

## Original Article

## Dose-dependent effects of fenugreek seed extract on the biochemical and haematological parameters in high-fat diet-fed rats



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### المخلص

**أهداف البحث:** تهدف الدراسة الحالية لتقييم تأثير مستخلص بذور الحلبة على مختلف العوامل الكيميائية والدموية لدى فئران تغذت على نظام غذائي عالي الدهون.

**طرق البحث:** تم تقسيم إناث فئران ويستار إلى خمس مجموعات (6 لكل مجموعة): 1- التحكم، 2- غذاء عالي الدهون التحكم، 3- تم تغذية مجموعات الغذاء عالي الدهون بمستخلص بذور الحلبة بجرعات 200 مجم/كجم/يوميًا، و 300 مجم/كجم/يوميًا و 400 مجم/كجم/يوميًا لمدة 12 أسبوعًا. تم جمع الدم لتقدير العوامل الكيميائية والدموية باستخدام عداد خلايا الدم البيطري لتحديد مؤشرات الدم، وحجم الكرية الوسطي، وهيموغلوبين الكرية الوسطي، والتركيز الوسطي لهيموغلوبين الكرية، وعرض التوزيع، والهيموجلوبين، والهيماتوكريت وعدد الصفائح الدموية. تم تشغيل عينات الدم باستخدام جهاز الطرد على 3000 دورة في الدقيقة لمدة 10 دقائق على حرارة الغرفة للحصول على مصلى لتقدير مستوى الدهون، ومستويات إنزيمات الكبد.

**النتائج:** أظهرت الفئران التي تم تغذيتها مع 400 مجم/كجم/يوميًا زيادة كبيرة في عدد كريات الدم الحمراء، والهيموجلوبين، والهيماتوكريت، وحجم الكرية الوسطي، وانخفاض كبير في عدد كريات الدم البيضاء، وعدد الخلايا الليمفاوية. مستويات الكوليسترول الكلي، والدهون الثلاثية، والبروتينات الدهنية منخفضة الكثافة زادت بنسبة كبيرة عند الفئران التي تغذت على نظام غذائي عالي الدهون

بالمقارنة بمجموعات التحكم. هذه المستويات انخفضت بشكل كبير عند الفئران التي عولجت بمستخلص بذور الحلبة 400 مجم/كجم/يوميًا.

**الاستنتاجات:** تقترح نتائج البحث الحالي أن مستخلص بذور الحلبة أظهر مفعولا لتقليل الدهون وله تأثير كبير على إنزيمات الكبد، وتعدادات ومؤشرات الدم.

**الكلمات المفتاحية:** مستخلص بذور الحلبة؛ مستوى الدهون؛ العوامل الدموية؛ مؤشر كتلة الجسم؛ غذاء عالي الدهون

### Abstract

**Objective:** The present study was carried out to assess the effects of fenugreek seed extract on various biochemical and haematological parameters in high-fat diet (HFD)-fed rats.

**Methods:** Female Wistar rats were allocated into five groups (n = 6): 1) control rats, 2) HFD-fed control rats 3) rats fed with HFD and fenugreek (FG) seed extract at doses of 200 mg/kg/day, 300 mg/kg/day, and 400 mg/kg/day for 12 weeks. Blood was collected to examine the biochemical and haematological parameters using a veterinary blood cell counter; blood indices such as MCV, MCH, MCHC, red blood cell distribution width, haemoglobin (Hb) levels, haematocrit, and platelet counts were measured. Blood samples were centrifuged at 3000 rpm for 10 min at room temperature to obtain serum for the estimation of lipid profiles, and aspartate transaminase and alanine transaminase levels.

**Results:** Rats fed with FG at a dose of 400 mg/kg/day showed a significant increase in the red blood cell count, Hb levels, haematocrit, and MCV, and a significant decrease in

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the lymphocyte count. The total cholesterol, triglyceride, and low-density lipoprotein levels increased significantly ( $p < 0.05$ ) in rats from the HFD control group, compared to those in the normal control group, but decreased significantly in rats fed with 400 mg/kg/day of FG.

**Conclusion:** The results of the current study suggest that FG seed extract exhibits hypolipidaemic activity and significantly improves the activity of hepatic enzymes, and the blood counts and indices in rats with HFD-induced obesity.

