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EFFECT OF FEEDING WITH MORINGA SEEDS ON SOME BIOLOGICAL AND BIOCHEMICAL PARAMETERS IN EXPERIMENTAL RATS

Hashish, A .S.; Sayed, Raya A. and Muhamed Thoraya A.
Food Tech. Res. Institute, Agric. Res. Center, Giza, Egypt.

ABSTRACT

Moringa species are important multi-purpose tropical crops, as human foods and for medicine and oil production. The present investigation was carried out to determine functional properties of moringa seeds flour, and to study the effect of different technological treatments of *Moringa oleifera* seeds on some biological and biochemical parameters in rats. Rats were fed on basal diet containing 10% raw Moringa seeds (RMS), soaked Moringa seeds (SMS), blanched Moringa seeds (BMS), roasted Moringa seeds (TMS) or germinated Moringa seeds (GMS). After feeding period (4weeks), some nutritional parameter as well as glucose, protein, cholesterol, triglyceride in the serum and blood hemoglobin were determined. Results show that moringa seeds flour had promising functional prosperities which may be used to give benefits to many products. The data also reveal that rats fed on (GMS) and (BMS) recorded the most biological and biochemical parameters in rats. While, Feeding with a diet containing 10% (RMS) showed loss of appetite, impaired growth, small liver, pancreas, kidneys, heart and spleen compared to rats fed on the control diet. The results suggested that the consumption of *M. oleifera* raw mature seeds should be viewed with some caution until suitable processing methods are developed to abolish the yet unknown adverse factors.

Keywords: Moringa seeds, Functional properties, Nutritional parameters, lipids patterns, blood profiles.

INTRODUCTION

Moringa oleifera Lam (Moringaceae) is a highly valued plant, distributed in many the tropics and subtropics countries. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta□-carotene, amino acids and various phenolics (Farooq *et al.,* 2007). It is an important food commodity which has had enormous attention as the natural nutrition of the tropics. In Philippines, it is known as 'mother's best friend' because of its utilization to increase woman's milk production and is sometimes prescribed for anemia (Estrella *et al.,* 2000; Siddhuraju and Becker, 2003).

The seeds of Moringa are considered to be antipyretic, acrid, bitter *(Oliveira et al., 1999)*. The seeds can be consumed fresh as peas; or pounded, roasted, or pressed into sweet, non-desiccating oil, commercially known as 'Ben oil' of high quality. The unique property is the ability of its dryness, crushed seed and seed pressed cake, which contain polypeptides, to serve as natural coagulants for water treatment *(Ndabigengesere and Narasiah, 1998).*

The main target of the present investigation was to determine functional properties of Moringa seeds flour, and to study the effect of different technological treatments of *Moringa oleifera* seeds on some biological and biochemical parameters in rats.

MATERIALS AND METHODS

Materials

- Moringa seeds were obtained from North Sinai Desert Station for Research and Extension, Egypt.

- Male albino rats (110-120g B.Wt.) Sprague Dawley strain was obtained from the Laboratory of Animal Colony, Fac. of Home Economics, Minufiya University, Egypt.

Methods

Preparation of *Moringa oleifera* seeds products

Wrinkled and mould seeds and foreign materials were removed. The Moringa seeds products (soaked Moringa seeds (SMS), blanched Moringa seeds (BMS), roasted Moringa seeds (TMS), and germinated Moringa seeds (GMS) as well as raw Moringa seeds (RMS) were prepared as shown in Figure (1) . After preparation, Moringa products were ground and screened to pass through a 80-mesh sieve (Brith Standard Screen), then packed in air tight kilner jars and kept at 4° C until used.

Diets: The basal diet consists of casein (12%), corn oil (10%), choline chloride (0.2%), cellulose (5%) and vitamin mixture (1%) according to (Campbell, 1963). Salt mixture (4%) Hegested (1941). Corn starch (up to 100 %) as shown in Table (1).

