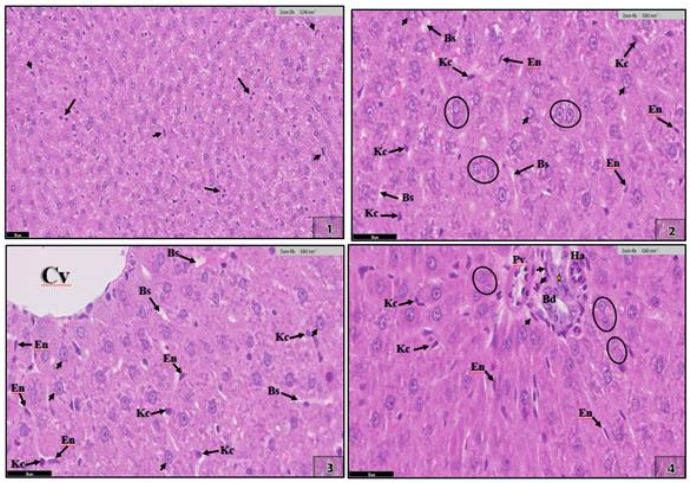

Fig A: liver sections of control group

Fig 1: the section of normal liver tissue shows hepatocyte strands which contains spherical central nuclei, blood sinusoids lining endothelial cells and fixed kupffer cells (H&E) (200×). Fig 2: A higher magnification of previous section showing the hepatocytes containing large, spherical central nuclei, most of the base pigmented chromatin is concentrated at the edge of the nuclear membrane, contain one or two nucleoli. Some hepatocytes are bi-nuclear. Blood sinusoids (Bs) lined with internal endothelial cells (En) and fixed Kupffer cells (Kc) (H&E) (400x). Fig 3: The section reveals normal structure of hepatocyte strands around central vein (Cv), containing large, spherical, central nuclei with one or two nucleoli. Also, the blood sinusoids (Bs) lined with normal endothelial cells (En) and fixed Kupffer cells (Kc) (H&E) (400x). Fig 4: The section of normal liver tissue shows the portal area contains of hepatic artery (Ha), portal vein (Pv), bile ductules (Bd), Myofibroblasts(*) and histiocytes. It also shows some bi-nuclear, with blood sinusoids contain normal endothelial cells (En) and fixed Kupffer cells (Kc) (H&E) (400x).

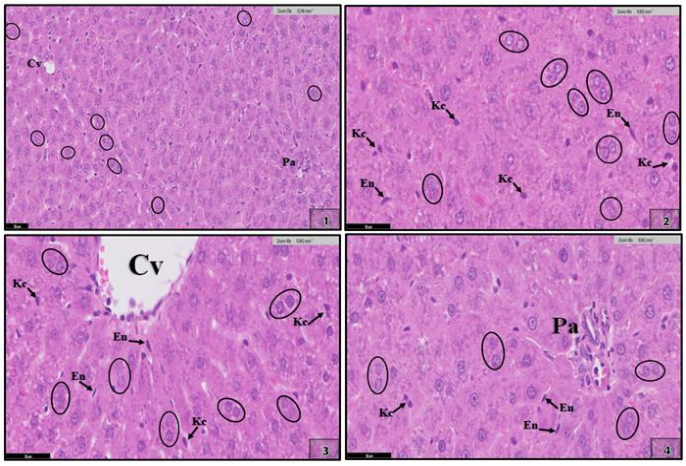

Fig B: liver sections of *Ocimum basilicum* group

Fig 1: Liver section shows normal arrangement of the hepatocyte's strands, and increase number of bi-nuclear hepatocytes (〇), around central veins (Cv), and portal areas (Pa) (H & E) (200x). Fig 2: A higher magnification of previous section shows normal arrangement of the hepatocytes strands, and increase number of bi-nuclear hepatocytes (〇), hypertrophy with proliferation of Kupffer cells (Kc) and elongation of endothelial cells (En) lining the blood sinusoids (H&E) (400x). Fig 3: Liver section shows the normal arrangement of hepatocytes in strands around the central vein (Cv) which lining with normal endothelium, increased number of bi-nuclear hepatocytes (〇). Blood sinusoids shows clarity of proliferation Kupffer cells (Kc), and elongation of endothelial cells (En) (H&E) (400x). Fig 4: Liver section shows normal arrangement of the hepatocytes strands around portal area (Pa), increased number of bi-nuclear hepatocytes (〇), hypertrophy with proliferation of Kupffer cells (Kc), and elongation of endothelial cells (En) (H& E) (400x)

TABLE 2: Serum Livre Functions

	Study Groups / Duration		Control Group	o. *basilicum* group
Alkaline Phosphatase -ALP	0 day	**Mean ± SD**	73.40 ± 34.566	158.40 ± 46.134
		Sig.	-	*P* =0.577
	8Weeks	**Mean ± SD**	180.80 ±57.652	175.20± 58.934
		Sig.	-	*P* =0.883
Alanine Aminotransferase - ALT	0 day	**Mean ± SD**	38.40 ± 6.387	35.40 ± 6.731
		Sig.	-	*P* =0.490
	8Weeks	**Mean ± SD**	45.80 ± 8.044	34.600 ± 3.975
		Sig.	-	*P* =0.598
Aspartate Aminotransferase - AST	0 day	**Mean ± SD**	90.00 ± 15.621	82.40 ± 7.503
		Sig.	-	*P* =0.355
	8Weeks	**Mean ± SD**	104.40 ±12.759	91.800± 13.179
		Sig.	-	*P* =0.163

Data are expressed as Mean +/- SD, SE.