**Keywords:** BMI; Fenugreek seed extract; Haematological parameters; High-fat diet; Lipid profile

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## Introduction

Obesity is an excessive accumulation of fat that impairs the well-being of an individual. It is associated with a wide range of chronic clinical complications. According to the World Health Organization (WHO), obesity is one of the most commonly neglected health problems in the developed and developing countries. By the year 2030, the estimated number of adults with obesity in the world is projected to be 573 million.<sup>1</sup> Previous studies have reported that in 2016, there were more than 1.9 billion adults who were overweight, of which over 650 million were obese (WHO fact sheet).<sup>2</sup> Excessive intake of high-calorie food and lack of adequate physical exercise is linked to numerous diseases such as hyperlipidaemia, type 2 diabetes, hypertriglyceridaemia, coronary heart diseases, and non-alcohol fatty liver diseases, which are the associated comorbid conditions.<sup>3</sup> The major approach in the prevention and treatment of obesity includes lifestyle modifications. The modern-day sedentary lifestyle has made it extremely difficult to overcome the outbreak of obesity. Although several anti-obesity drugs are available in the market, their untoward side effects have made the treatment of obesity rather difficult. Nowadays, complementary and alternative medicines for the treatment of obesity are preferred over the synthetic drugs available in the market. Herbal medicines are used for the treatment of chronic diseases including obesity. Fenugreek (FG) has been well established to have medicinal properties, such as anti-diabetic, anti-hyperlipidaemic, anti-inflammatory, anti-cancer, antioxidant, and neuroprotective activities.<sup>4,5</sup> FG contains active constituents like alkaloids, steroids, flavonoids, and saponins.<sup>6</sup> Alterations in haematological parameters have been observed among obese individuals.<sup>7</sup> However, the correlation of the body mass index (BMI) with various haematological parameters remains relatively unexplored, with very little clarity. Reviews of previously published scientific literature have revealed that the effects of FG seed extract on the haematological parameters of high-fat diet (HFD)-fed rats are yet to be identified. Hence,

the current study was designed to assess and document the effects of FG seed extract on various biochemical and haematological parameters in HFD-fed rats.

## Materials and Methods

### FG seed extract

One kilogram of 100% organic FG seeds (Pro Natural) was coarsely powdered and refluxed (three times) at 85 °C with 70% ethanol (1 l) to prepare the FG seed extract. The hydro-alcoholic extract was concentrated under vacuum after filtration. A freeze dryer was used to dry the final extract. The hydro-alcoholic extract was subjected to various qualitative tests for the identification of its different constituents by using standard qualitative methods,<sup>8</sup> and was subjected to high-performance thin-layer chromatography (HPTLC) to quantify the saponin compound diosgenin.

### Animals

For this study, 30 2–3-month-old healthy female Sprague–Dawley rats were obtained; they were handled according to the guidelines of the Committee for the Control and Supervision of experiments on animals, Government of India. The animals were housed in polycarbonate cages (three rats per cage) at the Central Animal research facility of the Manipal Academy of Higher Education, Manipal. They were fed with a standard pellet diet and water *ad libitum*, and were fed three different doses of FG seed extract (200, 300, and 400 mg/kg/day) for 12 weeks.

### Experimental design

Two weeks after acclimatisation, the 30 rats were divided into five groups comprising six animals in each group. Group 1 served as the control group; the rats in this group were fed with normal pellet diet. Rats in groups 2 to 5 were fed with high-fat diet (HFD) for 12 weeks. The HFD was prepared as per the standard procedure. The composition of the HFD was as follows (per 5 kg)<sup>9,10</sup>: Lard – 2 kg, Sucrose – 1/2 kg, Normal pellet (powdered) – 1 1/2 kg, Casein – 1/2 kg, Cholesterol – 125 g, Cholic acid – 2 g, Vitamin – 50 ml, and DL-methionine – 50 g. Group 2 served as the high-fat diet control group. The rats in groups 3, 4, and 5 were administered orally with 200, 300, and 400 mg/kg/day of FG seed extract, respectively. Treatment was carried out for 12 weeks. Body weight and food intake were measured every week. Fasting (12–16 h) blood samples (2.0 ml) were collected from the rats through tail-vein puncture or the puncturing the medial canthus of the eye in capillary tubes. Serum was obtained from these blood samples by centrifugation at 3000 rpm for 15 min at room temperature to estimate the biochemical parameters; 0.5 ml of blood was collected separately in EDTA-coated vials for the estimation of haematological parameters.

