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Protective effects of fenugreek (*Trigonella foenum-graecum*) on glucose homeostasis, lipid profile, and immune function in a Wistar rat model

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Abstract Fenugreek (*Trigonella foenum-graecum*) is well known worldwide as a spice and as an herbal remedy for combating high blood sugar. The present study investigated the effect of fenugreek on glycemic, lipidemic and immunomodulatory potential using Wistar rats *in vivo* model to compare the efficacy of two common methods of fenugreek administration, i.e. seed-soaked water (soaked overnight: traditional method) and fenugreek seed powder-soaked water (soaked for 48 hours). Phytochemicals in the prepared extracts were assessed qualitatively. Cholesterol-induced rats were orally administered with a dose of 1g/kg (body weight) of seed-soaked water (SS) and powder-soaked water (PS) (N=6 each), once daily for 28 consecutive days. A group of hypercholesterolemic rats without fenugreek treatment served as positive control (PC), while non-cholesterolemic rats without fenugreek treatment served as the negative control (NC) (n=6 each). Glycemic, lipidemic and immunomodulatory effects were investigated using standard procedures. Standard toxicity testing was conducted for oral consumption of fenugreek. Phytochemical analysis revealed proportionately higher presence of reducing sugars, tannins, saponins and flavonoids in the SS than PS extract. The traditional method of consumption (SS) reported the highest efficacy in significantly ($p < 0.05$) reducing both glucose (47.72 vs >137.56 mg/dL) and total cholesterol levels (59.75 vs >298.83 mg/dL compared to PC. Both treatments resulted in 8-10% elevation of lymphocyte counts compared to the controls, suggesting immunopotentiation, while no apparent toxic effects were observed in the rats. The results of the present study provide scientific validation for the traditional seed-soaked fenugreek remedy in controlling cholesterol and blood sugar, with potential extensions towards herbal drug leads.

Keywords: Antiglycemic, antilipidemic herbal, *in-vivo* model.

1 Introduction

Globally, non-communicable diseases (NCDs) have become the leading cause of death. The most alarming fact is that people with cardiovascular diseases and diabetes

have become the most vulnerable causing premature mortality worldwide. The occurrence of NCDs is associated with certain behavioral risk factors such as physical inactivity, unhealthy diet, smoking and harmful alcohol use (WHO 2018). It has always been a debate among the general public whether to use synthetic drugs or herbal remedies for treatments. At present, the interest of the scientific community in medicinal plants research, particularly plants traditionally used to treat diabetes in humans has increased remarkably. Pharmaceutical companies are also keen on large scale screening of herbal resources due to their potential for drug development (Mohammed 2007).

It is a popular belief in Sri Lanka that the use of complementary traditional plant medicine is more beneficial than synthetic drugs in respect of both economical aspect and minimum side effects (Medagama *et al*. 2014). The medicinal value of many plants remains under-studied (Punitha *et al*. 2006). The use of fenugreek (*Trigonella foenum-graecum*) seeds is common in various parts of Asia mainly in India and Sri Lanka. It is a leguminous plant belonging to the family Fabaceae. This plant is generally resistant to many crop infections, germinates within 3 days and grows to a sturdy, erect plant, and its leaves are compound, pinnate and trifoliate. Axillary white to yellowish flowers develop, and long, slender, pointed beaked pods of about 3-15 cm are produced containing about 10–20 oblong, greenish-brown seeds (Chatterjee and Pakrashi 1995).

Health benefits associated with fenugreek include hypoglycemic, hypolipidemic, antioxidant, and anti-inflammatory activities as reported in prior studies. Fenugreek seeds can reduce fasting plasma glucose, serum cholesterol, and triglycerides levels, especially in patients suffering from diabetes and hyperlipidemia (Rao *et al*. 1996, Srinivasan 2006, Roberts 2011). Such biological effects are due to the presence of various bioactive phytochemicals in the seeds of fenugreek, namely, saponins, flavonoids, alkaloids such as trigonelline, dietary fibers, and polyphenols (Srinivasan 2006). It is known that saponins and soluble dietary fibers inhibit intestinal lipid absorption and cholesterol metabolism, whereas flavonoids and alkaloids exhibit antioxidant and antidiabetic properties (Roberts 2011, Nagamma *et al*. 2019).

