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RESEARCH PAPER

Role of Moringa oleifera seeds and food supplement on some biochemical parameters in male rats

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A B S T R A C T:

The present study was investigated the antioxidant capacity of Moringa oleifera Seeds (MOS), its impact on the Male Albino rats, and its comparison with Food supplement (FS). The study provided 15 Rats, and according to different feeding foods, the rats are divided into three groups; MOS, FS, and normal Rat's food as a control group. Samples (urine and serum) were collected from each rat after intake MOS, FS and normal food for 85 days then biochemical analysis were performed such as liver enzymes (glutamic oxaloacetic transaminase (GOT), Glutamic Pyruvic Transaminase (GPT), and Alkaline phosphatase (ALP)), lipid profile (high-density lipoprotein (HDL), Triglycerides (TG), and Total Cholesterol (TC)), (Tp and Alb). Moreover, it determined mineral contents of MOS, FS, and normal rat's food by using X-ray fluorescence (XRF). It also detects total antioxidant capacities in MOS, which were evaluated through using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assays. Statistical analysis showed the final body weight significantly increased in FS group compared to MOS and control groups. The TP and Alb levels revealed a significant difference between groups in both serum and urine samples. Liver function parameters raised significantly in FS group when compared with Mos and control group. Moreover, it was evaluated of higher concentration of Potassium and Magnesium in MOS when compared with FS group and control group. PPH scavenging capacity in MOS. We conclude that MOS is healthy and rich with Potassium and Magnesium, which can be used as an antioxidant source. While FS has side effects on rats' kidneys and liver, although the FS increases body weight compared to other groups.

KEY WORDS: Moringa oleifera seeds; X-ray fluorescence; Experimental male rats; Total antioxidant; Food supplement.
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1. INTRODUCTION:

The World Health Organization (WHO) has stated that 80% of the developing world still benefits from the use of traditional medicines derived from medicinal plant and described medicinal plants as one of the potential sources of new drugs (Vaou et al., 2021). leave, root and seed of Moringa perform beneficial physiological and biochemical functions in humans (Hamza and Azmach, 2017).

The Moringa oleifera is an important medicinal plant belonging to the family Moringaceae and known as "Kelor" by Indonesians. It is the most cultivated plant in India and is famous for being a nutritional herb (Mohanty et al., 2021).

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Most of this therapy involves the use of plant extracts and their active components (Süntar, 2020). Thus, bioactive compounds present in

Universally, it is known as "horseradish plant" or "drumstick plant," which consists of biological exertion such as anticancer, antidiabetic, antihypertensive, treat malnutrition, and beneficial as a focus enhancer and as well as injury treatment enterprise (Fidrianny et al., 2021, Guterres et al., 2022). These plant seeds have nutritional advantages for muscle building and preserving body mass (Iliyasu et al., 2020, Gupta and Mishra, 2021). Additionally, these seeds are a source of omega-three, making them a good product to protect the body against cardiovascular disease. Therapeutic perspectives of them

discovered that seeds are a great source of antioxidants (Ezz El-Din Ibrahim et al., 2022), and are effective in lessening the levels of fasting blood glucose, total cholesterol, triglycerides, low-density lipoprotein, and liver function (Phimarn et al., 2021). Besides, high-density lipoprotein cholesterol levels were remarkably higher in these seeds (Phimarn et al., 2021). These seeds can decrease blood cholesterol and which of them namely lipids and reduce hazards to liver function (Ain et al., 2020, Akter et al., 2021). Mahajan et al, studied that the Moringa olifera ethanol extracted (MOEE) has anti-arthritic and antioxidant activity in treated rats (Mahajan et al., 2007).

Food supplements include a wide range of products that are designed to be taken because of their added nutrients and presumed health benefits (Fibigr et al., 2018). In the US, "food supplements" is the official term defined by the food and drug administration (FDA). According to the FDA definition, food supplements are products taken by mouth that contain a "dietary ingredient." Dietary ingredients include vitamins, minerals, amino acids, and herbs or botanicals, as well as other substances that can be used to supplement the diet (Fibigr et al., 2018). Food supplements are very helpful for nutrient deficiencies, enhance physical activity and sometimes to growth promotion (Savino et al., 2019, Fibigr et al., 2018). However, even taking too much of safe supplements like vitamins, proteins, and creatine, for a long time, can be harmful. For instance, creatine supplementation may lead to renal dysfunction and hepatotoxicity (Timcheh-Hariri et al., 2012).