Chemical analysis

Moisture, crude protein, crude fat, and ash contents were determined on dry weight basis according to A.O.A.C. (2000). While fibers were measured according to the method described by Pearson (1976). Total carbohydrates were determined by differences as 100-(protein+ total fat +fiber + ash).

Calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K) were determined according to the method described by Pearson (1976). While, Iron (Fe), Zinc (Zn), Manganese (Mn) and copper (Cu) were determined according to the method described by A.O.A.C. (2000).

Functional properties

Nitrogen solubility index was determined according to the method of Smith *et al.* (1959).

Water and oil absorption were determined according to the methods of Sosulski (1962) and Sosulski *et al.* (1976), respectively.

Emulsification capacity was measured by the method of Yasumatsu *et al.* (1972).

Foam capacity and stability were measured by the method of Huffman *et al.* (1975).

Biological investigations

Experimental design: Thirty six (36) Sprague-Dawley white male albino rats, weighting 110-120g for each, were used in this study. All rats were fed on the control (casein) diet for 7 consecutive days. Each, rat was housed in an

individual stainless steel cage under controlled condition. The first group (n= 6) was fed on the basal diet only as a control group(C). The second group (n=30) was divided into 5 subgroups as follows:

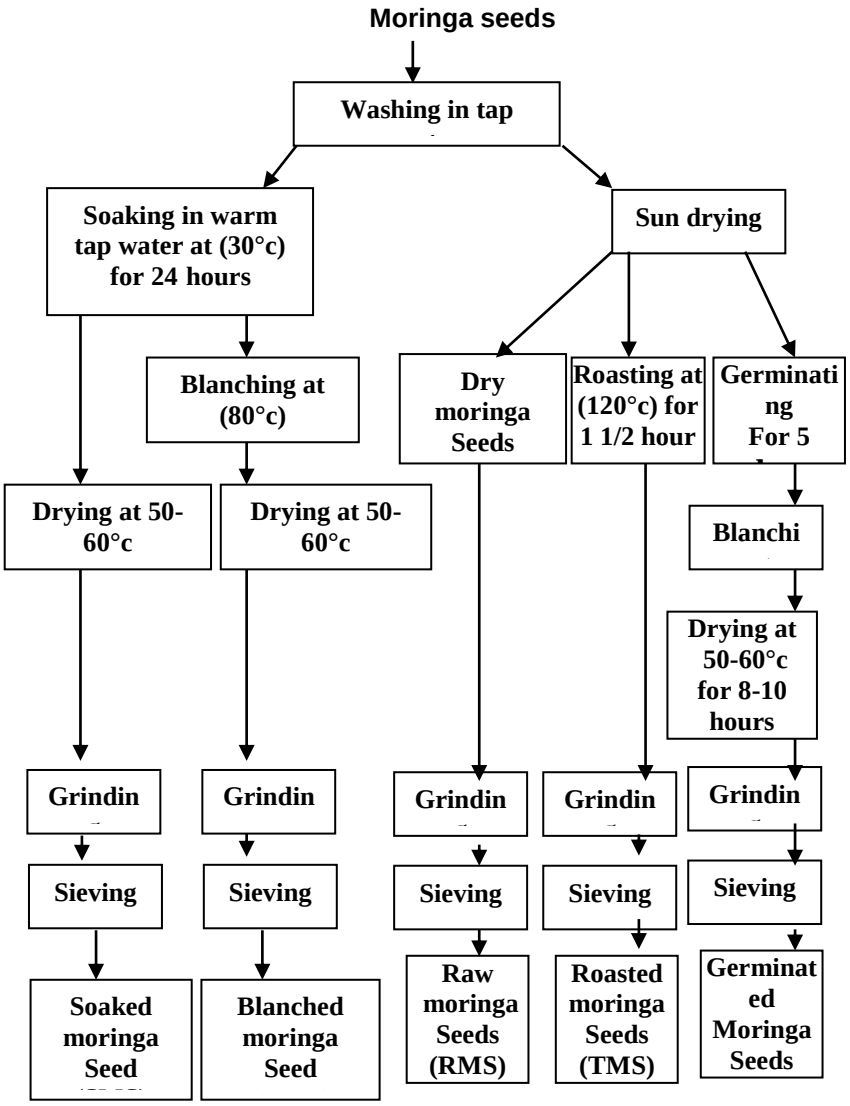

Fig. 1: Technological treatments of Moringa seeds

Table 1: Diet composition as gm/100 gm.