In vivo studies performed on rodents have revealed that fenugreek seeds have beneficial effects on serum lipid profile and glycemic index as well as provide protection against metabolic disorders (Mowla *et al*. 2009, Nagamma *et al*. 2019). Fenugreek seeds show immunomodulating properties affecting hemogram characteristics and immune response (Rao *et al*. 1996).

As for toxicology, fenugreek is considered to be relatively nontoxic at moderate doses since there is no hepato-renal toxicity in experimental animals (Sakr and El-Gamal 2011). Most studies have focused on powdered form of fenugreek seeds, its solvents, or commercial products, whereas few studies have considered the effects of home-made preparations based on fenugreek seeds. Consuming water from overnight fenugreek seed soaking is a popular method in Sri Lanka and some other Asian countries. There is also a lack of comparative studies involving seed-soaked preparation concerning glycemic, lipidemic, immunomodulating, and toxic effects. Thus, we focused on analyzing these parameters using an *in vivo* rat model to compare the health benefits of fenugreek seeds between two common methods of

administration, namely seed-soaked water and aqueous powdered-seed preparations. Furthermore, a qualitative phytochemical analysis of the tested preparations was conducted to evaluate their chemical profiles.

2 Material and Methods

2.1 Qualitative analysis of phytochemicals in fenugreek seed extractions

Using locally available fenugreek seeds, two different extractions were prepared by soaking raw fenugreek seeds overnight in water, and by soaking powdered form in water. In each case, fenugreek seeds or powder were diluted with the solvent in a 1:10 dilution ratio (by weight). In order to increase the extraction efficiency, powdered fenugreek extraction time was increased to 48 hours (Mowla *et al*. 2009).

Phytochemical screening was conducted by employing a series of qualitative chemical tests; Fehling's test for reducing sugars, Wagner's reagent for alkaloids, ferric chloride for tannins, foam test for saponins, alkaline reagent or Shinoda test for flavonoids, Keller–Killiani test for glycosides, Salkowski test for terpenoids and steroids, and Biuret test for proteins. The strength of the visual reaction was noted as a rough estimate of the relative abundance of tested compounds.

2.2 Experimental animals

Animal experiment was conducted under ethical approval (ERC IOBSL 202 12 2019) from the Ethical Review Committee of the Institute of Biology Sri Lanka (IOBSL; Jan 5, 2020), and followed the Organization for Economic Co-operation and Development (OECD) guidelines. Healthy male Wistar rats (strain: *Rattus norvegicus*) (n= 24, 190-220 g) obtained from the Medical Research Institute (MRI), Colombo, Sri Lanka were used as experimental animals *in vivo* model. The rats were housed in semitransparent plastic cages in the animal house of the Department of Zoology, The Open University of Sri Lanka, Nawala, under standard animal house conditions (temperature 28-31°C, photoperiod: approximately 12 hours of natural light per day, relative humidity: 50-55 %). Initially they were subjected to a one-week acclimatization period. All test rats were fed with pellet food daily and clear drinking water ad libitum during experimentation.

2.3 Induction of hypercholesterolemia in test rats

Eighteen out of the total 24 rats, were given a mixture of egg yolk and cheese 1% body weight continuously for a period of 2 weeks in addition to their standard pellet diet to induce hypercholesterolemia (Srinivasan 2006).

After the cholesterol induction period, a pre-bleed testing was carried out to estimate their cholesterol levels, glucose levels and blood cell counts prior to fenugreek treatment. To conduct the pre-bleed, the rats were carefully anaesthetized using diethyl ether, and about 1ml of blood was drawn out immediately using tail-vein nick method. Non-heparinized blood was collected separately into Eppendorf tubes for serum separation whereas anticoagulated blood (heparinized blood) was collected into EDTA tubes separately for blood cell count analysis.

Total serum cholesterol was measured using a colorimetric test kit (Centronic GmbH Am Kleinfeld 11 85456 Wartenberg/ Germany), following the procedure mentioned in the test kit. Then calculations were done using the equation and standard factor values provided with the kit, as

$$C = 200 \times \Delta A \text{ (sample)} / \Delta A \text{ (standard)} \text{ (mg/dl)}$$

where, $\Delta A = A(\text{sample}) - A(\text{sample blank})$, with absorbance (A) measured at a wavelength range of 495-525 nm using an ELISA reader (LisaScan II, Erba Mannheim, Germany).

