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BIOLOGICAL EVALUATION OF *SILYBUM MARIANUM* SEED OIL FOR NUTRITIONAL PURPOSES

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Silybum marianum seed oil was fed to forty-day old fifteen Swiss albino mice of equal body weight for six weeks. An increase of 6 %, 25 % and 31 % of the initial body weight was recorded at 0 %, 5 % and 10 % levels of added oil to the normal diets respectively. Higher feed intakes were also noted at 5 % and 10 % levels of the oil in the diet.

At the end of the experimental period (6 weeks) the average blood cholesterol value of 5 % and 10 % oil fed mice was noted to be 113.1 mg and 102.3 mg per 100 ml respectively. No microscopic pathological lesions were observed on liver, kidney, stomach and intestines of the mice at any levels of the dietery oil. Histopathological examinations showed no degenerative changes.

Key words: Cholesterol. Oil. Histopathology. Rat.

INTRODUCTION

Recently the chemical composition of the seed oil from *Silybum marianum* was studied [1]. As a result it was found that with a high percentage of oleic (36.5 %) and linoleic acid (42.1 %) in this oil it compared well with that of maize germ oil, oleic acid (33 %) and linoleic acid (42 %). Since *S. marianum* yields oilseeds both when growing in the wild as well as on cultivation it was, therefore, considered desirable to examine if the oil (25.7 %) can be used for edible purposes.

Feeding trials for this reason on *S. marianum* seed oil have been carried out by adding it at 0 %, 5 % and 10 % levels in the diets of Swiss albino mice for six weeks. It has been observed that there was an increase of 6 %, 25 % and 31 % of the initial body weights at 0 %, 5 % and 10 % levels of the oil in the normal diets respectively. Higher feed intakes were also noted at 5 % and 10 % levels of the added oil and it was inferred that the oil had an acceptable taste.

At the end of the sixth week the experiment was terminated and various internal organs of the mice were subjected to histopathological examination. No degenerative changes in any of the organs were observed. Similarly no microscopic pathological lesions were recorded on the liver, kidney, stomach and intestines of the mice at any levels of the added oil.

Physical appearance and general behaviour of all mice used in the experiment and fed on added oil in the diets were also observed showing no untoward symptoms. In the early days of the feeding trials, however, the mice showed roughened, erect and oily body hair both at 5 % and 10 % oil levels in the diets. In the duration of the experiment this state was variable and otherwise had no effect on the general body weight gain.

As a result of this study, therefore, it is proposed that the oil from *S. marianum* seeds be considered for edible purposes as it had no toxic effects on Swiss albino mice when fed at 5 % and 10 % levels admixed with the diets.

MATERIALS AND METHODS

Preparation of the oil. Seeds of wild growing *S. marianum* plants were collected from the Rawalpindi/Islamabad areas in the months of May and June. Cold expression of the oil was effected by *Kohlu* from clean and dry seeds (20 kg) yiedling 3.7 kg of a clear light yellow coloured oil. The oil so obtained was decanted into amber coloured bottles and kept at room temperature (26-28°). The physical characteristics of the oil, as determined in the laboratory [2], are given in Table 4.

Feeding of oil mixed diets to mice. Fifteen Swiss albino mice, of about 40-day old and approximately of

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equal body weight, were selected for the experiment. These mice were randomly divided into three groups. Mice of group I were kept on normal feed while those of group II and III were given feed with 5% and 10% added oil respectively. The feed and water were given *ad libitum* to the mice throughout the experiment.

Recording of observations. (i) All the mice were examined daily for any untoward symptoms. (ii) Collective body weight of the five mice in each group was taken initially as well as at the end of each week. (iii) Faecal samples were collected weekly throughout the experiment. (iv) Individual body weights, of all mice in three groups were taken at the end of the experiment, *i.e.* six weeks. (v) The mice were anaesthetised with chloroform after six weeks and their blood samples were collected either from the auxiliary vein or the heart directly. Blood plasma was separated from the mixture of blood and the anti-coagulant (1:1) by centrifugation at 3000 rpm for 20 min. (vi) Post mortem examinations of all mice were carried out and microscopic lesions on various internal organs were observed. (vii) Livers of all the mice were also removed and weighed separately. (viii) Kidneys were also removed and kept in 10 % formaldehyde solution along with liver specimens for histopathological processing.

Processing of the recovered materials. The feed, faecal material and blood plasma samples were examined as detailed hereunder:

Feed. The percentage of oil in the original feed as well as after admixture with *S. marianum* oil was determined by the standard Soxhlet extraction technique using hexane (b.p. 65-70°) as the solvent [2].

Faecal material. Estimation of oil in the faecal material was also conducted by the Soxhlet extraction procedure using hexane as the solvent (b.p 65-70°). The faecal material was collected weekly for the duration of the experiment.