Groups	Casein	Corn oil	Salts mixture	Vitamins mixture	Cellulose	RMS	SMS	BMS	TMS	GMS	Corn starch
(C)	12	10	4	1	5	-	-	-	-	-	68
RMS	12	10	4	1	5	10	-	-	-	-	58
SMS	12	10	4	1	5	-	10	-	-	-	58
BMS	12	10	4	1	5	-	-	10	-	-	58
TMS	12	10	4	1	5	-	-	-	10	-	58
GMS	12	10	4	1	5	-	-	-	-	10	58

C: Control diet group. **RMS: Raw Moringa seeds**
SMS: Soaked Moringa seeds
BMS: Blanched Moringa seeds **TMS: Roasted Moringa seeds**
GMS: Germinated Moringa seeds

Group (1) was fed on basal diet containing 10% of (RMS).
Group (2) was fed on basal diet containing 10% (SMS).
Group (3) was fed on basal diet containing 10% of (BMS).
Group (4) was fed on basal diet containing 10% of (TMS).
Group (5) was fed on basal diet containing 10% of (GMS).

Biological evaluation: All rats were weighted once weekly. At the end of the experiment, biological evaluation of the different diets was carried out by determination of food intake (consumption) daily, body weight gain% (BWG %), food efficiency ratio (FER) as described by Chapman *et al.* (1959). To calculate body weight gain (BWG) and food efficiency ratio (FER) the following equations were used:

$$\text{BWG \%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \qquad \text{ER} = \frac{\text{BWG}}{\text{Food Intake (g)}}$$

Blood sampling: At the end of the experiment (4 weeks), rats were fasted for 12 hour and then slightly anesthetized with ether. Two blood samples were taken from hepatic portal vein in two centrifuge tubes. Ethylene diamine tetra acetic (EDTA) was added to the first sample as anti-coagulant to be used in hematological analysis. The second blood sample was left for 15 minutes at room temperature. Then, blood was centrifuged for 20 minutes at 3000 r.p.m. to separate the serum. Serum was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (-20ºC) until the time of analysis. The organs (liver, kidney, Heart, Pancreas and Spleen) were removed and washed in saline solution, weighted and kept in formalin solution (10%) according to methods described by Drury and Wallington (1980), dried then weighted and relative weight of organs was calculated.

Biochemical analysis

The principle use of glucose determination according to Trinder, (1969). Total protein was carried out according to Gornal *et al.* (1949) was accomplished. Enzymatic calorimetric determination of alkaline phosphatase was carried out according to Belfield and Goldberg (1971). Enzymatic calorimetric determination of Triglycerides was carried out according to Fassati and Prencipe (1982), while Total cholesterol determination was according to Allain (1974).

Hemotological measurements included blood counting of red blood cells (RBC's) and platelets (Dacie and Lewis, 1984). Hemoglobin was measured by using sorine hemoglobin kits according to Corsby *et al.* (1954). Hematocret was measured using the method described by (Dacie and Lewis, 1984).

Statistical analysis

Statistical analysis were performed using computer program, Statistical Package for Social Science (SPSS, 1998), and compared with each other using the suitable tests.

RESULTS AND DISCUSSION

Chemical Composition of Moringa seeds

The chemical composition of Moringa seeds are shown in Table (2). Seeds are rich in energy as it contains mean value (raw seeds) 50.07% carbohydrates and 18.23% ether extract. Moreover, high protein, fiber and ash were found in Moringa seeds which recorded (22.34%), (5.49%) and (3.87%), respectively. Similar results were reported by Maleeha *et al.* (2007). Data of minerals contents are presented in Table (3). It shows that (K) potassium is the predominant mineral in (RMS) and (SMS) which recorded (3390 and 2980 mg/100gm sample, respectively) and (P) phosphorus is the most abundant mineral which recorded (1400 and 1600mg/100gm) in (RMS) and (SMS), respectively. On the other hand, Na and Mn of Moringa seeds are present in low quantities. Most of these results are in agreement with Ismaeil *et al.* (2004).

