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ORIGINAL ARTICLES

Effect of Moringa Oleifera on serum lipids and kidney function of hyperlipidemic rats

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ABSTRACT

The present work aims to study the potential effect of fortified bread with Moringa Oleiferato (*MO*) leaves powder under 5%, 10% & 15% concentrations to give more protection against hyperlipidemia .Thirty-two male albino rats were used in this experiment. These rats were put on ideal diet for two weeks before the performance of experiment. At the beginning, these rats were divided into four main groups and fed on diets for 45 days as follows: The first group fed on basal diet as a negative control. The second group fed on high fat diet (2% cholesterol) to induce hyperlipidemia as a positive control. The third and fourth groups fed on high fat diet containing fortified bread with 10% & 15% (*MO*) powder. At the end of the experimental period, blood samples were collected from the aorta to determine serum lipids and determine the kidney functions include serum uric acid, urea nitrogen and creatinine. Also, the kidney and heart were removed surgically for histopathological observation. From the obtained results we concluded that group of rats fed on high fat diet were considered as a major risk factor for hyperlipidemia disease. Our results could be summarized that bread fortified with *MO* at 10% & 15% were considered the best for causing a reduction of TC, TG, LDL and VLDL. Also, kidney function has been improved and there were significant reduction in uric acid serum, urea, and creatinine than that of positive control group. In fact, feeding bread fortified with *MO* improved the body weight gain and food intake. Histopathological observation proved that the last group diet is considered as a negative control group. We can conclude that fortified diet with 15% *MO* level powder realized the best effects on hyperlipidemia rats.

Key words: Moringa Oleifera - hyperlipidemia - serum lipids - Kidney function - histopathological.

Introduction

Hyperlipidemia is a powerful and extremely one of the major causes of the development of cardiovascular disorders (Morris & Ferdinand 2009 and Jain, *et al*., 2010). Over the last few years the changes in the type of diets have led to an increased frequency of lifestyle related disorders such as hyperlipidemia, diabetes mellitus and atherosclerosis, (Santoshkumar *et al*., 2013).

High fat diet is the term used to denote raised serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol, triglycerides, or both total cholesterol and triglyceride (combined hyperlipidemia) Luqman *et al*., (2012) and Sivaiah & Reddy (2012).

Moringa oleifera commonly known as "Miracle Tree" or "Mother's Best Friend" is the best known and most widely distributed species of Moringaceae family, having an impressive range of medicinal uses with high nutritional value throughout the world. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians. All parts of the Moringa tree are edible and have long been consumed by humans, and their anti-oxidant concentrations warrant the plant's image as a 'healthy' food source (Fahey *et al*., 2004 and Farooq *et al*., 2012).

Moringa tree has become an outstanding indigenous source of highly digestible protein, calcium (Ca), iron (Fe), amino acids and antioxidants, these nutritional characteristics of the plant may be, potentially beneficial to the developing regions of the world where undernourishment is a major concern showed by Mori *et al*., (2009); Ashfaq *et al*., (2011) and Tesfay *et al*., (2011). Leaves of Moringa can be eaten fresh in salads, cooked, or stored as dried powder for many months without refrigeration, and without loss of nutritional value (Bamishaiye *et al*., 2011).

Nutritional value of bread mainly depends of type of flour used in bread-making and application of other technological additives. Wheat flour, used in the production of bread, is usually characterized by a high carbohydrates and proteins, but this protein has a very low nutritional value, because it contains lower proportion of essential amino acids (Dewettinck *et al*., 2008). Bread is an Egyptian product that represents the main diet component for rich and poor Egyptian consumers. In Egypt, there is a big gap between wheat production and its consumption, where the total production of wheat grains covers only about 55% of the total

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needs, the way to overcome this problem is to search for the native sources which could be used with wheat flour bread making (Eissa *et al*., 2007 and Litwinek *et al*., 2013).

The aim of the present investigation was to improve the nutritional, healthy values and also, to evaluate the influence of bread fortified with MO at various ratios on the growth, food intake, serum lipids, and kidney function of hyperlipidemic rats on biochemical, biological and histopathological changes that may occur to rats fed on high fat diet.