Glucose levels in serum were measured using a test kit (Human laboratories, Germany) at the pre-bleed, following the procedure, calculation equation and standard factor values mentioned in the test kit. Serum glucose concentration C was calculated with absorbance (A) measured at 505 nm using the ELISA reader (LisaScan II, Erba Mannheim, Germany) as,

$$C = 100 \times \Delta A \text{ (sample)} / \Delta A \text{ (standard)} \text{ (mg/dl)}$$

where, ΔA is the difference in absorbance between sample and the sample blank.

To obtain the differential white blood cell counts, blood smears were prepared using a drop of anticoagulated blood on a clean, humidity-free slide immediately after blood collection. Blood smears were air dried, fixed with methanol, stained with 1% Giemsa, left for air drying and finally mounted. Different WBC types (lymphocytes, neutrophils, eosinophils, basophils and monocytes) were identified with the aid of a compound light microscope (400×; Optica, B-293, Italy), and the ratio of lymphocytes to neutrophils (LNR) was calculated.

2.5 Experimental setup and oral administration of fenugreek

After the induction of hypercholesterolemia, 18 cholesterol-induced rats were randomly assigned into three groups (n = 6 each): seeds-soaked group (SS), powdered seeds-soaked group (PS), and positive control group (PC). The rats in the SS group received the aqueous extract obtained from fenugreek seeds soaked overnight in water, while rats in the PS group received the aqueous extract prepared from powdered fenugreek seeds soaked in water. SS and PS extracts were orally administered to rats of the two treatment groups once daily for 28 consecutive days at a dose of 1 g/kg body weight via a gavage needle (equivalent to 270 mg of fenugreek). The PC group was composed of hypercholesterolemic rats that did not receive fenugreek treatment.

Additionally, six rats that were not treated with cholesterol or fenugreek were kept as the negative control group (NC).

Use of both negative and positive control groups allowed for comparisons to be made on the potential therapeutic effects of the fenugreek treatments under normal physiological conditions and cholesterol-induced hypercholesterolemia.

2.6 Final bleeding analysis

At the end of 28 days of treatment, the rats were subjected to final bleeding. The rats were anaesthetized with a high dose of anesthetic ether (diethyl ether) and about 3ml of blood was drawn by cardiac puncture. The anticoagulated blood (heparinized blood) was collected into EDTA tubes for obtaining cell counts. The coagulated blood was collected separately into Eppendorf tubes for serum separation.

Total cholesterol and glucose levels in serum

Total serum cholesterol and glucose levels were determined using commercial colorimetric assay kits (Centronic GmbH, Wartenberg, Germany; Human Laboratories, Germany) according to the manufacturers' instructions, following the same procedures used during the pre-bleed. Absorbance readings were obtained using an ELISA microplate reader (LisaScan II, Erba Mannheim, Germany) at wavelengths of 495–525 nm for total cholesterol and 505 nm for glucose.

Triglycerides levels in serum

The triglyceride levels in serum were measured using a colorimetric test kit (Centronic GmbH Am Kleinfeld, Germany), following the procedure specified in it and the absorbance was measured at a wavelengths of 500–546 nm using an ELISA microplate reader (LisaScan II, Erba Mannheim, Germany). Calculations were done using the equations and standard factor values provided with the kit as,

$$C = 200 \times \Delta A \text{ (sample)} / \Delta A \text{ (standard)} \text{ (mg/dl)}$$

where, ΔA is difference in absorbance between the sample and the sample blank.

Testing for toxicity in consuming fenugreek

Toxicity parameters were analyzed to find out whether there are detrimental health risks in consuming fenugreek. To evaluate the hepatotoxicity, the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum of all the tested rats were measured. The assays were conducted according to the procedure specified in the test kit, (RAL, Tecnica para el Laboratories, Spain). Absorbance was measured at 340 nm (ALT) and 505 nm (AST) using a UV-Visible spectrophotometer (Labomed, UVD-3000, USA). The enzyme activity was calculated using the following equation.

$$\text{Sample activity} = \Delta A / \text{minute} \times \text{factor}$$

To assess the nephrotoxicity, serum urea and serum creatinine levels of the treated rats were measured. Urea in serum was measured using a test kit (RAL, Tecnica para el Laboratories, Spain), and urea concentration was calculated as follows.