### Measurement of body mass index (BMI) and estimation of blood parameters

The BMI was calculated using the following formula  $BMI = \text{Body weight (g)} / \text{Body length (cm}^2\text{)}$ . Lipid

parameters such as serum total cholesterol (TC), triglycerides (TGs), and high-density lipoproteins (HDLs) were quantified using standard kits obtained from Aspen Biochem India. The TC level was estimated by the CHOD-POD method; 10 µl of the sample was mixed with 1000 µl of the kit reagent, and then, the reaction mixture was incubated at 37 °C for 10 min. The TG level was estimated by the GPO-POD method; 10 µl of the sample was mixed with 1 ml of the working reagent, and then, the reaction mixture was incubated at 37 °C for 10 min. To estimate the HDL levels, 50 µl of the sample was mixed with 1000 µl of enzyme chromogen, followed by incubation at 37 °C for 10 min; then, the absorbance of the samples was measured at 505 nm using a semi auto analyser. The concentrations of low-density lipoproteins (LDLs) and very-low-density lipoproteins (VLDLs) were calculated by using Friedewald's equation:  $VLDL-C = TG/5$ ;  $LDL-C = TC - HDL - VLDL$ . The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were estimated by using a commercially available ELISA kit (Aspen Biochem). The activities of AST and ALT were determined based on a UV kinetic method recommended by the International Federation of Clinical Chemistry (IFCC). First, 100 µl of the sample was mixed with 1 ml of the working reagent; then, the reaction mixture was incubated at 37 °C for 1 min. The change in absorbance (OD) was measured at the wavelength of 340 nm by using a semi-automated biochemical analyser. The results were expressed as U/L. For the estimation of haematological parameters, blood samples were analysed using an automated Veterinary Blood Cell Counter (Model PCE-210VET ERMA Inc.). The total, absolute, and differential leukocyte counts, red blood cell (RBC) counts, and indices such as the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haemoglobin (Hb) levels, haematocrit (HCT), and platelet counts were determined.

### Statistical analysis

Data analysis was performed using one-way analysis of variance (ANOVA), followed by Bonferroni's *post-hoc* test. Results are expressed as the mean  $\pm$  SEM, and p-values  $\leq$  0.05 were considered statistically significant.

### Results

The effects of FG seed extract on body weight and BMI are shown in Table 1. The body weight and BMI of the rats

increased significantly increased in rats from the HFD control group ( $P < 0.05$ ), and were maintained at normal levels in the FG seed extract-fed rats ( $P < 0.01$ ). The mean TC, TG, LDL, TC/HDL, AST, and ALT levels increased significantly ( $P < 0.05$ ) in the rats from the HFD control group. However, rats treated with FG seed extract at doses of 300 and 400 mg/kg/day showed a significant ( $P < 0.05$ ) normalisation of the TC, TG, LDL, AST, and ALT levels, and a significant increase in the HDL levels ( $P < 0.05$ ). The TC/HDL ratio was significantly reduced ( $P < 0.01$ ) in rats treated with the FG seed extract at a dose of 400 mg/kg/day (Table 2).

The RBC, Hb, and HCT counts of the rats from the HFD control group showed a significant decrease ( $P < 0.05$ ), compared to the case for those from the normal control group. The rats treated with FG seed extract at doses of 300 and 400 mg/kg/day showed a significant amelioration of the RBC count ( $P < 0.01$ ). The rats from the HFD control group showed a significant decline in the levels of MCV and MCH, compared to those from the normal control group, but treatment with 400 mg/kg/day of the FG seed extract significantly decreased the HCT and MCV (Table 3). The WBC count, i.e., lymphocyte number, showed a significant increase ( $P < 0.05$ ) in rats from the HFD control group, compared to the case for those from the normal control group (Table 4). Treatment with the FG seed extract at a dose of 400 mg/kg/day significantly normalised the monocyte counts, but the granulocyte counts showed no significant changes in the HFD-fed rats treated with the FG seed extract.