Material and Methods

Materials:

Moringa Oleifera powder and wheat flour (72% extraction) were obtained from Agriculture Research center Giza, Egypt. Casein, cholesterol, cellulose, all vitamins and minerals were obtained from El-Gomhoria Pharmaceutical Company, Cairo, Egypt. Corn oil and starch were obtained from the local market. Thirty two male albino rats (Sprague Dawley strain) weighting an average (130 ± 10g) were obtained from Helwan breeding farm. Cairo - Egypt. Kits used to determine serum cholesterol, triglycerides, HDL, VLDL, LDL, uric acid, urea nitrogen and creatinine produced by Egyptian American Company for laboratory service and supplied by Alkan Company.

Methods:

Chemical analysis of raw materials and bread:

Moisture, fiber, ash, protein and fat were determined according to the method outlined in AACC. (1994). Total carbohydrates were determined by difference as mentioned by Abd El-Latif (1990). Mineral contents including (Na, K, Ca, Fe, Zn, Cu, Mg & P) were determined according to the method described by Chapman & Pratt (1978). After complete digestion the minerals were determined using Unicam atomic absorption Spectrophotometer. Vitamins including (B_1, B_2, C, E, A and β -carotene) were assayed as recommended by J. Chrom. (2001 & 2005). Types and concentrations of polyphenolic compounds and flavonoids were estimated as recommended by J. Sci. Food Agric. (1999) & J. og. Agric. & Food Chem. (2000).

Diet composition and animal groups:

Diet composition:

Basal diet (Negative): prepared according to Reeves *et al*., (1993). The vitamin and mineral mixture had the prepared according to Campbell, (1963).

Experimental design:

Rats were adapted for two weeks prior to commencement of the experiment. Water was introduced ad-libitum. Rats were divided into four main groups and fed on diets for forty five days as follows:
Group 1: Negative control group (8 rats) fed on basal diet. Group2: Positive control group (8 rats) contained the same composition, in addition 2% cholesterol to induce (hyperlipidemia) according to Hassarajani *et al*., (2007). Group 3 and group 4: (16 rats) obtained the same composition as positive diet, in addition fortified bread with 10% & 15% MO leaves powder. During the experiment period, the quantities of diet, which were consumed and / or wasted, were recorded every day. In addition, rat's weight was recorded weekly, to determine Food Intake and Body Weight Gain % according to Chapman *et al*., (1959).

Blood Sampling:

At the end of the experiment period, the rats were fasted overnight then the rats were anaesthetized and sacrificed and blood samples were collected from the aorta. The blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till analysis at -20°C.

Biochemical analysis of serum:

Uric acid was determined in the serum according to the method described by Fossati *et al*., (1980). Urea nitrogen was determined according to Patton & Crouch, (1977). Creatinine was determined according to Bartels *et al*., (1972).

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Histopathological Studies:

Kidney and heart were prepared and the tissue stained by Hematoxylin and Eosin according to Bancroft *et al*., (1996).

Types of balady bread were classified into:

1-**Control bread:** was made from 100% wheat flour without any fortification.
2-**Different Formulas**: Were made from a mixture of wheat flour and *MO* powder at various concentrations (5%, 10% & 15%) were prepared at Agriculture Research Center according to the common method described by Khorshid *et al*., (1989). Then according to the panel test was detected the best two concentration.

Statistical analysis:

Results are expressed as the mean standard deviation ± SD. Data were statistically analyzed for variance "ANOVA" test at $P \leq 0.05$) according to Vandallen (1997), using SPSS statistical software, version 13.0 was used for these calculations.

Results and Discussion

Chemical constituents of raw materials: Wheat flour and *MO* Leaves were investigated on dry weight basis. The following parameters in Tables (1) pointed out that the highest moisture and carbohydrate contents were that of the wheat flour and the lowest content was that of MO Leaves (11.35, 75.31% and 07.52, 37.44%), respectively. Moreover, *MO* Leaves had the highest content of protein, ash, fiber and fat, the present results agreement with those Aberra (2011) and Dubey *et al*. (2013).

Table 1: Chemical composition of wheat flour and Moringa Oleifera as raw materials (g / 100g dry weight basis).