$\Delta A = [(A1- A2)]$ sample/calibrator

Urea (mg/dl) = (ΔA sample /ΔA calibrator) × concentration of calibrator (mg/dL)

Creatinine concentration (mg/dl) was calculated as

$\Delta A = [(A2 – A1)$ sample or calibrator$] – [(A2 – A1)$ blank$]$

Creatinine (mg/dL) = Δsample / Δcalibrator ×concentration of calibrator (mg/dl)

Absorbance was measured at 520-540 nm (urea) and 500-520 nm (creatinine) using a UV visible spectrophotometer (Labomed, UVD-3000, USA).

2.7 Effect of fenugreek on non-functional immunological parameters

Effects of the administration of fenugreek on blood cell counts were tested using the non-coagulated blood samples collected at the post-bleed. Total and differential white blood cells (WBC), red blood cell counts (RBC) and platelet counts were obtained using a Neubauer's improved Heamocytometer.

2.8 Statistical analysis of data

Data analysis was done by using IBM SPSS version software through the non-parametric, Kruskal Wallis H-test, followed by post-hoc analysis. All results were represented on bar charts with mean and standard errors (mean ± SEM). Means were compared, and considered statistically significant at $p<0.05$ (95% confidence limit).

3 Results

3.1 Qualitative analysis of phytochemicals in fenugreek extracts

Qualitative phytochemical analysis (Table 1) revealed the presence of some major phytochemical classes such as reducing sugars, tannins, glucosides, glycosides, terpenoids, flavonoids and saponins. However, among the phytochemicals detected, glycosides, glucosides and terpenoids were present in relatively low amounts. Reducing sugars, tannins, saponins and flavonoids were found in higher levels as indicated by reaction intensity. The extracts that contained fenugreek seeds soaked in water overnight and for 48 hours showed the presence of the highest number of phytochemicals.

Table 1. Qualitative phytochemical compositions of five different extracts of fenugreek.

Phytochemical	Extract 1 (Seeds soaked in water overnight)	Extract 2 (Seeds soaked in water kept in a shaker for 48 hours)	Extract 3 (powdered seeds in water kept in a shaker for 48 hours)	Extract 4 (powdered seeds in ethanol for 48 hours)	Extract 5 (powdered seeds in methanol for 48 hours)
Reducing sugars	+++++	+++	++++	++	++
Alkaloids	-	-	-	-	-
Glycoside	-	+	-	-	-
Glucoside	-	+	+	-	+
Tannins	+++	++++	++	+	++++
Steroids	-	-	-	-	-
Proteins	-	-	-	-	-
Terpenoids	-	++	-	-	-
Saponins	++	+++	+++	+	++
Flavonoids	+++++	+	+++++	++	++

+ Presence, - Absence of the relevant phytochemical. Repetition of the symbol indicates the intensity of their presence.

Serum glucose levels were significantly lower in the SS group compared to both NC and PC groups (Kruskal Wallis test, SS vs NC: H=9.00, p=0.016; SS vs PC: H=7.639, p=0.034). Although the PS group showed lower mean glucose values than the controls, these differences were not statistically significant (Figure 1a). There was no significant variation in glucose levels between SS and PS.

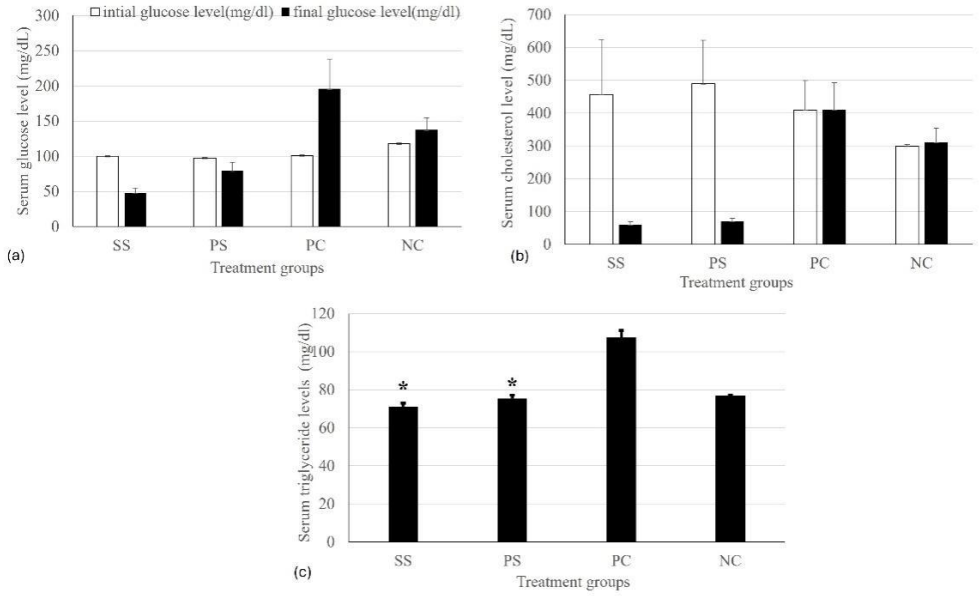

Fig 1. (a) Serum glucose levels, (b) Serum total cholesterol, and (c) Serum Triglyceride levels of hypercholesterolemic rats treated with or without fenugreek seed extract