The present study aimed to evaluate the role of MOS and FS on some biochemical parameters in male rats.

2. MATERIALS AND METHODS

2.1 Chemical substances used

In this study, Distilled water, (70%) Ethanol and Methanol Absolute (Spectrum Chemical, New Mexico, united states), Supplement food (Powerstar Food Nahrungsergänzungen GmbH, Homburg, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Santa Cruz Biotechnology, Inc., Santa Cruz, Canada), and Butylated hydroxytoluene (BHT) (Silverline Chemicals, India) are used.

2.2 Preparation of flours and extraction of plant seeds

Moringa Seeds and food supplement (that is the artificial protein used by sportsmen) are bought from Local markets of Koya City. Starting from grinding seeds into powder, then adding seeds powder and food supplements to standard components of the rat's diet by two doses (1.5 and 0.5g) per day, respectively. In this study, 20g of plant seed powders are extracted by adding 100ml of 70% ethanol to the shaker at 32oC for 48 hr. The extract solution is filtered by filter paper and then removed (70% ethanol) by using rotary evaporation at 45oC; after that, the dry extracted seeds are stored in the refrigerator for further analyses. Finally, all the chemicals used are obtained from chemistry department- Faculty of Science (Koya University, Kurdistan, Iraq) (Kwon et al., 2019, Araújo et al., 2020, Owon et al., 2021).

2.3 Experimental animal

This study was performed on 15 experimental male rats; whose weights were between (84-110g), and were obtained from the Hawler Medical Univeristy /College of Pharmacy, Erbil, Iraq. They were housed in plastic cages in a controlled temperature environment (25 ± 3 °C) and automatically controlled by a 12hr light-dark cycle. The rats were divided into three groups; each group contained (5) rats. Each Rat was colored code and received water and their respective experimental diets ad libitum. The first group received standard rat's diet the two other groups received MOS and FS (1.5g and 0.5g) per day respectively for 85 days.

2.4 Blood sampling and urine collection

After eighty-five days, all rats were individually kept in metabolic cages, from each of them, urine was collected, centrifuged, and stored at -20^{o}C till analyzed (Yin et al., 2022). Rats were overnight fasted and then sacrificed under ether anesthesia by cervical dislocation. 4 ml of blood was drawn from heart of each rat, then centrifuged at 4000 rpm for 10 minutes to obtain serum for

biochemical analysis (Arabsorkhi and Sereshti, 2018, Hasan et al., 2020).

2.5 Biochemical parameters

A few microliters of serum were added into dry slide reagents for each biochemical parameters and concentration of TP (slide lot No. 451908), Alb (slide lot No. 153502), HDL (slide lot No. 117902), LDL (slide lot No. 240114), TG (slide lot No. 238310), ALP (slide lot No. 177410), GOT (slide lot No. 449714) and GPT (slide lot No. 405706) were analyzed by using automatic chemical analyzer (DRI-CHEM NX500i/ FujiFILM).

2.6 Antioxidant activity

DPPH antioxidant measurement assay was used to determine the antioxidant activity (Flieger and Flieger, 2020). A solution of 0.1g Butylated hydroxytoluene (BHT) and Moringa seed was extracted; each was mixed with 100 mL 70% ethanol to form the solvent. And 0.004g DPPH reagent was mixed with (100ml) Absolute methanol to make a DPPH solution. Finally, different concentration (15,30,60,90,105,135,150,165 and 180 µg/ml of each (BHT)and extracted solution, with 3ml DPPH reagent were all mixed and kept at dark (25^{o}C) incubated for 30 min. At 517 nm wavelength, sample absorbance was recorded versus the control sample. Percentages of inhibition of plant extract were calculated using **Eq.1** given below. The % inhibition values obtained, by plotting against concentration, the values (IC50) of the plant extract that inhibit DPPH radical by 50% were determined **Eq.2**. BHT was used as a positive control (Das et al., 2019).

Eq.1.

% Of Inhibition = [(A control– A sample) /A control)] ×100

Eq.2

IC50 = (concentration – intercept) / slope

2.7 XRF analysis technique

X-ray fluorescence has been an extraordinary spectrometer technique since 1950, this method widely utilized in elemental composition examination investigation (Mortari et al., 2021). In this study, Energy Dispersive X-ray Fluorescence spectrometer type of (Rigaku NEX CG, Serial No.:CG1 194) was used to detect the mineral composition of the SF, MOS, and standard normal rat's food.