The correlation analysis revealed that the TC and TG levels were positively correlated with the BMI (TC Vs. BMI,  $P = 0.02$ ;  $r$  value = 0.86 and TG Vs. BMI;  $P = 0.002$ ;  $r$  value = 0.96; Figures 1A and B). A negative correlation was observed between the BMI and HDL level ( $r = -0.97$ ), but this was not significant ( $P = 0.07$ ). The correlation coefficient between the BMI and WBC count was 0.82 (positive correlation). By using linear regression plots, an equation that can predict the WBC count (y-axis) from the BMI [independent variable (x)] was developed ( $y = 35.191x - 7.68$ ). The P value was 0.04 (Figure 1C). The platelet count ( $10^3$  cell/ $\mu$ l) in rats from the normal control group was  $312 \pm 68.23$ ; there was no significant increase in the platelet count in rats from the HFD control group ( $319 \pm 34.5$ ). Treatment with the FG seed extract at doses of 200, 300, and 400 mg/kg/day slightly decreased the platelet count ( $315 \pm 78.2$ ,  $311 \pm 23.98$ , and  $308 \pm 61.6$ , respectively), but these decreases were not significant.

**Table 1: Effect of fenugreek seed extract on the body weight of rats from the normal control, HFD control, and HFD+FG seed extract (200, 300, and 400 mg/kg/day) groups.**

Bodyweight (g)	Control	HFD	HFD+FG (200 mg/kg/day)	HFD+FG (300 mg/kg/day)	HFD+FG (400 mg/kg/day)
Before Supplementation	120.06 $\pm$ 2.4	119.41 $\pm$ 3.6	125.8 $\pm$ 2.1	120.32 $\pm$ 4.2	118.32 $\pm$ 3.9
After Supplementation	176.23 $\pm$ 4.7	211.01 $\pm$ 3.9*	192.1 $\pm$ 2.7	188.56 $\pm$ 5.3 <sup>a</sup>	180.2 $\pm$ 6.1 <sup>a</sup>
BMI	0.43 $\pm$ 0.01	0.62 $\pm$ 0.02*	0.58 $\pm$ 0.04	0.51 $\pm$ 0.03 <sup>a</sup>	0.47 $\pm$ 0.01 <sup>a</sup>

Values are expressed as the mean  $\pm$  SEM, HFD: High-Fat Diet; \*Control vs. HFD control; <sup>a</sup>HFD control vs. HFD+FG seed extract groups,  $P \leq 0.05$ .

**Table 2: Effect of fenugreek seed extract on lipid profiles, and AST and ALT levels in rats from the normal control, HFD control, and HFD+FG seed extract (200, 300, and 400 mg/kg/day) groups.**

Parameters	Control	HFD	HFD+FG (200 mg/kg/day)	HFD+FG (300 mg/kg/day)	HFD+FG (400 mg/kg/day)
Cholesterol (mg/dl)	63.85 ± 3.5	112.8 ± 3.7*	102.4 ± 2.6	89.87 ± 2.5 <sup>a</sup>	71.36 ± 6.3 <sup>a</sup>
Triglycerides (mg/dl)	64.67 ± 3.7	105.8 ± 4.4*	95.09 ± 3.7	73.21 ± 2.8 <sup>a</sup>	61.9 ± 2.5 <sup>a</sup>
LDL (mg/dl)	21.56 ± 2.2	68.72 ± 3.4*	55.47 ± 2.7	44.4 ± 4.4 <sup>a</sup>	24.46 ± 6.6 <sup>a</sup>
HDL (mg/dl)	40.71 ± 3.8	22.3 ± 2.8*	26.24 ± 2.3	29.15 ± 2.5	36.79 ± 3.8 <sup>a</sup>
TC/HDL ratio	1.56 ± 0.9	5.05 ± 1.3*	3.9 ± 1.1	3.09 ± 1	1.9 ± 1.6 <sup>a</sup>
AST (IU)	31.02 ± 2.6	69.86 ± 1*	59.6 ± 1.9	47 ± 1.4 <sup>a</sup>	35 ± 4.5 <sup>a</sup>
ALT (IU)	40.2 ± 3.9	79 ± 5.3 *	66.1 ± 4.3	54.7 ± 3.9 <sup>a</sup>	46 ± 3.2 <sup>a</sup>

Values are expressed as the mean ± SEM, HFD: High-Fat Diet; \*Control vs. HFD control; <sup>a</sup>HFD control vs. HFD+GF seed extract groups,  $P \leq 0.05$ .

