

EFFECT OF DIETARY SUPPLEMENTATION WITH DRUMSTICK TREE (*MORINGA OLEIFERA*) LEAVES POWDER AND HIGH FAT DIET ON LIVER AND KIDNEY FUNCTION OF CHICKENS

Afia Amin^a, Irum Naureen^a, Aisha Saleem^{b*}, Dilshad Ali^c, Malaika Javed^a, Zohaib Hassan^a

^aSchool of Biology, Minhaj University Lahore, Pakistan.

^bState Key Laboratory of Organic-Inorganic Composites, Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, North Third Ring Road 15, Chaoyang District, Beijing 100029, P. R. China.

^cState Key Laboratory of Chemical Resource Engineering, Beijing Engineering Centre for Hierarchical Catalysts, Beijing University of Chemical Technology, No. 15 Beisanhuan East Road, Chaoyang District, Beijing, 100029, China.



***Corresponding Author: Aisha Saleem**

State Key Laboratory of Organic-Inorganic Composites, Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, North Third Ring Road 15, Chaoyang District, Beijing 100029, P. R. China.

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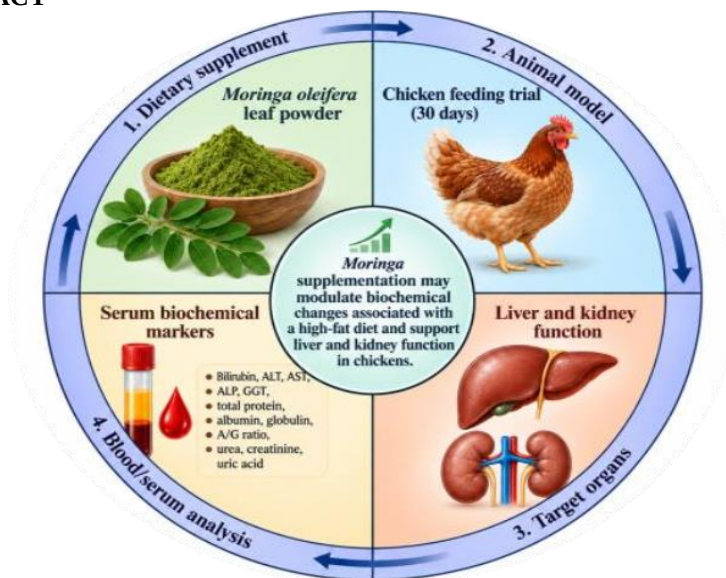
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ABSTRACT

Moringa oleifera is an important tropical food plant with potential applications in industrial, therapeutic, and agricultural uses, as a natural feed supplement in poultry production. The purpose of the study was to analyze the effect of *Moringa oleifera* with a high fat diet on the liver and kidney function of chickens and to evaluate the beneficial effect of *Moringa oleifera* on the function of the liver and kidney. Twenty egg-laying hens of the same size were divided into four groups of five birds each. Group 1 (control) received a standard diet; Group 2 received the diet + oil; Group 3 received the diet + oil + *Moringa*; and Group 4 received the diet + *Moringa* alone. Body weight gain was recorded after 30 days of the experiment relative to the initial body weight. Group 2 showed changes in liver and kidney oxidative stress markers due to the fatty diet; the induction of fat by oil was associated with metabolic disturbance arising from inflammation and oxidative stress that adversely affected liver and kidney function. However, Group 3, which received *Moringa oleifera* extract together with oil, showed reduced severity of nephrotoxicity and hepatotoxicity. Serum levels of creatinine, urea, SGPT and SGOT in the *Moringa*-treated chickens were lower than in the control group. It was concluded that chickens exposed to a fatty diet showed increased fat accumulation, but *Moringa* supplementation could control fat accumulation, hepatotoxicity and nephrotoxicity.