(Macronutrients)	Wheat flour	Moringa Oleifera
Moisture	11.35	07.52
Protein	10.05	27.81
Ash	00.57	07.83
Carbohydrate	75.31	37.44
Crude Fibre	01.45	17.10
Fat	01.27	02.30

Table 2: Minerals and vitamins composition of wheat flour (WF) and Moringa Oleifera as raw materials (mg/100g dry weight basis).

(Micronutrients) Minerals	Wheat flour	Moringa Oleifera
Sodium (Na)	02.25	01.16
Potassium (K)	110.0	1320.00
Calcium (Ca)	20.42	945.25
Iron (Fe)	01.81	24.70
Zinc (Zn)	01.20	01.03
Copper (Cu)	00.12	00.19
Magnesium (Mg)	35.05	88.32
Phosphorus (P)	102.00	218.04

Vitamins		
Vitamin (B_1)	00.09	00.65
Vitamin (B_2)	00.06	04.94
Vitamin (C)	Not detected	409.73
Vitamin E (ppm)	00.6	36.61
Vitamin A (μ 100g)	Not detected	221.074
β -carotene (mg/100g)	Not detected	106.603

The following parameters for wheat flour and *MO* were determined for the content of minerals including, (Na, K, Ca, Fe, Zn, Cu, Mg and P). Results in Table (2) indicated that *MO* leaves had the highest contents of these vitamin and minerals except Na and Zn compared with wheat flour which had the lowest content of Potassium, Calcium, Iron, Copper, Magnesium and Phosphorus. However, these data are more or less in accordance with those reported by Barminas *et al*., (1998) they showed that 454.00 mg/100g calcium and 450.60 mg/100g magnesium in Moringa leaf powder. Also, Reddy *et al*., (2012) and Sengev *et al*., (2012) those reported that the high levels of iron (Fe), copper (Cu), magnesium (Mg) and calcium (Ca) contents in *MO* leaves of 8.30, 3.10, 244.00 and 442.20 mg/100g respectively. In fact, minerals content of Moringa shows variation in composition may be related to changes in location, or genotype and / or in the extracted method.

The following parameters for wheat flour and *MO* Leaves powdered were determined for the contents of vitamins including (B_1, B_2, C, E, A and β-carotene). Results indicated that *MO* had the highest contents of these vitamins compared with wheat flour which had the lowest content of these vitamins. These results in agreement with the previous authors Sánchez-Machado *et al*., (2006); Lako *et al*., (2007) and Coppin (2008) they published that Moringa leaves rich in carotenoids, tocopherols and vitamin C which have health-promoting potential in maintaining a balanced diet and preventing free-radical damage that can initiate many illnesses.

The results given in Tables (3) indicated that *MO* leaves contained considerable amount of polyphenolic compounds with an average from 4119.91 to 018.04 (ppm). It is evident from the data that Ellagic acid; Salicylic and Catechol were the predominant polyphenolic present in Moringa leaves powder, comparing with other polyphenolic compounds present in moderates concentrations such as Caffeic acid; Chlorogenic acid; Protocatechuic and Caffiene. While data in the same table revealed that Vanillic; Gallic acid and Cinnamic (ppm) were present in the lowest abundant levels. Chlorogenic acid also, is a major phenolic acid in M. oleifera leaves showed by (Bennett *et al*., 2003 and Amaglo *et al*., 2010) and it's an anti-dyslipidemic properties are more evident as its dietary supplementation has been shown to significantly reduce plasma TC and TG in obese rats (Cho *et al*., 2010 and Mbikay 2012).

From results in Table (4) it can be showed that the types and concentrations of flavonoid compounds in *MO* leaves powder. It indicated that Quercitrin and Querctin were the abundant flavonoid compounds, which were at concentration of 4135.00 and 1804.40 (ppm), respectively. While, Rutin 276.78 (ppm) was the moderate abundant flavonoid compounds in Moringa leaves powder and the lowest abundant were Rosmarinic acid 33.51 and Kampferol 28.514 (ppm) respectively. These findings are in agreement previously with (Bennett *et al*., 2003) they revealed that, in addition, to the provitamins, Moringa leaves are also considered a rich source of polyphenols, flavonoids (Siddhuraju & Becker 2003 and Lako *et al*., 2007) these essential nutrients can help decrease the nutritional deficit and combat many chronic diseases.