Table 2: Chemical composition of raw Moringa seeds (RMS) and soaked Moringa seeds product (SMSP).

Nutrients Sample	Moisture content (%)	Protein* (%)	Ether extract* (%)	Fiber (%)	Ash* (%)	Total Carbohydrates** (%)
(RMS)	10.13	22.34	18.23	5.49	3.87	50.07
(SMS)	12.96	18.87	17.89	5.41	4.13	53.70

*** On dry weight basis.** ****By difference**

Table 3: Minerals contents of raw Moringa seeds (RMS) and soaked Moringa seeds (SMS).

Nutrients / Samples	Macro-minerals (mg/100g)					Micro-minerals (mg/100g)			
	Ca	P	Mg	Na	K	Fe	Zn	Cu	Mn
(RMS)	390	1400	320	52	339	69	38	40	21
(SMS)	340	1600	290	56	2980	74	33	46	19

*** On dry weight basis**

Functional properties of Moringa seeds

Functional properties of Moringa seeds are important to know how to use effectively them in food products. Water, oil absorption, emulsification capacity as well as foaming properties have some utilizations of the functional properties of (RMS) and (SMS) flour are presented in Table (4). The high values of the flour may be due to the presence of carbohydrates which absorb both water and oil. The emulsification capacity of the treated flour (SMS) (65.90ml oil/gm sample) was highly compared to the untreated sample (RMS) (54.80ml oil/gm sample). In general, Moringa seeds flour had promising functional prosperities which can be used to give in many food products, such as bakery products and meat products.

Table 4: Functional properties of raw Moringa seeds (RMS) and soaked Moringa seeds (SMS).

Groups / Functional properties	(RMS)	(SMS)
Protein solubility index using		
Distilled water	54.60	62.90
5% Nacl	57.50	66.60
0.02 NaoH	59.70	71.50
Water absorption (ml H_2O/100gm)	90.60	99.80
Oil absorption (ml oil/100gm)	150.90	130.70
Emulsification capacity(ml oil/gm)	54.80	65.90
Foam capability in H_2O (% volume increase)	52.00	58.00
Foam stability in water after 15 min	32	36
30 min	29	32
45 min	26	29
60 min	24	27

Biological evaluation

The average changes in body weight gain, food intake and food efficiency ratio of rats are summarized in Table (5). It could be observed that

(GMS) group was more effective for the growth of rats compared with (C) group. While, (RMS) group recorded the highest decrease in both of body weight gain and food intake followed by (TMS) group. This may be due to the loss of appetite of (RMS) and (TMS) groups. These reflect the food efficiency ratio which showed negative figures as a result of body weight loss and also the decrease in food intake. The obtained results are in the line with Oliveira *et al.* (1999) who reported that feeding rats with a diet containing the seed meal showed loss of appetite, impaired growth in comparison with rats fed on a control diet.

Table 5: Influence of Moringa seeds and its products on nutritional parameters of rats.

Parameters / Groups	Initial body weight (g/rat)	Final body weight (g/rat)	Body weight gain		Daily body weight gain (g/rat)	Daily food intake (g/rat)	Food efficiency ratio (FER)
			g/rat	%			
C	117.4	155.0	37.50	31.94±3.66	1.34	13.62	0.098±0.011
RMS	117.8	126.50	8.70	7.38±2.95***	0.31	12.21	0.025±0.009***
SMS	112	159.30	47.30	42.23±3.67	1.69	13.56	0.125±0.100
BMS	116	175.2	59.20	51.03±4.95*	2.11	14.56	0.145±0.099
TMS	118	139.30	21.39	18.13±2.81**	0.76	12.86	0.059±0.013*
GMS	117.6	196.0	78.40	66.67±5.79***	2.80	15.68	0.178±0.019**