2.8 Statistical analysis

All data are studied by one-way analysis of variance (one–way ANOVA) using GraphPad Prism 9 statistical package, version 9.3.1 software. The results are conveyed as means ±SD, and values are considered statistically significant at $P \leq 0.05$ Duncan test was used to compare between all groups (Gupta et al., 2019).

3. RESULTS

3.1 Body weight gain

As revealed in Table 1, administration of MOS, FS and control group did not show any significant body weight gain. In all groups However, compared to MOS, administration of FS caused a significant increase in body weight.

Table 1: Mean±SD of body weight gain in control and different treated groups.

Parameters	Animal groups		
	Control	MOS	FS
1nitial body weight (g)	126±15.524	126±16.077	126±13.095
Final body weight (g)	342.6±50.21	327.6±36.54	365.2 ±39.55 [b]

Body weight gain (g)	207.6±33.77	210.2±26.50	232.8±33.36
b: Significant when compared with MOS group.			

3.2 Serum and urine TP and Alb levels

The data presented in Table 2 showed serum and urine, TP, and Alb in the control and different treated rat groups. Administration of FS group significantly increased serum TP, urine TP and urine ALB with significant reduction in serum ALB when compared to MOS. In MOS group serum TP decreased significantly when compared with control group, but it didn't show any significant changes in the other reported parameters.

Table 2: Serum and urine, TP and Alb levels in control and different treated groups.

Parameters	Animal groups		
	Control	MOS	FS
Serum TP (g/dl)	6.780±0.2588	5.960 ±0.3286 [a]	7.080 ±0.6496 [b]
Urine TP (g/dl)	0.4100±0.03674	0.3880±0.04147	1.124 ±0.2555 [ab]
Serum Alb (g/dl)	2.986±0.1550	2.926±0.1016	2.606 ±0.08620 [ab]
Urine Alb (g/dl)	0.3860±0.0181	0.3740±0.03050	0.9700 ±0.1032 [ab]
a: Significant when compared with the control group. b: Significant when compared with the MOS group.			

3.3 Lipid Profile

As indicated in Table 3, Level of serum high density lipoprotein and cholesterol increased non-significantly in MOS and FS group when compared with control group, while triglyceride decreased significantly in FS group with nonsignificant increase in MOS group compared to control group.

Table 3: Lipid profile in control and different treated groups.

Parameters	Animal groups		
	Control	MOS	FS
HDL (mg/dL)	2.184±1.860	4.414±4.838	3.034±4.123
TC (mg/dL)	5.943±4.003	6.233±4.539	6.397±2.867

TG (mg/dL)	31.60±8.041	40.28±9.478	29.08 ±10.45 [b]
b: Significant when compared with MOS group.			

3.4 Liver function test

As it can be seen from the obtained data, Table 4, presents serum alkaline phosphatase (ALP), GOT and GPT activities. The administration of FS increased serum level of ALP, AST and ALP significantly compared with MOS and control groups. However, in MOS group the level of ALP decreased significantly when compared to the control group, with non-significant increase in AST and ALT when compared with control group.

Table 4: ALP, GOT and GGT activities in control and different treated groups.

Parameters	Animal groups		
	Control	**MOS**	**FS**
ALP(U/L)	143.8±11.39	89.00 ±13.84 [a]	174.2 ±15.53 [ab]
GOT(U/L)	165.0±44.99	171.8±20.39	407.0 ±12.69 [ab]
GPT(U/L)	45.00±5.385	45.80±7.050	253.6 ±16.30 [ab]
a: Significant when compared with the control group. b: Significant when compared with the MOS group.			

3.5 DPPH radical scavenging capacity

Inhibition percentages(%Inhibition) corresponding to different MOS extract concentrations and BHT are given in figures (1-3) respectively. In addition, the Inhibition concentration (IC50) values that inhibit DPPH radical by 50% with the highest inhibition percentages of MOS extract and positive control are given in Table 5, which is calculated from the inhibition plot; BHT is used as a positive control to compare with plant seed extracted.

Table 5: The highest inhibition (in percentages) and concentrations IC50 values that inhibit the DPPH radical by 50% of M.oleifera (%70)ethanol extract plant seeds and positive control.