According to Lako *et al*., (2007) published that the flavonol quercetin is found at concentrations as high as 100 mg/100 g of dried *MO* leaves, also known as isoquercitrin or isotrifolin (Atawodi *et al*., 2010). Quercetin is a potent antioxidant with multiple therapeutic properties (Bischoff 2008 and Zhang *et al*., 2011). It has shown anti-dyslipidemic, hypotensive, and anti-diabetic effects in the obese rat model of metabolic syndrome (Mbikay 2012).

Chemical composition of the balady bread prepared from wheat flour with various levels of powdered Moringa Oleifera Leaves:

Nutrients of balady bread made from wheat flour (control) compared with the other types of bread after fortification at 5%, 10% and 15% *MO* leaves are shown in Table (5). The results of bread samples are shown that the moisture content decreased from 31.80 in 100% wheat flour (WF) to 26.61%, 25.49% and 25.08% in 95% WF:5% *MO*; 90% WF:10% *MO* and 85% WF:15% *MO* composite bread. This could be due to the low moisture content of *MO* leaf powder used in the blends and might have implications in terms of the texture quality of bread processed with added Moringa leaf powder this data reported similar values with Olaoye *et al*., (2006) and Sengev *et al*., (2012). Our results indicated that the addition of *MO* at 10% and 15% improved the macro and micronutrients profile of the balady bread.

In this concept, balady bread made using 15% *MO* had 13.14% protein, 02.90% fat, 02.39% ash, 02.02% fiber and 54.47% carbohydrates, compared with the same kind of bread (control sample), the values recorded were 10.87%, 01.65%, 01.68%, 00.98 % and 53.02% respectively. In fact, the control sample of balady bread had the lowest percentage of nutrients than that of bread made using 5%, 10% and 15% Moringa Oleifera.

Furthermore, balady bread supplementation in our study with *MO* at different levels resulted more vitamins and minerals content as compared with control sample. It could be noticed that from Tables (6) that addition of *MO* powder improved the contents of B_1, B_2, β –carotene, K, Ca, Fe, Zn and Cu than the corresponding control sample. These results are in harmony, with those obtained by Sengev *et al*., (2012) they found that the mineral and βeta-carotene contents of bread supplemented with Moringa leaf powder increased as the levels of supplementation increased caused by the high levels of iron (Fe), copper (Cu), magnesium (Mg) and calcium (Ca) contents in Moringa leaf of 8.30, 3.10, 244.00 and 442.20 mg/100 g respectively.

Table 3: Types and concentrations of polyphenolic compounds (ppm) of Moringa Oleifera Leaves.

Phenolic compounds (ppm)	Moringa Oleifera Leaves
Gallic acid	098.18
Protocatechuic	354.72
Catechol	1120.47
Chlorogenic acid	747.81
Caffeic acid	906.99
Vanillic	183.37
Caffiene	316.02
Salicylic	1292.81
Ellagic acid	4119.91
Cinnamic	018.04

Table 4: Types and concentrations of flavonoids (ppm) of Moringa Oleifera Leaves.

Flavonoids (ppm)	Moringa Oleifera Leaves
Rosmarinic acid	33.51
Rutin	276.78
Quercitrin	4135.00
Querctin	1804.40
Kampferol	28.514

Table 5: Chemical composition of the balady bread prepared from wheat flour (72% extraction) without and with various levels of Moringa leaves powder (g / 100g dry weight basis).

Chemical composition	Control wheat flour	*M.O. 5%*	*M.O. 10%*	*M.O. 15%*
Moisture	31.80	26.61	25.49	25.08
Protein	10.87	12.02	12.95	13.14
Fat	01.65	01.86	02.36	02.90
Ash	01.68	01.94	02.17	02.39
Carbohydrate	53.02	56.35	55.44	54.47
Curd fiber	00.98	01.22	01.59	02.02

Table 6: Effect of various levels of Moringa leaves powder and wheat flour on the vitamin, mineral and β-carotene contents of the balady bread (g/100g dry weight basis).