*** Differences are significant at 5 % (P<0.05).**
**** Differences are significant at 1 % (P<0.01).**
***** Differences are significant at 0.1 % (P<0.001).**
C: Control diet group.
RMS: Raw Moringa seeds SMS: Soaked Moringa seeds
BMS: Blanched Moringa seeds TMS: Roasted Moringa seeds
GMS: Germinated Moringa seeds

Table (6) demonstrates that kidney, spleen and pancreas weights for rats which were fed on basal diet containing 10% (RMS) revealed a significant (P<0.001) decrease compared with the control being (1.38±0.16, 0.20±0.06 and 0.48±0.01, respectively). It could be shown that no significant changes in the mean values of heart, kidney, spleen, and pancreas (g) for other different groups of rats. These results are in the line with Oliveira *et al.* (1999).

Table 6: Influence of Moringa seeds and its products on organ weight of rats.

Organs / Groups	kidney	Liver	Heart	Spleen	Pancreas
C	1.38±0.16	4.66±0.25	0.40±0.08	0.20±0.06	0.48±0.01
RMS	0.70±0.18***	2.67±0.31**	0.27±0.09*	0.10±0.05***	0.25±0.04***
SMS	0.94±0.20*	4.34±0.15	0.58±0.10	0.30±0.05	0.46±0.07
BMS	1.24±0.18	5.84±0.19	0.76±0.06	0.46±0.09	0.78±0.06
TMS	1.02±0.17	4.70±0.20	0.50±0.09	0.30±0.09	0.50±0.09
GMS	1.30±0.16	6.04±0.11	0.80±0.08	0.65±0.07	0.92±0.08

*** Differences are significant at 5 % (P<0.05). ** Differences are significant at 1 % (P<0.01). *** Differences are significant at 0.1 % (P<0.001).**
C: Control diet group.
RMS: Raw Moringa seeds SMS: Soaked Moringa seeds
BMS: Blanched Moringa seeds TMS: Roasted Moringa seeds

GMS: Germinated Moringa seeds

Rats fed on 10% (RMS) and (TMS) showed a significant increase (P<0.01) of serum glucose while the other groups had slight changes as compared with the control group in Table (7). Also the same group of rats receiving 10% (RMS) had a decrease in protein (45.37gm/l) and an increment of triglycerides (0.84mm/l) when compared with the control group(55.66gm/l and 0.37mm/l protein and triglyceride, respectively). In the same table, it could be observed that phosphatase kinase and triglycerides for all rats groups had slight changes in the values except for rats fed on 10% (RMS) and (TMS) showed significant increased levels in comparison with the control group. No significant changes were shown for serum cholesterol levels except rats fed on 10% (GMS) observed significant decrease value (P<0.01) as compared with the control group. These results are in accordance with those obtained by Farooq *et al.* (2007).

Results in Table (8) show the effect of feeding rats with basal diet containing 10% Moringa seeds and its products for 28 days on red blood cell, hemoglobin, hematocrit and platelets. The data show that no significant differences could be detected among all groups in red blood cells and hematocrit as compared to control group. While significant differences (P<0.05) were found in hemoglobin and platelets for rats fed on basal diet containing 10 %(RMS).

Table 7: Influence of Moringa seeds and its production serum glucose, protein, phosphate kinase, cholesterol triglyceride.

Parameters Groups	Glucose (mm/l)	Protein (gm/l)	Phosphatase kinase (u/l)	Cholesterol (mm/l)	Triglycerides (mm/l)
C	6.74±2.32	55.66±5.91	198.7±10.51	2.34±0.25	0.37±0.05
RMS	10.44±3.85*	45.37±6.35	243.3±9.51*	2.89±0.22	0.84±0.07**
SMS	6.89±3.34	56.74±7.25	208.7±11.32	2.27±0.32	0.54±0.06
BMS	7.43±2.62	58.43±7.35	199.6±12.45	2.25±0.44	0.32±0.06
TMS	9.83±3.63*	54.37±6.21	234.7±11.36*	2.63±0.91	0.73±0.04**
GMS	6.69±2.31	64.32±4.21	190.2±12.44	1.45±0.25**	0.28±0.02

*** Differences are significant at 5 % (P<0.05). ** Differences are significant at 1 % (P<0.01).**
***** Differences are significant at 0.1 % (P<0.001).**
C: Control diet group.
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GMS: Germinated Moringa seeds

Table 8: Influence of Moringa seeds and its products on blood profile of rats.