Sample	% Inhibition	IC50 (µg/mL)
Moringa oleifera seeds	44.37±1.059	419.49
BHT	73.95±17.34	21.16

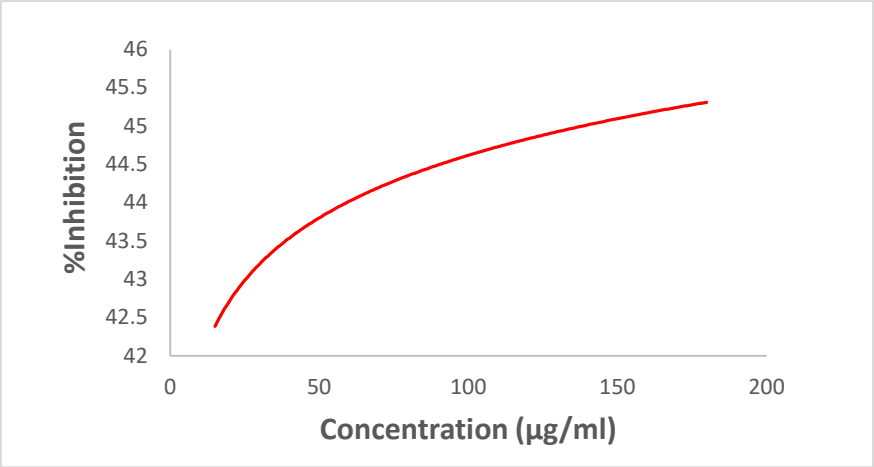

Figure 1: DPPH radical % inhibition observed at different concentrations of the (%70) ethanol extract of Moringa oleifera seeds.

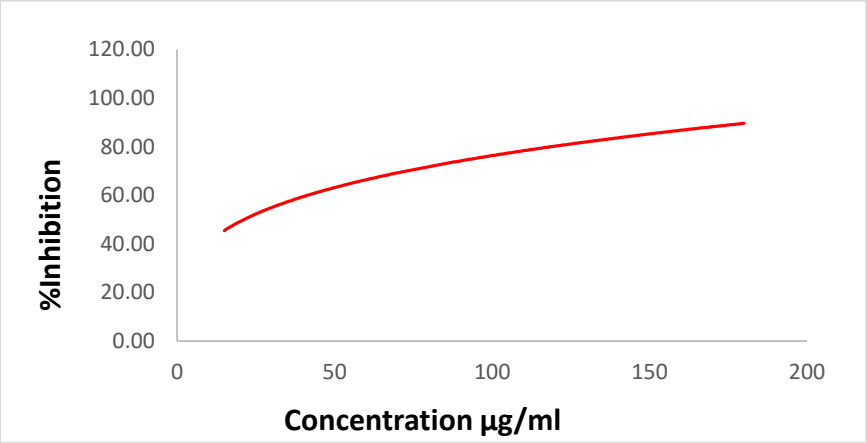

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Figure 2: DPPH radical % inhibition observed at different concentrations of BHT.

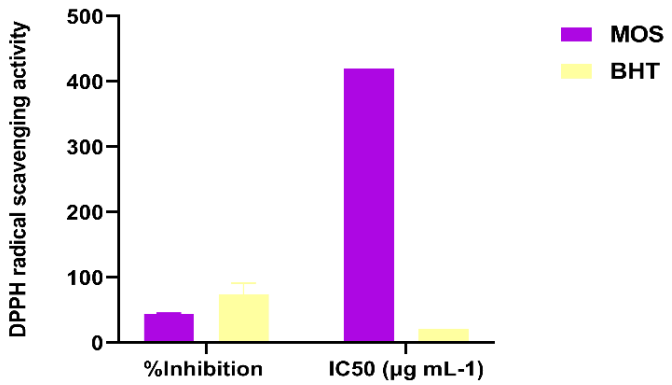

Figure 3: Graph showing the change in % inhibition and IC50 values of DPPH radical for M.oleifera seeds and BHT.

3.6 Elemental analysis

Small portion of the powdered sample was used for the elemental analysis using an energy dispersing X ray fluorescence (EDXRF) transmission emission technique, at the physical lab, Koya University, Iraq. As indicate table 8 Analyses of the MOSs, FS and normal food produced a total of (18,15 and 18) elements respectively, as shown in Table 6.