Minerals & vitamins	Control	*M.O. 5%*	*M.O. 10%*	*M.O. 15%*
Vitamin B_1	00.12	00.126	00.139	00.164
Vitamin B_2	00.09	00.098	00.112	00.149
β -carotene	01.12	04.12	08.63	09.27
Na	89.00	109.64	112.28	119.94
K	225.00	282.33	376.66	413.80
Ca	014.00	19.07	23.24	24.12
Fe	021.00	21.79	22.19	22.97
Zn	00.24	00.48	00.63	00.98
Cu	12.00	00.14	00.19	00.21
Mg	20.00	22.78	23.06	23.18
P	96.00	96.18	96.35	97.17

Effect of feeding hyperlipidemia rats on Moringa fortified bread at different ratios on food intake and body weight gain %:

Data presented in Table (7) showed the mean value of food intake (g/day for each rat) and body weight gain % of hyperlipidemia groups. The mean value of food intake decreased in the positive control group than that of the negative control group (09.523 *vs*. 11.250 g/day for each rat), respectively. Increasing amounts of *MO* used in fortification of the bread led to gradual increase in food intake in hyperlipidemia rats. Furthermore, there was a significant decrease in body weight gain % for control positive group (8.12±2.67), as compared to the negative control group (26.42±3.04). Moreover, BWG% of all treated hyperlipidemia groups with fortified bread (10% and 15% MO) significant increased as compared to the positive control group, this is in agreement with the previous studies done somewhere else Mekonnen *et al*., (2007) who reported that M. stenopetala contains important nutrients such as vitamins, proteins, minerals, carbohydrates and fats. This data confirmed by Ghebreselassie *et al*., (2011) they showed that the mice treated with the extract *MO* leaf on body weight showed increase in their body weight compared to the control group. The increase in weight might be due to the fact that Moringa is a good source of nutrition.

Results in Table (8) indicated that there were significant changes in the serum levels of total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and high density lipoprotein of hyperlipidemic rats when compared with negative control group. Rats which fed on high fat diet fortified with *MO* at 10% and 15% had lower mean values of TC, TG, LDL-C and VLDL-C compared with the positive

control group. On the other hand, all treated groups with fortified bread with different levels of *MO* had higher mean values of HDL-c comparison with those of the positive control group.

Table 7: Effect of feeding Moringa fortified bread at different ratios on food intake and body weight gain % of hyperlipidemia rats.

Parameters / Groups	Body Weight Gain (BWG%)	Food Intake (FI%)
Control negative (B-)	26.42±3.04^{bc}	11.250
Control positive (B+)	8.12±2.67^{e}	09.523
Bread + 10% *M.O.*	44.73±5.30^{a}	14.480
Bread + 15% *M.O.*	31.87±5.61^{b}	14.010

All results are expressed as mean ± SD. BWG%: Body Weight Gain %.
Values in each column which have different letters are significantly different (p<0.05).

The best results in lipid fractions for all treated groups was noticed in hyperlipidemia group fed on basal diet containing fortified bread with 15% *MO*, because these treatment improved levels of serum lipids than other treated groups. These results are in harmony, with those obtained by Ghasi *et al*., (2000) they mentioned that the crude extract of Moringa leaves has a significant cholesterol lowering action in the serum of high fat diet fed rats which might be attributed to the presence of a bioactive phyto-constituents, i.e. β-sitosterol confirmed by Dubey *et al*., (2013). Moreover, Chumarka *et al*., (2008) investigated the hypolipidaemic and antiatherosclerotic activities of *MO* leaf extract. They found that in hypercholesterol-fed rabbits, at 12 weeks of treatment, the water extract of the plant significantly lowered the cholesterol levels and reduced the atherosclerotic plaque formation to about 50% and 86%, respectively.

Table 8: Effect of Moringa fortified bread on lipid profile of hyperlipidemia rats.

Parameters / Groups	mg/dl				
	Cholesterol	Triglyceride	HDL-C	LDL-C	VLDL-C
Control negative (B-)	83.86^{b} ±3.250	105.857^{bc} ±3.610	75.571^{a} ±1.931	12.882^{b} ±2.351	21.171^{bc} ±0.901
Control positive (B+)	100.857^{a} ±6.240	122.006^{a} ± 7.160	25.510^{d} ±2.474	50.946^{a} ±4.892	24.401^{a} ±1.613
Bread + 10% *M.O.*	73.285^{d} ±1.454	107.875^{b} ±2.324	38.714^{c} ±3.481	12.486^{b} ±3.831	22.085^{b} ±0.405
Bread + 15% *M.O.*	79.66^{c} ±5.830	103.332^{c} ±4.485	47.125^{b} ±2.368	11.869^{c} ±2.492	20.666^{c} ±0.869

All results are expressed as mean ± SD.
Values in each column which have different letters are significantly different (p<0.05).

