Nat. Volatiles & Essent. Oils, 2021; 8(4): 6754-6763

Estimate Effect Of Ocimum Basilicum L. Seeds Extract On Lipid Profile & Minerals In Hypomagnesemic Of Female Rats

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Abstract

The current study was performed to investigate the effect of the alcoholic extract of Basil seeds (**Ocimum basilicum L**.) in remission of metabolic syndrome in hypomagnesemia which is induced experimentally in female rats. Fifty female rats were randomly assigned to 5 equal groups and treated daily for 56 days as follows: Control group (G1) received tap water, hypomagnesemia group (G2) treated with KCl (1.39mg/kg Bw) orally, (G3) orally KCl (1.39mg/kg BW) orally with (250mg/kg BW) alcoholic extract of Basil seeds, group treated with (250mg/kg BW) alcoholic extract of Basil seeds (G4) Finally hypomagnesemia group treated with MgSo4 injection (0.1 mg/kg BW) (G5). Fasting blood samples were collected by heart puncture technique at 56 days of experiment to assess the lipid profile (LDL-c, VLDL-c, HDL-c, total cholesterol and TAG) and Minerals. The result indicated that magnesium and calcium ion concentration decreased significantly in hypomagnesmia group and increased significantly in groups treated with oral alcholic basil seeds and injection $MgSo_4$. Potassium concentration: Groups with oral intubation of basil seeds causes significant decrease in K^+ as compared with hypomagnesmia group. In addition, $MgSo_4$ injection causes significant decrease in K^+ concentration. Lipid profile (LDL-c, VLDL-c, total cholesterol and TAG) showed significant increase in group which treated with KCl only, while decrease in groups with oral intubation of alcoholic extract of basil seeds, also decrease in group with $MgSo_4$ injection. HDL-c level showed significant decrease in hypomagnesmia group which treated with KCl, while increase in groups with oral intubation of basil seeds, also $MgSo_4$ injection in show significant increase in HDL-c. The concluded that obtained from these results revealed that $MgSo_4$ supplementations are beneficial in returning the minerals to the normal level.

Key words: - Basil seeds, Ocimum basilicum L., Metabolic syndrome, hypomagnesemia, lipid profile, Minerals.

Introduction

Metabolic syndrome is defined by a constellation of interconnected physiological, biochemical, clinical and metabolic factors that directly increase the risk of cardiovascular disease, type 2 diabetes mellitus, all-cause mortality, Insulin resistance, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, genetic susceptibility, elevated blood pressure, hypercoagulable state, and chronic stress are the several factors which

constitute the syndrome **(Piuri et al., 2021).** Normal serum magnesium levels are between 1.46 and 2.68 mg/dL, Hypomagnesemia is an electrolyte disturbance caused when there is a low level of serum magnesium (less than 1.46 mg/dL) in the blood, hypomagnesemia can be attributed to chronic disease, alcohol use disorder, gastrointestinal losses, renal losses, and other conditions, signs and symptoms of hypomagnesemia include anything from mild tremors and generalized weakness to cardiac ischemia and death **(Gragossian et al., 2020).**

Proximate composition of basil seeds contains 9.6% moisture, 14.8% crude protein, 13.8% crude fat, 7.7% ash, 22.6% fiber and 63.8% carbohydrates, total phenolic contents in basil seeds are 63.78±1.75 mg /100g, Mg contents were found in high amount 31.55 mg/100 g as compared to other determined minerals. Also, Zn, Fe and Mn were found in a higher rate (**Munir et al., 2017**). Many authors reported that Ocimum basilicum L. seeds (Basil) can be extensively used for the treatment of different metabolic syndrome such as diabetes mellitus-II (**Chaudhary et al., 2016**); hyperlipidemia **(Kadhim, 2016)**; hypertension **(Umar et al., 2010)**; Ocimum basilicum seed also has antiviral activity **(Al-Amri , 2015).**

Materials and Methods

Experimental Animals:

Fifty female Wister albino rats, four weeks of age and weighed about ranged between 110-140 g. The rats were randomly divided into five main groups each group have 10 animals and housed in stainless steel wire mesh cages on a bedding of wood shavings. Animals in all stages of the experiment were housed in plastic cages 70×50×15 cm in conditioned room Ambient temperature was controlled at (25 ± 3 C) with relative humidity of 50% ± 15% and a light/ dark cycle of 12 hrs. /12 hrs. Food and water were provided at all times except before drugs administration (1/2 hrs. before administration).