Table 6: Elemental list of MOS, FS and normal rats' food

No.	Name of elementals	MOS (ppm)	FS (ppm)	Normal food(ppm)
1	K	552000	5420	36800
2	Ca	154000	5910	25700
3	S	64000	15500	1050
4	Zn	61000	483000	176
5	Fe	50800	42000	632
6	Sn	23700	1130	76.6
7	P	19400	4890	436
8	Sr	18700	-	-
9	Rb	15300	-	26.1
10	Mn	14200	190000	179
11	Cu	10400	-	44.5
12	Br	5660	-	8.31
13	Mg	3580	-	931000
14	Ni	3040	-	32.8
15	Al	1430	-	-
16	Cl	1000	2270	2880
17	Ta	485	46300	-
18	Se	260	-	-
19	Hf	-	100000	-
20	Au	-	82100	-
21	Pt	-	17700	-
22	Si	-	901	-
23	Al	-	828	-
24	Sr	-	-	46.5
25	V	-	-	8.17
26	Ti	-	-	38.3
27	Cr	-	-	6.67

4. DISCUSSION

As presented in Table 1, administration of FS-fed rats group increased body weight compared to MOS and normal control group this result is agree with Andoyo et al 2021 (Penggalih and Solichah, 2019, Andoyo et al., 2021). Serum and Urine, albumin Alb, and TP are important biomarkers to assess, monitor, and determine treatment and prognosis for people who suffer from chronic kidney and liver disease (Miller et al., 2019). The data revealed in Table 2 showed serum and urine, total protein and albumin in FS rats group increased compared to MOS and Control group. According to this study, intake the FS-fed caused liver and kidney injury (Miller et al., 2019).

GPT and GOT are enzymes generally found in the liver and red blood cells, cardiac cells, muscle tissue, and many organs. (GPT, GOT) are also called (ALT, AST) (alanine aminotransferase, aspartate aminotransferase). GOT or GPT and ALP levels are mainly respected assistance in determining liver disease or liver damage released into the bloodstream. The amount of GPT and GOT in the blood is directly related to the level of tissue damage (Mohammed, 2021). As evidenced by the data obtained; in Table 4, the administration of feeding FS rats increased in the mentioned parameters compared with MOS and the control groups.

The tested extract's free radical scavenging capacities were measured by DPPH˙ assay, and the results are shown in Table 5. According to the results obtained, the activity in (%70) ethanol extract of Moringa Olifera defatted was found active with IC50 value 419.49 µg/ml. A lower IC50 value indicates higher antioxidant activity. IC50 values of the

synthetic antioxidant BHT were 21.16µg/ml. Usually, antioxidant properties of plant extracts is attributed to the existence of polyphenolic compounds, which have great potential as antioxidant agents (Paesa et al., 2022). It is known that phenolic and flavonoid contents are directly related to the properties of antioxidant (Hossain et al., 2022). MOSs are potential sources of phytochemicals that have been found to counteract free radicals due to their antioxidant activity (Rehman et al., 2018).

Content of mineral elements in M. oleifera seed as can be seen from Table 6, M. oleifera seed contains potassium (K), calcium (Cl), sulfide (S), and other elements, of which the highest potassium content is 552000 (ppm), and the lowest Selenium (Se) content is 260 (ppm). The main elements of M.oleifera seeds (MOS) have high concentrations compared to food supplement (FS) and normal food rats (NF). The previous studies similar the main mineral element but decreased the amount and numbers according to their device used and sample collection (Fred-Ahmadu et al., 2018, Liang et al., 2019, Susanto et al., 2019, Zaid and Ghazali, 2019). A high intake of fruits and vegetables greatly lowers the risk of stone formation because of their high potassium and magnesium content, which helps reduce urinary calcium excretion; the MOS has a high concentration of potassium and magnesium minerals (Elgharabway et al., 2018).

5. CONCLUSIONS

This study indicates FS administration increased body weight gain in male rats. In addition, the feeding of FS rats increases level of liver enzymes causing liver disease. So, Total protein and Albumin have side effects on the kidney and body weight of the rats compared with the MOS and control group. The extracted MOS showed the antioxidant activity in DPPH free radical scavenging assay in vitro; MOS can be used as an antioxidant source. A high concentration of potassium and magnesium in the MOS compared to the control and FS groups, which greatly reduces the risk of stone formation and helps in reducing urinary calcium excretion.

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