KEYWORDS: Bilirubin; Creatinine; *Moringa oleifera*; Mustard oil; Hepatotoxicity.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Poultry production has a vital role in the economy of developing countries and plays an important role in poverty alleviation through income generation and household food security. It provides income to about 1.5 million people in Pakistan. Poultry is a very important part of agriculture because it provides protein, contributing up to 28.4% of the total meat production of the country. Chicken production is regarded as an essential part of many families' income, and the share of poultry in the gross domestic production of Pakistan is about 1.12%. The major expenses of poultry production are feed and disease control.^[1,2] The poultry sector has been growing very rapidly in Pakistan. Poultry keeping is a source of income generation in rural areas worldwide, particularly in Pakistani villages, where it supplies food and provides a part-time business, as desi poultry is closely associated with organic production. Desi birds are always a source of higher income and have gained importance as they are regarded as a medicinal feast; current estimates indicate that desi poultry exceeds 85 million birds.^[3-5]

Moringa oleifera, commonly known as drumstick, is the most widely cultivated species of the *Moringaceae* family. It is an ornamental, rapidly growing plant with slender, softwood-free branches.^[6] The application of natural herbs to enhance production performance and health status has created substantial demand in poultry production, and *Moringa oleifera* leaves are used in poultry feed to enhance nutritional value.^[7-9] Nutraceuticals are important in poultry because of the nutritional and favorable health effects of their components; apart from providing nutritional essentials, they have pharmacological effects that help establish normal physiological status, prevent disease, and enhance production performance, and thus play an essential role in protecting animals against disease. *Moringa* has provided many benefits to humans for

centuries, both medicinal and nutritional.^[10] The leaves of *Moringa oleifera* carry vitamin C, which has the potential to counter causative agents of cold and flu, and vitamin A, which is helpful for skin disease, diarrhea, eye and heart disorders, and various other infections. *M. oleifera* Lam. is an abundant, multipurpose plant with many industrial uses and is considered a high-value crop owing to its nutritive, curative and preventative properties, which are linked to a high content of potent bioactive compounds. This plant exhibits therapeutic properties such as anti-cancer, anti-inflammatory, ulcer-healing and antioxidant activity; the antioxidant and reactive-oxygen-species-clearing properties of *M. oleifera* are attributed to its phenolic content.^[11]

Moringa has been used for centuries to treat a range of conditions, including abnormal blood pressure, swelling, ear and skin infections, headaches, glandular disorders, blood impurities, fever, asthma, anaemia, anxiety, cough, eye infections, conjunctivitis, bronchitis, chest infection and congestion, blackheads, diarrhoea, hysteria, cholera, lactation support, joint pain, semen deficiency, psoriasis, pregnancy-related complaints, pimples, respiratory disorders, scurvy, tuberculosis, sore throat, diabetes, sprains, and intestinal worms.^[12] Growth acceleration in animals and birds fed *Moringa oleifera* leaves has been reported by many researchers, and it has therefore been suggested for incorporation into poultry feed to reduce production costs, as its pods and leaves are highly nutritious, easily accessible and free of cost. *Moringa oleifera* controls lipid peroxidation and reactive oxygen species in kidney tissue and may play a key role in reducing gentamicin-induced kidney and liver damage in chickens.^[13-15] Although *Moringa oleifera* is most often applied to manage diabetes rather than liver and kidney disease, it can reduce blood cholesterol and other lipids, reduce hazards to liver and kidney function, and improve feed intake and body weight gain.^[16,19] The objective of the present study was therefore to explore the beneficial

effect of *Moringa oleifera* on chickens at the lowest possible cost in Pakistan; the findings are intended to inform the future use of herbal supplements in poultry production.

2. MATERIALS AND METHODS

2.1 Animal collection

Twenty hens of 28 to 30 weeks of age (Golden Misri breed) were purchased from the Poultry Research Institute, Lahore, and housed in cages in the animal house of the Department of Zoology, Minhaj University, Lahore, at room temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$). After five days of acclimatization, the birds were divided into four groups; three groups served as experimental groups and one as the control group.

2.2 Dosage

Control group: sample diet, feed 13.

Experimental group I: feed 13 and oil.

Experimental group II: feed 13, oil and *Moringa oleifera*.

Experimental group III: feed 13 with *Moringa oleifera*.

2.3 Preparation of *Moringa oleifera* leaf meal

Leaves of the *Moringa* plant were carefully removed from the stem, sun-dried until crisp, and ground using a hammer mill to obtain a powder that was stored in polythene bags until use. Trials were conducted during the winter season, and the moisture content of the leaves was found to be 10% after drying, obtained by subtracting the dried leaves' weight from the fresh leaves' weight using the following formula:

$$\text{Moisture \%} = [\text{Weight of fresh leaves (g)} - \text{Weight of dried leaves (g)}] / \text{Weight of fresh leaves (g)} \times 100$$

2.4 Collection of tissues and blood serum

After 30 days of treatment, body weight was recorded and all animals were sacrificed. Blood serum from each animal was collected in clean, dry centrifuge tubes and allowed to clot to separate the sera. The weight of each liver and kidney was also measured.