Jain *et al*., (2010) found that the serum cholesterol, triacylglyceride, VLDL, LDL, and atherogenic index were reduced by *M*. Oleifera but HDL level was increased as compared to the corresponding high fed cholesterol diet group (control). *MO* was also found to increase the excretion of fecal cholesterol. Thus, it can be concluded that *MO* possesses a hypolipidemic effect. Confirmed by Reddy *et al*., (2012) they showed reduction in cholesterol levels in rats on oral supplementation of *MO* leaves powder. It was concluded that the leaves of *MO* have definite hypocholesterolemic activity and that there is valid pharmacological basis for employing them for this purpose.

Effect of Moringa fortified bread on kidney function of hyperlipidemia rats:

Mean values and standard deviation of serum uric acid, urea nitrogen and creatinine (mg/ dl) for the groups of rats are illustrated in Table (9). From these results, it could observe that there was non-significant difference (P< 0.05) between positive control group (4.014 ± 0.108) and group fed on Moringa fortified bread at 10%. While, there was significant difference between positive control group and group fed on Moringa fortified bread at 15%, which reduced the uric acid level significantly. The concentration of uric acid was reduced by 2.84% and 18.41% respectively. It appears from our results that high concentration of *MO* fortified bread at 15% was safe and improves renal functions.

High fat diet induced hyperlipidemia rats had the higher values of serum urea nitrogen and creatinine reached to 22.371 ± 3.342 and 1.168 ± 0.172 compared with negative control group 15.371 ± 1.162 and 1.094 ± 0.004 mg/dL. From table (9) showed that the level of urea nitrogen and creatinine decreased gradually according to the concentration of *MO*, the ratios reached to 17.371 ± 2.742 and 0.931 ± 0.152 with Moringa fortified bread at 10%, while, with Moringa fortified bread at 15%, the ratios reached to 16.862 ± 1.912 and 0.832 ± 0.057 mg/dl respectively. Our results confirmed that *MO* at high concentration improved the nutritional value and realized the best effect on kidney functions. On the contrary, in a previous study by Ghebreselassie *et al*., (2011) they concluded that the administration of the mice treated with doses of 600, 750 and 900 mg/kg bw of M. stenopetala aqueous leaf extract did not show significant change on urea, creatinine, total protein and uric acid as compared to the control group.

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Table 9: Effect of Moringa fortified bread on kidney function of hyperlipidemia rats.

Parameters Groups	mg/dl		
	Uric acid	Urea nitrogen	Creatinine
Control negative (B-)	3.442^{c} ±0.121	15.371^{c} ±1.162	0.529^{c} ±0.004
Control positive (B+)	4.014^{a} ±0.108	22.371^{a} ±3.342	1.168^{a} ±0.172
Bread + 10% *M.O.*	3.900^{ab} ±0.180	17.371^{b} ±2.742	0.931^{ab} ±0.152
Bread + 15% *M.O.*	3.275^{c} ±0.151	16.862^{b} ±1.912	0.832^{b} ±0.057

All results are expressed as mean ± SD.
Values in each column which have different letters are significantly different ($p<0.05$).

Histopathological results:

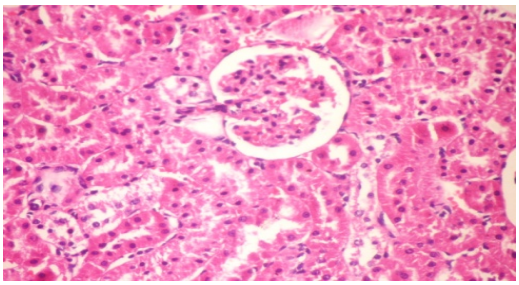

Fig. 1: Section through kidney of control untreated rat showing normal histological structure of renal parenchyma (H & E x 400).