Experimental Design:

Fifty rats randomly divided in to five equal group (10 rats per group) and these were treated daily along the experimental period 56 days as follows: Control group (G1): - The animal in this group were gave oral gavage of distilled water. G2: rats in this group were treated with KCl solution at dose of 1.39 g/kg BW (administered orally by gavage needle) (Obaid, 2019), G3: rats in this group were treated with KCl solution at dose of 1.39 g/kg BW. Plus treated with basil seed 250 mg/kg body (Chaudhary et al.,2016), G4: rats in this group were treated with basil seed 250 mg/kg body (Chaudhary et al .,2016), G5: rats in this group were treated with KCl

solution at dose of 1.39 mg/kg BW orally and Mg sulphate at dose 0.1mg / Kg. BW Subcutaneous Injection (Constable et al., 2016).

Materials

Plant Materials

Ocimum basilicum L. seeds which were obtained from local market were collected from the Baghdad. The classification of the plant was done in the collage of science, university of Baghdad, Iraq.

Extraction

Ocimum basilicum L. seeds left to dry in open air away from direct sunlight. They were crashed in a coffee grinder for 2 min, but during this time the grinding will halted for 15 sec at periodic intervals to prevent heating of the sample. The samples were wrap and stored at -18^{o}C until the extraction was performed. The extract of Ocimum basilicum L. seeds were obtained by ethanol 70% with ratio 8:1 of liquid to solid at (50C^{o}) for 3 hours by magnetic stirrer. Then put in rotary evaporator to dry extract then stored in the freezer (-18^{o}C). The extract was semi gelatinous texture, brown color and extraction ratio was (100g/12g).

Preparation of KCl Solution:

According to manufacturer instructions (Science Lab, Sarjapur, India), KCl solution was prepared as following:

1. The powder of KCl was weighted, carefully, using of an electronic analytical balance and then put into a flask.

2. To obtain 10% KCl solution, 100 g of KCl powder was dissolved in 1000 ml (1liter) distilled water at room temperature (25C^{o}), completely.

3. KCl solution was transferred into dark bottle and kept at room temperature (25C^{o})

Blood Samples Collection:

At the end of period (56 day) of the experiment (before sacrificing), animals were prepared for blood samples collection. Blood was obtained via heart puncture according to **Parasuraman et al.,** (2010), from each anesthetized rat's intramuscular injection of Ketamine (100mg/Kg BW) and xylazine (40mg/kg BW) using disposable syringes. Serum was isolated after centrifugation at a speed of 3000 revolution/minute (rpm) for 20 minutes, and then serum samples were stored in freezer at (-18^{o}C) until used for Serum HDL-c, VLDL-c, total cholesterol, triglyceride (Linear chemicals, Barcelona (Spain)), and LDL-c was calculated by Friedewald formula **(Friedewald et al., 1972);** LDL-c (mmol/l) = TC - (HDL-c + VLDL-c)**.**

Determination of Potassium, Calcium and Magnesium in serum:

Magnesium (Mg^{+2}) measured by using the Xylidyl blue with ATCS methode in an enzymatic colorimetric method. Potassium (K^{+1}) measured by using the TPB NA methode in an enzymatic colorimetric method, using kit (BIOLABO) for K^{+1}, according to (**Tietz , 2005**) and according to manufacture company. Calcium (Ca^{+2}) measured by using the modified OCPC method in an enzymatic colorimetric method, using kit (BIOLABO) for Ca^{+2}, according to **(Bigss et al., 1974)** according to manufacture company.

Statistical Analysis

System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study. Ethics The handling of animals and the experimental protocol are performed for being sure that animals do not suffer at any stage of the experiments.

Results and Discussion

Effect of Basil seeds alcoholic extract (Ocimum basilicum L.), KCl and $MgSO_4$ on lipid profile (mg/dl) for 56 days in female rats.

The effect of alcoholic extract of Basil seeds alcoholic extract (Ocimum basilicum L.), KCl and $MgSO_4$ on mean value of serum Cholesterol concentration, TAG concentration, LDL, HDL and VLDL is illustrated in table (1). After 56 days, induced hypomagnesmia by KCl causes significant ($p<0.05$) increase in Cholesterol concentration of G2 (128.00±1.45) as compared with other groups. Of relevance, treatment hypomagnesemia in rats G3 (92.16±0.83) with Basil seeds alcoholic extract leading to significant ($p<0.05$) decrease in Cholesterol concentration as compared with G2 (128.00±1.45) and also decrease in G4 (84.46±1.16) that treated with Basil seeds alcoholic extract group and G5 (87.28±1.05) that treated with KCl and Mg sulphate. In addition, there were no significant differences between G4 (91.20±0.54) and a control group.

TAG concentration in serum was showed a significant increase ($P<0.05$) in G2 (87.30±1.31) as compared with other groups. Meanwhile, treatment of hypomagnesmia in rats G3 (66.68±0.78) with Basil seeds alcoholic extract leading to a significant decrease in TAG concentration also decrease in G4 (65.04±0.76) that treated with Basil seed alcoholic extract group and G5 (63.62±1.70) that treated with KCl and Mg sulphate. Also, there was no significant difference between G4 (65.04±0.76) and a control group.