Blood plasma. Cholesterol levels in the blood plasma, as obtained in (v) above, were determined spectrophotometerically. The colours produced by the Lieberman Burchard reagent [3] were measured at 560-580 nm. Separate readings were observed for the standard and test samples and cholesterol concentrations were directly worked out by the equation:

$$\text{Cholesterol concentration} = A_s \frac{300}{A_{st}} \text{ mg/dl}$$

Where A_s = absorbence of the sample
A_{st} = absorbence of the standard

Liver and kidney specimens. Both liver and kidneys from each mice at the end of the experiment were removed and preserved as described in (vi) and (vii) above. These specimens were processed by the paraffin method of embedding for histological examination. Histological sections of the paraffin embedded specimens were made at 4-5 μ and stained with haemotoxylin and eosin method of staining and then examined microscopically as usual.

RESULTS AND DISCUSSION

Data regarding feed intake and increase in body weight of the three groups of mice fed on *S. marianum* seed oil mixed diet are given in Table 1. At the end of the first week a rapid increase in the body weight of mice was recorded in groups II and III which were fed on feed mixed with 5 % and 10 % oil respectively. Increase in body weight first gradually slowed and then stopped after the 4th and 5th weeks in these two groups respectively. A slight increase in body weight was however, again observed in group III during the 6th week.

An overall increase of 25 % for group II and 31 % for group III was recorded whereas only 6 % increase in body weight was recorded in the control group (I). Moreover, a gradual increase in body weight was found in group I during the first three weeks but it decreased during the last 3 weeks.

The rapid and continuous increase in body weight of groups II and III may be due to extra supply of energy to the mice which resulted into depositions of body fat.

Feed intake. Feed intake was much higher in groups II and III of mice fed on 5 % and 10 % added oil as compared with control group I (Table 1). However, feed intake began to decrease after the 3rd week in groups I and II and after the 4th week in group III. Higher feed intake in groups II and III suggested that the taste value of the oil at 5 % and 10 % levlels were quite good. Decrease in feed intake in all the three groups may be attributed to sufficient storage of energy during the first few weeks.

Roughened, erect and oily hairs. Mice of groups II and III on 5 % and 10 % added oil respectively showed roughened, erect and oily body hairs during the first three days. During the 4th and 7th days of the experiment mice of group III showed the same hair posture when the skin was also visible under the hairs, and an oily smell was coming out from their bodies. Although this state varied during the course of the experiment, yet it had no affect on the general weight gain. Mice of group I (control) showed normal hair posture throughout experiment.

Liver (Percent) body weight. Liver percent body weight and liver weight of individual mouse have been shown in Table 2. Average liver weights were similar in three groups (2.5, 2.6 and 2.54 g. in the case of groups I, II and III respectively). However, average percent liver body weight was higher in control group (8.16 %) as compared with groups II and III (6.56 % and 6.82 %) respectively. A similar average liver weight in these groups indicated that there was no fat deposit in the liver of mice and that they equally utilized the added oil in feed.

Fat deposits. A moderate to excessive deposition of fat in group II and only moderate in group III was found around the kidneys and on the peritonium. However, no fat deposit was seen in the control group I.

Table 1. Feed intake and increase in body weight

Group	Number of mice	% Oil added	Observations	Body weight (g.) weeks							Increase in body weight (g.)	
				0	1	2	3	4	5	6	Overall	% Increase
I	5	0 %	Body weight	144	149	160	162	156	157	153	–	–
			Increase/*** Decrease in body weight	–	+ 5	+ 11	+ 2	– 6	+ 1	– 4	+ 9	6 %
			Feed intake	–	120	136	144	108	90	98	–	–
II	5	5 %	Body weight	158	169	176	181	188	202	198	–	–
			Increase/Decrease in body weight	–	+ 11	+ 7	+ 5	+ 7	+ 14	– 4	+ 40	25 %
			Feed intake	–	160	192	201	153	130	120	–	–
III	5	10 %	Body weight	142	155	166	176	183	182	186	–	–
			Increase/Decrease in body weight	–	+ 13	+ 11	+ 10	+ 5	– 1	+ 4	+ 44	31 %
			Feed intake	–	168	196	239	201	108	128	–	–