**Table 3: Effect of fenugreek seed extract on the RBC count and related indices in rats from the normal control, HFD control, and HFD+FG seed extract (200, 300, and 400 mg/kg/day) groups.**

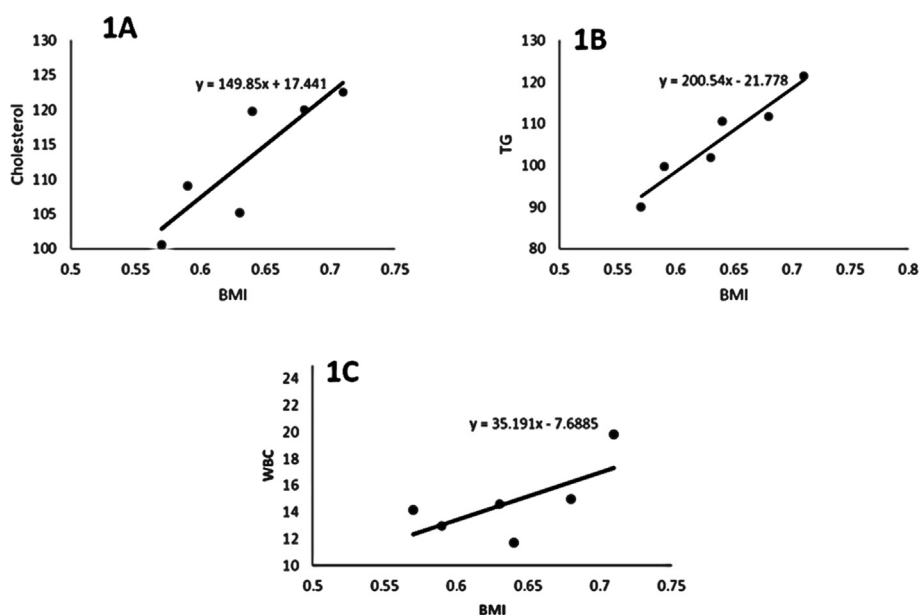
Parameters	Control	HFD	HFD+FG (200 mg/kg/day)	HFD+FG (300 mg/kg/day)	HFD+FG (400 mg/kg/day)
RBC ( $10^6$ cells/ $\mu$ l)	7.81 ± 0.46	5.43 ± 1.83 *	6.23 ± 1.12	6.68 ± 0.34 <sup>a</sup>	7.29 ± 0.68 <sup>a</sup>
Hb (g/dl)	14.43 ± 0.23	11.56 ± 0.92*	12.03 ± 1.23	12.98 ± 0.86 <sup>a</sup>	13.98 ± 1.1 <sup>a</sup>
HCT (%)	36.81 ± 1.3	30.28 ± 0.78*	31.12 ± 0.71	32.5 ± 0.76	36.76 ± 0.05 <sup>a</sup>
MCV (fl)	50.3 ± 0.23	42.78 ± 1.2*	45 ± 2.7	45.99 ± 0.2	47.34 ± 1.09 <sup>a</sup>
MCH (pg)	16 ± 3.2	14.2 ± 1.1*	14.9 ± 0.9	15.1 ± 0.75	15.48 ± 0.86
MCHC (g/dl)	31.2 ± 2.9	29.2 ± 1.02	29.98 ± 0.3	29.34 ± 1.1	30.32 ± 1.5

Values are expressed as the mean ± SEM, HFD: High-Fat Diet; RBC: Red blood cell, Hb- Haemoglobin, HCT-haematocrit, MCV-mean corpuscular volume, MCH-mean corpuscular haemoglobin, MCHC-mean corpuscular haemoglobin concentration. Values are expressed as the mean ± SEM, \*Control vs. HFD control; <sup>a</sup>HFD control vs. HFD+FG seed extract groups,  $P \leq 0.05$ .