P: significance versus Negative Control group, [1]*P*: significance versus Positive Control group, using One-way Anova test.[2]*P*: significance versus Positive Control group, using One-way Anova test. Significant levels: $P > 0.05$ not significant: $P \leq 0.05$ significant.

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TABLE 3: Serum Lipid Profile

	Study Groups / Duration		Control Group	o. *basilicum* group
Low-Density Lipoprotein LDL	0 day	**Mean ± SD**	0.182 ± 0.030	0.146 ± 0.043
		Sig.	-	*P* =0.160
	8Weeks	**Mean ± SD**	0.164 ±0.034	0.164 ±0.034
		Sig.	-	*P* =0.733
High-Density Lipoprotein HDL	0 day	**Mean ± SD**	1.74 ± 0.084	0.944 ± 0.132
		Sig.	-	0.059
	8Weeks	**Mean ± SD**	1.034 ±0.095	0.878± 0.102
		Sig.	-	*P* =**0.037***
Total Cholesterol TC	0 day	**Mean ± SD**	1.00 ± 0.148	0.736 ± 0.120054
		Sig.	-	*P* =**0.015***
	8Weeks	**Mean ± SD**	0.908 ±0.123	0.742± 0.129
		Sig.	-	*P* =0.071
Triglyceride TG	0 day	**Mean ± SD**	0.752 ± 0.174	0.576 ± 0.118
		Sig.	-	*P* =0.099
	8Weeks	**Mean ± SD**	0.752 ±0.174	0.576± 0.118
		Sig.	-	*P* =0.099

Data are expressed as Mean +/- SD, SE.
P: significance versus Negative Control group, [1]*P*: significance versus Positive Control group, using One-way Anova test.[2]*P*: significance versus Positive Control group, using One-way Anova test. Significant levels: $P > 0.05$ not significant: $P \leq 0.05$ significant.

IV. Discussion

The liver is the main organ in the body. It performs many vital functions, including secreting bile, removing toxins, manufacturing many components of blood plasma, in addition to storing glycogen and oxidizing fatty substances. The liver is also a regulator of all metabolic processes, as well as a number of drugs are metabolized through it by reactions that include cytochrome P450 enzymes. Although the liver is not directly connected to the components, it is affected indirectly as a result of its direct contact with the blood, which exposes it to pathological changes and metabolic disorders in many mammals (Vaja and Rana 2020). Traditional health practitioners have often prepare herbal formulations for the treatment of several diseases (Thring and Weitz 2006). Furthermore, higher pharmacological activity is generally observed in ethanolic, ethyl acetate, and methanolic extractions of plants (Dhanani et al. 2017, Hummdi, Qahl., 2020, Aisha *et al.*,2022).*Ocimum basilicum* L has one of the oldest, richest, and most diverse cultural living traditions associated with the use of medicinal plants (Brown 1980). Hence methanolic extractions of basil were selected for this study. No changes were observed in serum liver function of *Ocimum basilicum* group compared with control group. In general; when there is hepatopathy, the extent of hepatic damage is evaluated by the increased level of cytoplasmic enzymes such as ALT, AST, ALP, and LDH, these marker enzymes leak into the blood. This has been associated with massive centrilobular necrosis, balloon degeneration, and liver cell infiltration (Gram, Atasever, and Eren 2018; Mahmoodzadeh, Mazani, and Rezagholizadeh 2017).

The present study reports have also established that the treatment with *Ocimum basilicum* did not affect the lipid metabolism of liver. No changes in TC, TG, and LDL levels were observed. In contrast moderate decrease in HDL levels was observed. These results confirm the non-toxic effect of *Ocimum basilicum.* It has been reported that hepatotoxicity was characterized by significant elevation in TC, TG, and LDL levels and marked decrease in HDL (Touiss et al. 2021; Ben Hsouna et al. 2019).

Results obtained in the present study indicate that administration of *Ocimum basilicum* for 8 weeks has no effect on liver histology. In this fact, it has been reported that *O. basilicum* have an hepatoprotective effect (Yacout, Elguindy, and El Azab 2012). Furthermore, many studies proved that basil suppressed hepatic fibrosis and protected liver against parenchymal damage (Sakr et al. 2011; Falowo et al. 2019; Ogaly et al. 2015).

V. Conclusion

According to the results obtained, the sweet basil methanolic extract appears to be nontoxic. The study demonstrated the potential of *Ocimum basilicum* to maintain liver parameters as well as liver histology during 8 weeks of treatment.

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