2.5 Body weight, liver weight, kidney weight and physical parameters

Body weight of each animal was recorded at the beginning (initial weight) and at the end of the experiment (final weight). Liver, kidney, breast, thigh, wing, heart, pancreas and spleen weights were recorded after dissection.

2.6 Assessment of liver and kidney functions

Liver and kidney function tests help diagnose organ disease, infection or damage; elevated or reduced levels of these substances can indicate a problem. The substances assessed were ALT, AST, ALP, GGT, total protein, albumin, globulins and the A/G ratio for liver function, and urea, creatinine and uric acid for kidney function; these markers were quantitatively estimated in serum samples.

Liver function tests were performed using a colorimetric assay (total protein, bilirubin, indirect bilirubin, ALP, AST, ALT), the diazo method for direct bilirubin, a kinetic colorimetric assay (Jaffe method) for creatinine, an enzymatic colorimetric assay for GGT and uric acid, and a kinetic urea/glutamate dehydrogenase test for urea.

2.7 Statistical analysis

Data were analyzed using GraphPad Prism (version 5 and version 7). Mean, standard deviation and P-values for each parameter were determined using the unpaired t-test.

3. RESULTS

3.1 Body weight

Body weight and size increased gradually across all groups, and normal water and feed intake were observed throughout. Weight gain was limited in Experimental group 2 (diet + oil) relative to the control and *Moringa*-treated groups, and a substantial proportion of feed remained uneaten in the cages of this group. Co-administration of *Moringa oleifera* leaf powder with mustard oil also produced weight gain, and birds in the *Moringa* groups appeared healthier and consumed larger quantities of water. Chickens fed a diet combined with *Moringa oleifera* leaf powder showed a consistent positive response in appearance and general health (Table 1). Liver and kidney weight showed no significant difference among the treated groups relative to the control group (Tables 2 and 3).

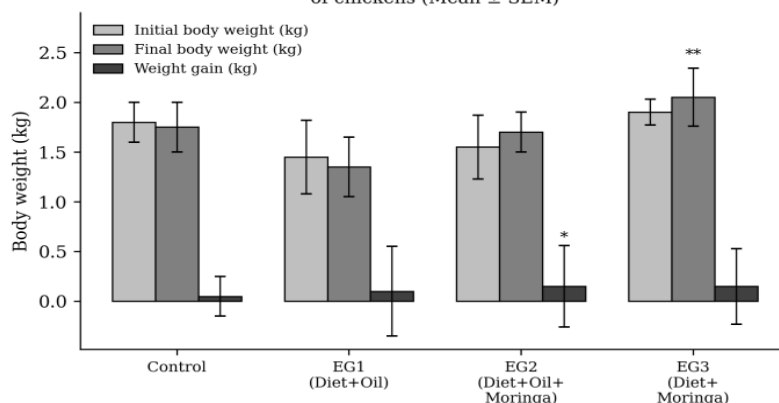
This study examined the effect of *Moringa oleifera* combined with a high-fat diet on liver and kidney function in chickens. Blood parameters were compared with the control group and included bilirubin, ALT, AST, ALP, GGT, total protein, albumin, globulins, the A/G ratio, urea, creatinine and uric acid. Statistical analysis showed significant differences ($P < 0.05$) in weight gain, ALP, GGT, total protein, globulins, A/G ratio and creatinine values, while liver weight, kidney weight, total bilirubin, direct and indirect bilirubin, ALT/SGPT, AST/SGOT, albumin, urea and uric acid did not differ significantly ($P \geq 0.05$) among the experimental groups relative to the control (Tables 4 and 6).

For the liver function parameter ALP, Experimental group 1 showed a significant change, Experimental group 2 showed no significant change, and Experimental group 3 showed a highly significant change relative to the control group. GGT differed significantly in Experimental group 1, and highly significantly in Experimental groups 2 and 3, relative to the control. All three experimental groups showed highly significant differences in total protein, globulins and the A/G ratio relative to the control (Table 5). Experimental groups 2 and 3 also showed significant changes in creatinine (Table 6).