**Table 4: Effect of fenugreek seed extract on the WBC count and related indices in rats from the normal control, HFD control, and HFD+FG seed extract (200, 300, and 400 mg/kg/day) groups.**

Parameters	Control	HFD	HFD+FG (200 mg/kg/day)	HFD+FG (300 mg/kg/day)	HFD+FG (400 mg/kg/day)
WBCs ( $10^3$ cells/ $\mu$ l)	9.53 ± 0.75	12.34 ± 0.1*	11.98 ± 1	11.02 ± 3.9	10.01 ± 0.7 <sup>a</sup>
Lymphocytes ( $10^3$ cells/ $\mu$ l)	11.2 ± 1.4	16.23 ± 3.6*	15.01 ± 2.3	14.98 ± 1.5 <sup>a</sup>	12.09 ± 1.01 <sup>a</sup>
Monocytes (%)	12.12 ± 0.1	12.68 ± 2.1	12.42 ± 0.9	12.21 ± 1.2	12 ± 0.05
Granulocytes (%)	14.45 ± 0.4	15.43 ± 1.6	15.04 ± 0.1	14.98 ± 6.2	14.54 ± 2.1

Values are expressed as the mean ± SEM, HFD: High-Fat Diet; \*Control vs. HFD control; <sup>a</sup>HFD control vs. HFD+FG seed extract-fed treated groups,  $P \leq 0.05$ .



**Figure 1:** Pearson's correlation coefficient between the BMI and biochemical and haematological parameters of rats from the HFD control group.

## Discussion

The rat model of high-fat diet-induced obesity is the most commonly used rat model of obesity. In the current study, intake of HFD for 12 weeks significantly augmented the BMI. BMI is a predominant indicator of the fat stores in the body.<sup>11</sup> In our study, a significant reduction in body weight and BMI was observed following the treatment of the rats with 300 and 400 mg/kg/day of the FG seed extract; this indicates that the FG seed extract notably suppressed the weight gain and BMI in obese rats.

Obesity is one of the independent risk factors for dyslipidaemia. In this study, the TC, TG, and LDL levels increased significantly in the rats from the HFD control group. Treatment with 300 and 400 mg/kg/day of the FG seed extract caused hypolipidemic effects by lowering the TC, TG, and LDL levels, compared to the case for the rats in the HFD control group. The HDL levels improved significantly in the rats fed with 400 mg/kg/day of the FG seed extract. The hypolipidaemic effect of FG can be attributed to: (i) the presence of dietary fibres and gum (fibres restrict the absorption of lipids by the small intestine),<sup>12</sup> (ii) the steroidal saponins present in the FG extract, which slow down the absorption of cholesterol, as well as the production of cholesterol by the liver, by decreasing the activity of the regulatory enzyme of the cholesterol synthesis pathway, i.e.,  $\beta$ -hydroxy  $\beta$ -methylglutaryl CoA,<sup>13</sup> (iii) the 4-hydroxy isoleucine in FG, which acts on liver cells and adipocytes, leading to decreased cholesterol and TG synthesis, in addition to enhanced LDL receptor-mediated LDL uptake, and (iv) the increase in the activity of lecithin cholesterol acyltransferase, thereby favouring the incorporation of a greater amount of cholesterol in HDL and increased cholesterol uptake by the liver.<sup>14</sup> Our results concur with those of the study by Praveen Kumar et al., which has suggested that supplementation of 0.5 and 1 g/kg/day of FG seed aqueous extract for four weeks significantly decreased the TC, TG, and LDL levels, and increased the HDL levels.<sup>15</sup> Another study has reported that treatment with a dose of 200 mg/kg/day of a FG seed extract along with choline-docosahexaenoic acid significantly decreased the TC and LDL levels, and increased the HDL levels.<sup>16</sup> Previous literature has reported that administering an ethanolic extract of FG (seed) (400 mg/kg body weight/day) for four weeks to HFD-fed rats lowered the levels of TC, TG, and LDL, and concurrently increased the HDL levels.<sup>17</sup>

The TC/HDL ratio, and AST and ALT levels increased significantly in rats from the HFD control group. These changes indicate that the risk of cardiovascular and hepatic disorders is augmented in HFD-fed rats. However, the FG seed extract-fed rats showed a reduction of the TC/HDL ratio, and AST and ALT levels, compared to the rats from the HFD control group, which is suggestive of decreased lipid accumulation in the liver and reduced risk for cardiovascular and hepatic disorders.