LDL concentration in serum was showed a significant increase (P<0.05) in G2 (94.50±1.07) as compared with other groups. Meanwhile, treatment of hypomagnesmia in rats G3 (50.48±1.00) with Basil seed alcoholic extract leading to a significant decrease in LDL concentration also decrease in G4 (49.46±1.29) that treated with Basil seed alcoholic extract group and G5 (51.42±1.66) that treated with KCl and Mg sulphate. Also, there was no significant difference between G4 (49.46±1.29) and a G5 group.

HDL concentration in serum was showed a significant decrease (P<0.05) in G2 (18.97±0.39) as compared with other groups. Meanwhile, treatment of hypomagnesmia in rats G3 (30.04±1.24) with Basil seed alcoholic extract leading to a significant increase in HDL concentration also increase in G4 (32.8±1.48) that treated with Basil seed alcoholic extract group and G5(31.3±0.32) that treated with KCl and Mg sulphate.

VLDL concentration in serum was showed a significant increase (P<0.05) in G2 (15.37±0.14) as compared with other groups. Meanwhile, treatment of hypomagnesmia in rats G3 (13.61±0.16) with Basil seed alcoholic extract leading to a significant decrease in VLDL concentration also decrease in G4 (13.22±0.14) that treated with Basil seed alcoholic extract group and G5 (12.80±0.08) that treated with KCl and Mg sulphate.

Table 1. **Effect of Basil seed alcoholic extract (Ocimum basilicum L.), KCl and MgSO4 on lipid profile (mg/dl) for 56 days in female rats.**

Parameter / Groups	Cholesterol (mg/dl)	TAG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
G1 Control	82.23±2.06 D	65.92±0.93 B	45.30±0.61 C	33.34±0.76 A	13.51±0.20 B
G2 (KCl)	128.00±1.45 A	87.30±1.31 A	94.50±1.07 A	18.97±0.39 D	15.37±0.14 A
G3 KCl & Basil seeds alcoholic extract	92.16±0.83 B	66.68±0.78 B	50.48±1.00 B	30.04±1.24 C	13.61±0.16 B
G4 Basil seeds alcoholic extract	84.46±1.16 CD	65.04±0.76 B	49.46±1.29 B	32.8±1.48 AB	13.22±0.14 BC
G5 KCl & Mg Sulphate	87.28±1.05 C	63.62±1.70 B	51.42±1.66 B	31.3±0.32 BC	12.80±0.08 C
LSD	4.07	3.41	3.48	2.82	0.45

Means with a different letter in the same column are significantly different (P<0.05).

G: control, G2: potassium chloride, G3: potassium chloride &Basil seeds alcoholic extract, G4: Basil seeds alcoholic extract, G5: Magnesium sulphate & potassium chloride.

In the basil seed alcoholic extract group showed that restoration effect to it normal value due to significant hypolipidemic effect and that concordance with the study by **(Harnafi et al ., 2013)** decreasing plasma total cholesterol and triacylglycerol levels and increase in LDL level this finding suggests that the plant extract effect may be due to the improvement of reduced activity of hepatic LDL receptor site, the underlying mechanisms of this hypotriglyceridemic effect may be due to an increased stimulation of the lipolytic activities of plasma lipoprotein lipase (LPL) and hepatic lipase (HL) and due to presence of Mg in its phytochemical profile as well as vitamins, minerals, fiber, flavonoids and polyphenols that is support it beneficial evidence to improve lipid profile level to it normal range (**Kiczorowska et al., 2015**). In fact, tannins, flavonoids a group of ubiquitous plant polyphenols, have a variety of pharmacological activities including the hypolipidemic effect **(Bursill et al., 2007)** the possible mechanism underlying this effect of these phytochemicals results from that these phenolic compounds increase hepatic LDL receptor activity.

In addition, tannins and flavonoids are known to reduce cholesterol synthesis via the suppression of hydroxy methyl glutary L CoA (HMG-COA) reductase **(Jung et al., 2006)** Finally, these molecules inhibit the major enzyme involved in cholesterol metabolism, acyl CoA cholesterol acyl transferase (ACAT) (Hanafi **et al., 2009**).

In the present study we identified alterations in the lipid profile, a significantly raised total cholesterol and LDL-c and non-HDL, representing a state of atherogenic dyslipidemia in hypomagnesemia rats. Magnesium deficiency has been linked to the increased risk of development the major cardiovascular risk factors like dyslipidemia, metabolic syndrome, atherosclerosis, diabetes and and hypertentionsion **(Reffelmann et al., 2011)**.