Table 1: Effect of mustard oil, *Moringa oleifera*, and their combination on the final body weight and weight gain of chickens (*Gallus domesticus*).

Groups	Initial body weight (kg)	Final body weight (kg)	Weight gain (kg)
Control group	1.8 ± 0.20	1.75 ± 0.25	0.05 ± 0.20
Experimental group 1	1.45 ± 0.37	1.35 ± 0.3	0.1 ± 0.45
Experimental group 2	1.55 ± 0.32	1.7 ± 0.20	0.15 ± 0.41*
Experimental group 3	1.9 ± 0.13	2.05 ± 0.29**	0.15 ± 0.38

a = Control vs treated group, b = Feed + Oil vs Feed + Oil + Moringa, c = Feed + Oil + Moringa vs Feed + Moringa. *P<0.05, **P<0.01, ***P<0.001. Values are expressed as Mean ± SEM.

Figure 1. Effect of Moringa oleifera and mustard oil on body weight of chickens (Mean ± SEM)**Figure 1: Effect of Moringa oleifera and mustard oil on body weight of chickens (Mean ± SEM). *P<0.05, **P<0.01 vs control.****Table 2: Effect of mustard oil, *Moringa oleifera*, and their combination on liver weight (g) of chickens (*Gallus domesticus*).**

Control group	Experimental group 1	Experimental group 2	Experimental group 3
38.95 ± 4.05	32.40 ± 8.59	32.94 ± 6.30	41.93 ± 9.38

a = Control vs treated group, b = Feed + Oil vs Feed + Oil + Moringa, c = Feed + Oil + Moringa vs Feed + Moringa. *P<0.05, **P<0.01, ***P<0.001. Values are expressed as Mean ± SEM.

Table 3: Effect of mustard oil, *Moringa oleifera*, and their combination on kidney weight (g) of chickens (*Gallus domesticus*).

Control group	Experimental group 1	Experimental group 2	Experimental group 3
3.15 ± 0.39	2.84 ± 1.38	2.45 ± 0.03	2.84 ± 1.13

a = Control vs treated group, b = Feed + Oil vs Feed + Oil + Moringa, c = Feed + Oil + Moringa vs Feed + Moringa. *P<0.05, **P<0.01, ***P<0.001. Values are expressed as Mean ± SEM.

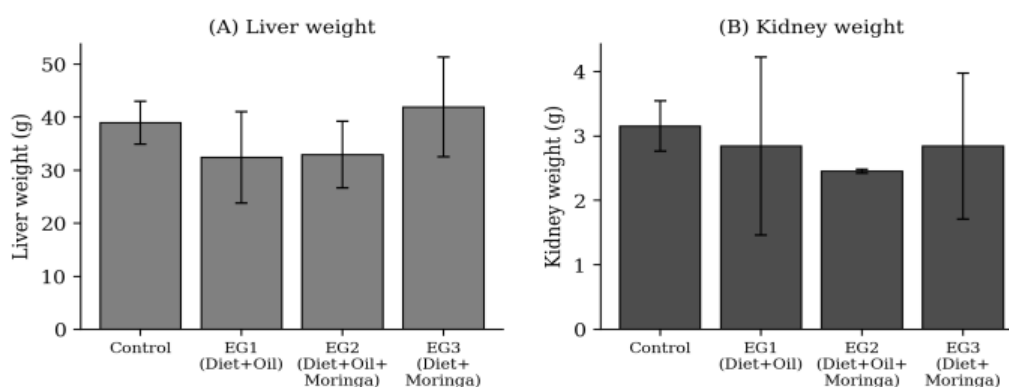
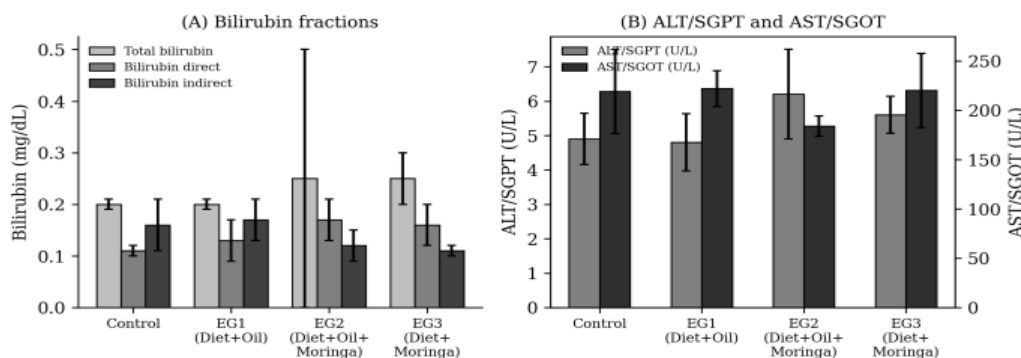
Figure 2. Effect of Moringa oleifera and mustard oil on liver and kidney weight of chickens (Mean ± SEM)**Figure 2: Effect of Moringa oleifera and mustard oil on liver and kidney weight of chickens (Mean ± SEM).**