The rats in the HFD control group showed a significant decrease in the RBC counts, Hb levels, HCT, MCV, and MCH. These changes can be attributed to the RBC dysfunction occurring due to obesity induced by a diet rich in saturated fats.<sup>18</sup> Hyperlipidaemia is known to trigger the generation of free radicals and reactive oxygen species; a weak antioxidant

defence mechanism favours lipid peroxidation, which may lead to the damage of cellular organelles, enzymes, and DNA.<sup>19</sup> FG seed extract contains flavonoids and polyphenols, which exhibit antioxidant properties. The free radical-scavenging activity of the antioxidants decreased, causing increased oxidative stress, which may damage the RBC membrane and lead to the lysis of RBCs. Haemoglobin released from the lysis of RBCs is further known to turn highly toxic because of the oxidative properties of haeme.<sup>20</sup> Previous studies have reported that hypercholesterolaemia is associated with alterations in the RBC cholesterol and fluidity of membranes in humans.<sup>21</sup> Previous literature has also reported contrasting findings, whereby the haematological parameters of rats fed with HFD for six weeks showed no change.<sup>22</sup> One study has reported that anaemic mice supplemented with gold nanoparticles comprising FG seeds showed a significant increase in the RBC counts, and Hb and HCT levels.<sup>23</sup> This effect of FG may be due to its iron content (approximately 33.5 mg of iron/100 g of FG seeds). A study conducted by Jagadeesh et al. has stated that feeding 200 mg/kg body weight/day of FG extract to ovariectomised rats for four weeks significantly increased the RBC counts, and the Hb and HCT levels.<sup>24</sup>

Our study reveals that the number of WBCs, especially, lymphocytes, increased in HFD-fed rats, compared to the rats in the normal control group. HFD-induced obesity is associated with low-grade inflammation, and is one of the causes for an increase in the WBC count. A previous study has put forth a hypothesis proposing that the exposure of the bone marrow to high concentrations of leptin can stimulate the proliferation of myeloid stem cells, which leads to an increase in the WBC count.<sup>25</sup> A study conducted by Faizania et al. has reported an increase in the WBC count in obese rats. Female Sprague–Dawley rats have been reported to show decreased WBC counts after the administration of atorvastatin.<sup>26</sup> A study performed by Effraim et al., in 1999 has reported a significant increase in the Hb levels, WBC counts, and PCV after seven days of treatment with FG seed extract at doses of 300–900 mg/kg/day for 7 days, followed by a decrease in these parameters after 14 days of the treatment.<sup>27</sup> On the contrary, data from a previous study have suggested that short-term HFD feeding (6 weeks) does not cause significant alterations in the biochemical and haematological parameters.<sup>22</sup>

Analysis of the effects of obesity on platelet counts has revealed inconsistent results. A pathological increase in platelet count has been reported to be associated with a risk of thrombotic complications. Studies have suggested a positive correlation between healthy middle-aged men with coronary heart disease and a high platelet count.<sup>28</sup> In our study, no significant change in platelet count was observed in rats from the HFD control group, compared to those from the normal control group or those fed with the FG seed extract. Our results are in agreement with those of the study by Youssef et al., who have reported that no significant changes in platelet count and platelet aggregation were observed in obese rats, compared to control rats.<sup>29</sup>

## Conclusion

The current study reveals that an FG seed extract dose of 400 mg/kg/day shows a positive effect with regards to the

reduction of hyperlipidaemia and normalisation of the hepatic enzyme profile. The anti-hypolipidaemic effect of the FG extract in ovariectomised rats was attributed to the presence of various phenolic compounds. Our study also highlights the effects of FG whereby it increased the RBC counts and haemoglobin content, and significantly decreased the WBC count, which is suggestive of an overall positive effect on the haematological scenario. Effects on haematological changes may be attributed to the presence of iron in the extract and a protective effect against the RBC membrane.

#### Source of funding

This project was self-funded by the institute as this is a PhD project. Hence, the authors thank the University for providing the infrastructure and facilities for this research.

#### Conflict of interest

The authors have no conflict of interest to declare.

#### Ethical approval

The experiments on rats were conducted as per the recommendations made by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) in advance (Letter No. IAEC/KMC/16/2016).

#### Authors' contributions

YN, PU, CDN, SUK, and NT conceived and designed the study, conducted the research, and performed the analysis and interpretation of the data. AK and NT conducted the research and performed the analysis and interpretation of the data. All authors contributed equally and significantly to the draft of the article and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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