Magnesium has been shown to reduce triglycerides and increase HDL-c by enhancing lipoprotein lipase activity. in addition, it also inhibits HMG_CoA reductase because it’s the ATP-Mg complex for this enzyme in cholesterol synthesis. therefore, it’s called a natural statin (**Manaswini et al , 2017**), it's considered necessary for the action of lecithin cholesterol acyl transferase activity, lowering LDL-c, TG and increasing HDL-c levels, thus, magnesium deficit led to a dyslipidemic state (**Dey et al., 2015**). Further, we also observed that low serum magnesium correlated positively with the presence of metabolic syndrome. Hence, magnesium supplementation might have a role in alleviating the dyslipidemia.

Effect of Basil seeds alcoholic extract (Ocimum basilicum L.), KCl and $MgSO_4$ of minerals for 56 days in female rats.

The effect of alcoholic extract of Basil seeds alcoholic extract (Ocimum basilicum L.), KCl and $MgSO_4$ on mean value of serum Mg^+ concentration, K^+ concentration and Ca^+ concentration is illustrated in table (2). after 56-day, induced hypomagnesmia by KCl causes significant ($p<0.05$) decrease in Mg^+ concentration of G2 (1.78±0.005) as compared with other groups. Of relevance, treatment hypomagnesemia in rats G3 (2.33±0.02) with Basil seeds alcoholic extract leading to significant ($p<0.05$) increase in Mg concentration as compared with G2 (1.78±0.005) and also increase in G4 (2.66±0.14) that treated with Basil seeds alcoholic extract group and G5 (2.58±0.09) that treated with KCl and Mg sulphate.

K^+ concentration in serum was showed a significant increase ($P<0.05$) in G2 (7.74±0.15) as compared with other groups. Meanwhile, treatment of hypomagnesmia in rats G3 (3.80±0.07) with Basil seeds alcoholic extract leading to a significant decrease in K^+ concentration also decrease in G4 (3.87±0.04) that treated with Basil seed alcoholic extract group and G5 (3.65±0.10) that treated with KCl and Mg sulphate.

Ca^+ concentration in serum was showed a significant decrease ($P<0.05$) in G2 (6.27±0.05) as compared with other groups. Meanwhile, treatment of hypomagnesmia in rats G3 (9.81±0.12) with Basil seeds alcoholic extract leading to a significant increase in Ca concentration also increase in G4 (10.86±0.19) that treated with Basil seed alcoholic extract group and G5 (9.97±0.02) that treated with KCl and Mg^+ sulphate.

Table 2. Effect of Basil seeds alcoholic extract (Ocimum basilicum L.), KCl and MgSO4 of minerals for 56 days in female rats.

Parameter / **Group**	Mg **(mg/dL)**	K **(mmol/l)**	Ca **(mg/dL)**
G1 Control	2.16±0.01 **C**	3.90±0.13 B	10.15±0.18 B
G2 (KCL)	1.78±0.005 D	7.74±0.15 A	6.27±0.05 A
G3 KCL & Basil seeds alcoholic extract	2.33±0.02 B	3.80±0.07 B	9.81±0.12 B
G4 Basil seeds alcoholic extract	2.66±0.14 A	3.87±0.04 B	10.86±0.19 A
G5 KCL & Mg Sulphate	2.58±0.09 B	3.65±0.10 C	9.97±0.02 B
LSD	0.23	0.32	0.39

Means with a different letter in the same column are significantly different ($P<0.05$).

G1: control, G2: potassium chloride, G3: potassium chloride &Basil seeds alcoholic extract, G4: Basil seeds alcoholic extract, G5: Magnesium sulphate & potassium chloride.

In this study, hypomagnesemia was induced by administration of potassium chloride result in decrease of serum magnesium and calcium and increase potassium **(Eby et al., 2010)**. potassium administration directly depressed the circulating level of divalent action; magnesium, calcium and phosphorous, because of the increased intra cellular potassium level, increased excretion of magnesium or increased cellular uptake of it. Hence the decline in serum calcium level in response to magnesium deficiency may have result from increased urinary calcium excretion with a coincident decrease in calcium re absorption from bone and similar change in magnesium (**Goff et al., 2014**) . The depletion of plasma Mg and Ca probably precludes the occurrence of tetany in animals. Therefore in grassing animal when feed on pasture with heavily fertilized and high level of soil K are produce tetany (**Friend al et., 2018).** Seeds are one of the most important sources of medicines. The medical plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance (**Alamgir al et., 2017)**.

Basil Seeds as a Novel Food, Source of Nutrients and Functional Ingredients with Beneficial Properties Intake of seeds is well known to have positive effects on health and has correlated to decreased risk of most of metabolic syndrome. basil seeds is reported to be a good source of minerals such as magnesium levels of 31.55 mg/100 g percent constitute a safe level to prevent magnesium deficiency (**Bravo et al., 2021**).

Conclusion

The obtained results revealed that $MgSo_4$ supplementations are beneficial in returning the minerals to the normal level.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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