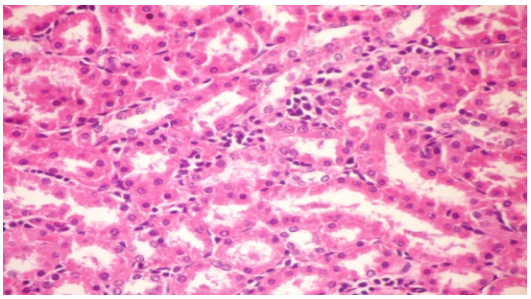

Fig. 2: Kidney of control positive rat showing peritubular inflammatory cells infiltration (H & E X 400).

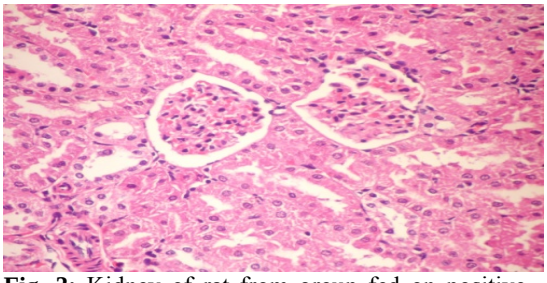

Fig. 3: Kidney of rat from group fed on positive diet with 10% MO showing focal area of leucocytic cells aggregation. Moreover, examined sections of rat revealed no histopathological changes (H & E x 400).

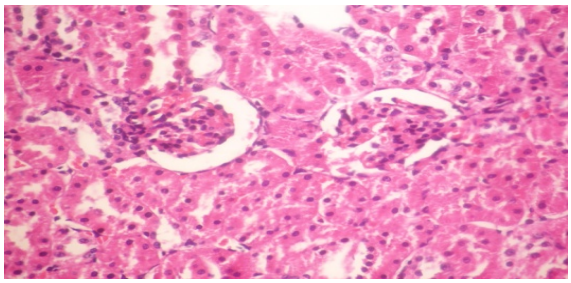

Fig. 4: Kidney of rat from group fed on positive diet with 15% MO showing no histopathological changes, and examined kidneys of rat revealed apparently normal renal parenchyma (H & E x 400).

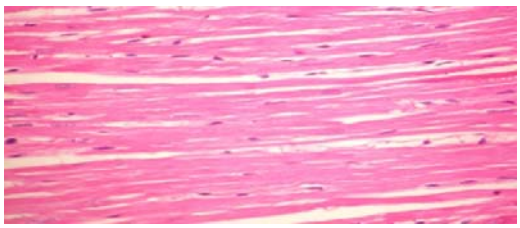

Fig. 5: Heart of control, untreated rat showing normal cardiac myocytes (H & E X 400).

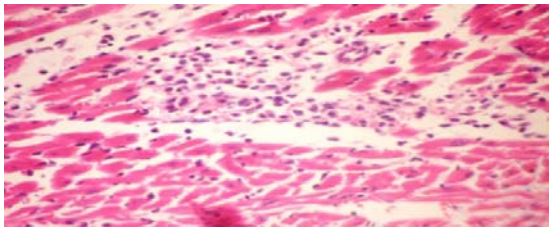

Fig. 6: Heart of control positive rat showing focal myocarditis with inflammatory cells infiltration between cardiac myocytes (H & E X 400).

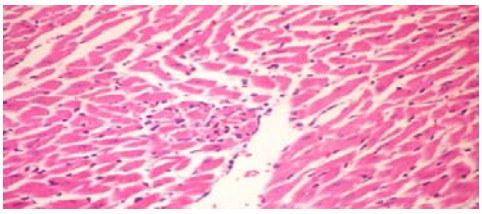

Fig. 7: Heart of rat from group fed on positive diet with 10% MO showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).

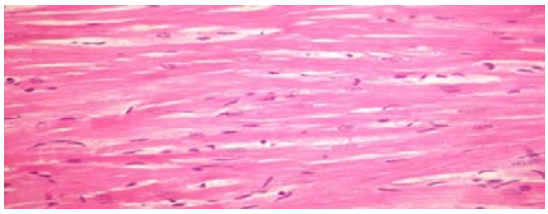

Fig. 8: Heart of rat from group fed on positive diet with 15% MO showing no histopathological changes (H & E X 400).

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