Table 4: Effect of mustard oil, *Moringa oleifera*, and their combination on liver function (bilirubin fractions, ALT/SGPT, AST/SGOT) of chickens (*Gallus domesticus*).

Groups	Total bilirubin	Bilirubin direct	Bilirubin indirect	ALT/SGPT	AST/SGOT
Control group	0.20 ± 0.01	0.11 ± 0.01	0.16 ± 0.05	4.9 ± 0.74	219 ± 42.61
Experimental group 1	0.20 ± 0.01	0.13 ± 0.04	0.17 ± 0.04	4.8 ± 0.83	222 ± 18.14
Experimental group 2	0.25 ± 0.25	0.17 ± 0.04	0.12 ± 0.03	6.2 ± 1.30	184 ± 10.21
Experimental group 3	0.25 ± 0.05	0.16 ± 0.04	0.11 ± 0.01	5.6 ± 0.54	220 ± 37.69

a = Control vs treated group, b = Feed + Oil vs Feed + Oil + Moringa, c = Feed + Oil + Moringa vs Feed + Moringa. *P<0.05, **P<0.01, ***P<0.001. Values are expressed as Mean ± SEM.

Figure 3. Effect of *Moringa oleifera* and mustard oil on liver function (bilirubin, ALT/SGPT, AST/SGOT) of chickens (Mean ± SEM)**Figure 3: Effect of *Moringa oleifera* and mustard oil on liver function — bilirubin fractions, ALT/SGPT and AST/SGOT (Mean ± SEM).****Table 5: Effect of mustard oil, *Moringa oleifera*, and their combination on liver function (ALP, GGT, total protein, albumin, globulins, A/G ratio) of chickens (*Gallus domesticus*).**

Groups	ALP	GGT	Total protein	Albumin	Globulins	A/G ratio
CG	989 ± 81.4	28.2 ± 2.8	5.4 ± 0.82	2.16 ± 0.26	3.44 ± 0.63	0.52 ± 0.08
EG1	1819 ± 989**	24.8 ± 1.30*	3.64 ± 0.55***	1.98 ± 0.21	1.92 ± 0.58***	1.24 ± 0.54**
EG2	254 ± 71.47	14.4 ± 1.67***	5.28 ± 0.36***	2.02 ± 0.13	3.26 ± 0.35**	0.58 ± 0.08**
EG3	1902 ± 407***	24.4 ± 1.14***	5.58 ± 0.19***	2.21 ± 0.08	3.36 ± 0.33**	0.67 ± 0.04*

a = Control vs treated group, b = Feed + Oil vs Feed + Oil + Moringa, c = Feed + Oil + Moringa vs Feed + Moringa. *P<0.05, **P<0.01, ***P<0.001. Values are expressed as Mean ± SEM.