*** + = Increase in body weight. – = Decrease in body weight.

Table 2. Liver percent body weight and microscopic lesions on internal organs

Group	% Oil added	Mice No.	Body weight (g.)	Liver		Microscopic lesions				Fat deposits	
				Weight (g.)	% Body wt.	Liver	Kidney	Stomach	Intestine	Peritonium	Kidney
I	O %	1	31.8	2.5	7.87	N	D	SC**	N	–	–
		2	35.0	3.0	8.53	N	D	N	N	–	–
		3	27.0	2.5	9.25	N	D	N	N	–	–
		4	26.2	2.0	7.63	N	D	N	N	–	–
		5	33.0	2.5	7.57	N	D	N	N	–	–
	Average:		30.6	2.5	8.16	–	–	–	–	–	–
		1	48.5	3.0	6.18	N	D	N	N	+++	+++
		2	34.6	2.0	5.78	N	D	N	N	++	++
		3	36.0	2.0	5.44	N	D	N	N	+++	+++
		4	43.7	3.0	6.86	SC	D	N	N	+++	+++
		5	34.5	3.0	8.69	SD	D	N	N	+	++
	Average:		39.6	2.6	6.56	N	–	–	–	–	–
		1	37.5	3.0	8.00	N	SD	N	N	++	++
		2	39.0	2.7	6.90	N	SD	N	N	++	++
		3	35.5	2.5	7.04	N	SD	N	N	++	++
		4	37.5	2.0	5.33	N	SD	N	N	++	++
		5	36.5	2.5	6.84	N	SD	N	N	++	++
	Average:		37.2	2.54	6.82	–	–	–	–	–	–

** = Pyloric end, N = Normal in colour, C = Congested, D = Dark in colour, S = Slightly
+++ = Excessive, ++ = Moderate, + = A little, – = Nil

Table 3. Microscopic changes in liver and kidney

Group number	Number of mice	% Oil added	Lesions in liver (%)							Lesions in kidney (%)				
			Abnormal nuclei	Cloudy swelling	Granulation	Fatty infiltration	Proliferation	Cellular reaction	Glomerular changes	Cloudy swelling	Granulation	Fatty infiltration	Proliferation	Celular reaction
I	5	0	–	–	–	–	–	–	–	40	40	–	–	–
II	5	5	100	100	100	–	–	–	–	60	60	–	–	–
III	5	10	100	100	100	–	–	–	–	40	40	–	–	–

The pattern of fat deposition in groups II and III showed that whatever energy was in excess to the requirement of the mice become deposited in the form of peritonial or kidney fat. A possible reason for moderate to excessive fat deposits in group II may be due to maximum utilization of added oil in this group, whereas some wastage of undigested oil may be there in group III (10 % added oil).

Microscopic lesions on internal organs. Normal brownish liver colour was seen in all the mice of three groups. However a slight congestion or darkness was found in two mice of group II, probably due to the retention of blood during bleeding.

Kidneys of mice of groups I and II were of normal dark colour whereas those from group III were only slightly dark in colour. This variation in colour may be due to complete bleeding in this group. No lesions of degenerative charges were however, seen in both organs.

The stomachs and intestines of all mice included in the experiment also showed no microscopic pathalogical lesions. However, a slight non-specific congestion over the pyloric end of the stomach was seen in one mouse of the control group.

Histological examination of liver and kidney. Histological sections of liver and kidney specimens were examined under light microscope. Mice of control group I did not show any pathological change in the liver sections. The liver parenchymatous cells were arranged well in columns around the central veins. The cells were flattened having normal centrally placed nuclei. There was no indication of degenerative changes at any stage. Liver sinuses were clear and had no deposits in them. Some of the portal veins showed aggregation of red blood corpuscles. No cellular reaction was present in any liver section (Table 3).

Kidney sections of 60 % mice of control group I revealed normal histological picture. Glomeruli and renal tubules did not show any deposits or proliferation. Cells lining the tubules were normal in shape and showed no indication of degeneration. However, some of the blood vessels were found to be engorged with red blood corpuscles. Kidney sections of 40 % control mice showed early lesions of a non-specific cloudy swelling (Table 3).

Histological examination of liver tissue specimens of mice fed on 5 % and 10 % added oil (groups II and III) showed a similar histological picture (Table 4). The liver parenchymatous cells were in columns, but somewhat dearranged. These cells had no clear boundaries and looked as swollen (cloudy swelling). Uneven staining of cytoplasm and its granular appearance was seen in these cells. The intensity of granulation was more in group III as compared with group II. The nuclei were not much sharp in their outline though centrally placed and showed haziness. Liver sinuses were, however, clear with no deposits. Some of the portal veins showed aggregations of r.b.c. There was no indication of fat degeneration/infiltration or cellular reactions in all the liver specimens examined in both groups (II and III).

Table 4. Physico-chemical characteristics of *Silybum marianum* seed oil.

Yield	=	25.7%
Refractive Index	=	1.4429
Specific gravity	=	0.8900
Acid value	=	2.0
Iodine value	=	108
Saponification equivalent	=	225

Lesions like cloudy swelling and granular appearance of renal tubules were found in 60 % and 40 % of kidney specimens in group II and III, respectively (Table 3). However, the rest of the kidney specimens did not show any pathological changes in both groups.

The presence of cloudy swelling like lesions even in the

control group may be attributed to anaesthetising the mice with chloroform.

CONCLUSION

The results of the present studies, therefore, indicate that the feeding of *S. marianum* seed oil both at 5 % and 10 % levels as the constituent of the diet had no toxic effects on Swiss albino mice. On the contrary the mice fed on 5 % and 10 % oil levels in the diets showed a 4 and 5 times more weight gain respectively when compared to the control.

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