Parameters / Groups	Red blood cells (mm/l)	Hemoglobin (gm/dl)	Hematocrit (%)	Platelets (mm/l)
C	9.08±2.34	15.88±3.13	45.63±4.63	1269±31
RMS	7.38±1.31	10.42±2.75*	40.38±5.83	1144±25*
SMS	9.13±3.33	16.75±2.59	48.38±5.63	1283±27
BMS	9.42±3.04	17.38±3.88	50.27±6.33	1305±29
TMS	8.41±2.55	14.53±3.49	42.53±7.91	1267±22
GMS	9.79±3.98	18.72±4.15	51.33±8.42	1335±27

*** Differences are significant at 5 % (P<0.05). ** Differences are significant at 1 % (P<0.01).**
***** Differences are significant at 0.1 % (P<0.001).**
C: Control diet group.
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تـأثير التغذيـة ببـذور المورينجـا علـى بعـض المقـاييس البيولوجيـة والبيوكيميائيـة بفئران التجارب

عبد المنعم سامي حشيش ، ثريا عبد الغني محمد و رية علي سيد
معهد بحوث تكنولوجية الأغذية - مركز البحوث الزراعية - الجيزة - مصر

نبـات المورينجـا مـن المحاصـيل الاسـتوائية الهامـة المتعـددة الأغـراض فـي تغذيـة الإنسـان والعلاج الطبي وإنتاج الزيت. تهدف هذه الدراسة لتقييم الخواص الوظيفية لبذور المورينجا ودراسة تأثير المعاملات التكنولوجية المختلفة لبذور المورينجا على بعض المقاييس البيولوجية والبيوكيميائية في فئران التجارب. استخدم ستة وثلاثون فأرا من الذكور البيضاء زنة (١١٠-١٢٠جم) قسمت لستة مجـاميع بعـد تغـذيتها لمـدة أسـبوع علـى غـذاء قياسـي،خلال فتـرة التجربـة تغـذت المجموعـة الأولـى (الضابطة) على الغذاء القياسي بدون إضـافات ، أمـا المجـاميع الخمسـة الأخـرى فتغذت علـى غـذاء قياسي يحتوى علـى ١٠%مـن بـذور المورينجا(الخـام، المنقوعـة، المسلوقة، المحمصـة، المنبتـة)علـى التوالي. فـي نهايـة التجربـة تـم حسـاب الغـذاء المتنـاول، وزن الجسـم ومعـدل كفـاءة الغـذاء، بالإضـافة لهيموجلوبين الدم ، الجلوكوز، البروتين، الكولسترول، والجليسريدات الثلاثيـة فـي السيرم. أوضـحت النتائج أن بذور المورينجا ذو خواص وظيفية عالية والتي يمكن الاستفادة بهـا فـي التصـنيع الغذائي. أظهـرت الدراسـة أن بـذور المورينجـا المنبتـة كانـت ذات التـأثير الأكثـر ايجابيـة لمعظـم المقـاييس البيولوجية والبيوكيميائية يليها المسلوقة. بينما سبَب تناول بذور المورينجا الخام فقدان الشهية وتأخر في النمو وانخفاض وزن الأعضاء الداخلية مع صغر الحجم. خلصت الدراسة أيضا إلى أن استهلاك بذور المورينجـا الخـام مرتبط ببعض المخـاطر والتحـذيرات، لـذا يفضـل تناولـه بعد استخدام وسـائل التصنيع المناسبة والتي تبطل العوامل الغير ملائمة.