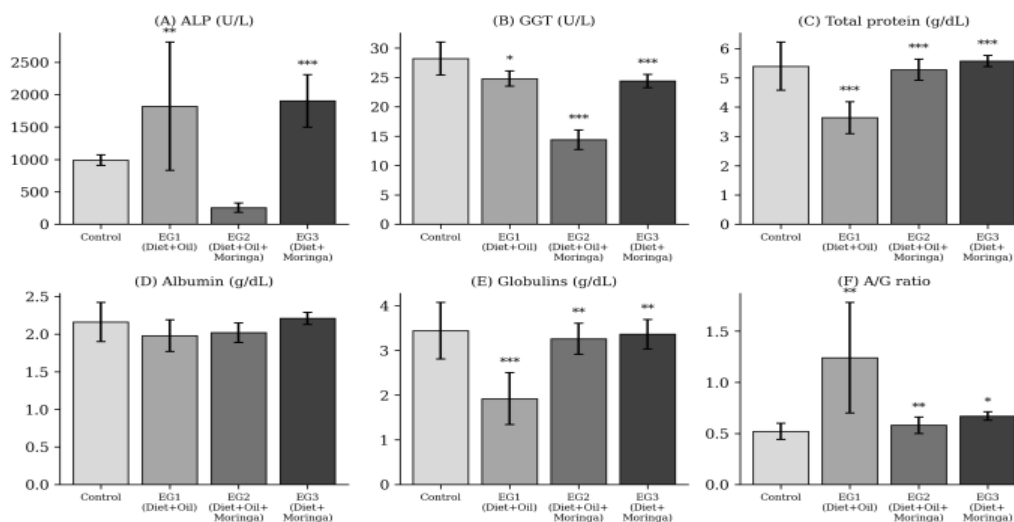
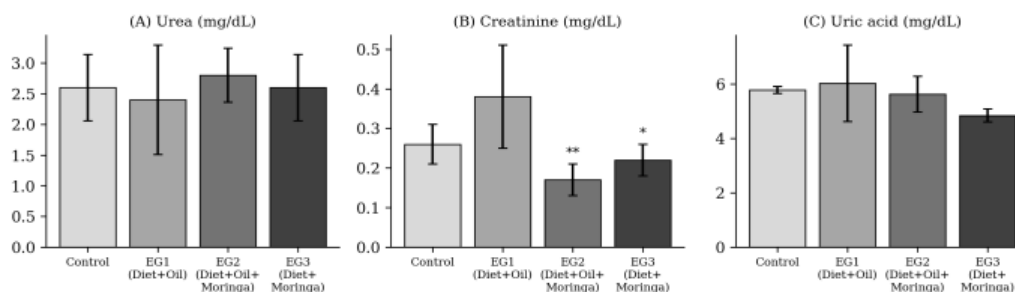
Figure 4. Effect of *Moringa oleifera* and mustard oil on liver function markers (ALP, GGT, total protein, albumin, globulins, A/G ratio) of chickens (Mean ± SEM)**Figure 4: Effect of *Moringa oleifera* and mustard oil on liver function markers — ALP, GGT, total protein, albumin, globulins, A/G ratio (Mean ± SEM). *P<0.05, **P<0.01, ***P<0.001 vs control.**

Table 6: Effect of mustard oil, *Moringa oleifera*, and their combination on kidney function (urea, creatinine, uric acid) of chickens (*Gallus domesticus*).

Groups	Urea	Creatinine	Uric acid
Control group	2.6 ± 0.54	0.26 ± 0.05	5.78 ± 0.13
Experimental group 1	2.4 ± 0.89	0.38 ± 0.13	6.02 ± 1.40
Experimental group 2	2.8 ± 0.44	0.17 ± 0.04**	5.62 ± 0.66
Experimental group 3	2.6 ± 0.54	0.22 ± 0.04*	4.84 ± 0.24

a = Control vs treated group, b = Feed + Oil vs Feed + Oil + *Moringa*, c = Feed + Oil + *Moringa* vs Feed + *Moringa*. *P<0.05, **P<0.01, ***P<0.001. Values are expressed as Mean ± SEM.

Figure 5. Effect of *Moringa oleifera* and mustard oil on kidney function (urea, creatinine, uric acid) of chickens (Mean ± SEM)**Figure 5: Effect of *Moringa oleifera* and mustard oil on kidney function — urea, creatinine and uric acid (Mean ± SEM). *P<0.05, **P<0.01 vs control.**

4. DISCUSSION

The present study examined the effects of dietary *Moringa oleifera* leaf powder on body weight and selected liver and kidney function indices in chickens receiving a mustard-oil-supplemented diet. Initial and final body weights were recorded for all groups before and after the experiment, and weight gain was observed after 30 days relative to the initial body weight. Feed intake in the oil group decreased significantly relative to the control, while weight gain and fat mass increased owing to the higher energy content of the oil supplementation, consistent with higher energy intake driving weight and fat gain in the oil group.^[7, 22] Experimental groups 2 and 3, which received *Moringa*, showed higher total and direct bilirubin than the control and oil groups. ALT/SGPT was higher in Experimental group 2 (*Moringa* + oil) and, to a lesser extent, in Experimental group 3 (*Moringa* alone) than in the control and oil groups, although this difference was not statistically significant. ALP and GGT levels in Experimental group 2 were comparatively low, and total protein was reduced in this group, indicating a negative effect of oil on protein levels; globulin levels were similarly reduced in this group. These findings indicate that a high-fat diet can adversely affect liver function.

The biochemical findings indicate that the effects of oil and *Moringa* supplementation on liver-related parameters were marker dependent. Total, direct and indirect bilirubin, ALT/SGPT and AST/SGOT did not differ significantly among the experimental groups. In contrast, significant differences were observed for ALP, GGT, total protein, globulin and the albumin/globulin (A/G) ratio. Notably, the oil + *Moringa* group showed lower ALP and GGT concentrations and total protein and

globulin values closer to the control than the oil-only group. Furthermore, recent work using *M. oleifera* leaf polysaccharides demonstrated improvements in body-weight gain and increases in serum total protein and globulin in broilers, together with changes in antioxidant indices.^[23] Kidney function parameters (urea, creatinine and uric acid) also reflected the effects of *Moringa oleifera* and oil: urea was higher in the *Moringa* groups and lower in the oil group, whereas creatinine and uric acid were higher in the oil group than in the other three groups. In the present study, kidney function parameters based on serum ALT, AST, ALP and other biochemical indices are commonly used to evaluate hepatic responses in poultry. Recent studies have shown that dietary *Moringa oleifera* supplementation can influence liver-associated biochemical markers without adversely affecting hepatic function. Ousmane et al.^[24] reported significant reductions in serum ALT and AST in *Moringa*-supplemented broilers, while Akib et al.^[25] observed no significant adverse change in ALT following supplementation with *Moringa leaf* powder. Comparable results were reported by Islam et al.^[20], who showed that intake of mustard oil in Wistar rats increased serum LDL cholesterol and triglycerides and decreased HDL, leading to non-alcoholic fatty liver disease accompanied by a significant increase in liver enzymes (ALT, AST, ALP).

After dissection, liver and kidney weights were recorded for all groups. Experimental group 3 (*Moringa*) showed greater organ weight than the oil-treated groups, which showed reduced weight, indicating a negative effect of oil on chicken organs; the *Moringa* and control groups also showed greater kidney weight than the oil group. A fatty diet therefore appeared to have adverse effects on

chicken organs, whereas *Moringa* had a positive effect on chicken health, reflected in body weight gain. Soliman et al.^[21] reported comparable ameliorative effects of *Moringa oleifera* leaf extract, reducing total protein, albumin and globulin changes induced by methotrexate. Mustard oil contains approximately 60% monounsaturated fatty acids (12% oleic acid and 42% erucic acid), 21% polyunsaturated fats (15% omega-6 linoleic acid and 6% omega-3 alpha-linolenic acid), and 12% saturated fats. The A/G ratio was also higher in the oil group than in the other groups, and liver and kidney oxidative stress markers were altered by the fatty diet.

5. CONCLUSION

Dietary supplementation with *Moringa oleifera* leaf powder showed a beneficial influence on selected biochemical responses. Chickens receiving a fatty diet showed increased fat accumulation, and liver and kidney oxidative stress markers were altered accordingly. The induction of fat by oil was associated with metabolic disturbance arising from inflammation and oxidative stress that adversely affected liver and kidney function. However, *Moringa oleifera* extract administered together with oil reduced the severity of nephrotoxicity, hepatotoxicity and the adverse effects of oil by normalizing serum levels of ALP, ALT, AST, albumin, total protein, urea and creatinine. In conclusion, *Moringa* supplementation could help control fat accumulation, hepatotoxicity and nephrotoxicity in poultry fed a high-fat diet.

AUTHORS' CONTRIBUTIONS

Afia Amin: conceived and designed the study, conducted the experiment, analyzed the data and drafted the manuscript. Irum Naureen: supervised the research and critically reviewed the manuscript. Aisha Saleem: assisted with sample collection, laboratory analysis and data compilation. Dilshad Ali: writing, formal analysis, and data curation. Malaika Javed: formal analysis and data curation. Zohaib Hassan formal analysis and data curation. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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