(Note: SS-Seed soaked in water, PS-powdered seeds soaked water, PC-positive control, NC-negative control, at the pre-bleed and final bleed. Results are given as Mean ± SE (N=6 rats/group) (**significant alteration compared to PC & NC and *significant compared to the PC only; Kruskal-Wallis test, $p<0.05$)).

As represented in Figure 1(b), fenugreek treated SS and PS groups had very low serum cholesterol values (59.75 ± 9.20 and 70.65 ± 9.23 mg/dL, respectively), compared to PC (409.677 ± 82.59 mg/dL) and NC (298.83 ± 5.70 mg/dL) groups. The reduction of the cholesterol levels by SS were significant compared to the controls (Kruskal-Wallis test, SS/NC: H=9.00, p=0.016; SS/PC: H=7.639, p=0.004). However, the levels reported by PS were not significantly different compared with the controls (Kruskal-Wallis test, p>0.05). SS and PS showed no significant variations in their total cholesterol levels.

As shown in Figure 1(c), the fenugreek-treated SS and PS groups showed a marked reduction in triglyceride levels (71.17±1.77 and 75.38±1.79 mg/dL, respectively) compared to the controls (PC: 107.41±3.87 mg/dL and NC:76.81± 0.47 mg/dL). This reduction was significant in both SS and PS compared to the positive control (Kruskal-Wallis test, SS/PC: H=7.639, p=0.034; PS/PC: H=12.00, p=0.003) though not significant compared to the untreated NC (Kruskal-Wallis test, p>0.05). The SS and PS showed no significant variations in their triglyceride levels.

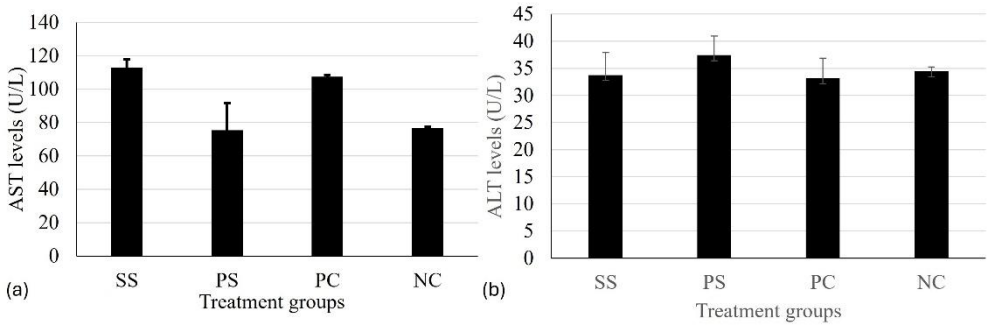

Fig 2. (a) -Serum Aspartate aminotransferase (AST) and (b) - Serum Alanine aminotransferase (ALT) levels of experimental groups of rats.

(Note: SS-Seed soaked in water, PS-powdered seeds soaked water, PC-positive control, NC-negative control) at final bleed. Results are given as Mean ± SE (N=6 rats/group) (Kruskal-Wallis test, p>0.05)).

As denoted in Figure 2 (a), even though the AST levels of SS and PS treated groups varied, they showed no significant variation compared to the controls. Similarly, the variations of ALT levels were not significant when compared with the controls (Kruskal-Wallis test, p>0.05, Figure 2(b)).

Serum urea concentrations remained within the range of 50–60 mg/dL and were not different markedly between the treated and control groups. In contrast, the SS and PS groups exhibited lower creatinine levels (7.52 and 8.10 mg/dL, respectively) compared with their corresponding control groups. A statistically significant difference was observed when the SS group was compared with the NC group (Kruskal–Wallis test, H = 9.00, p = 0.016) and when the PS group was compared with the PC group (Kruskal–Wallis test, H = 12.00, p = 0.003; Figure 3b).

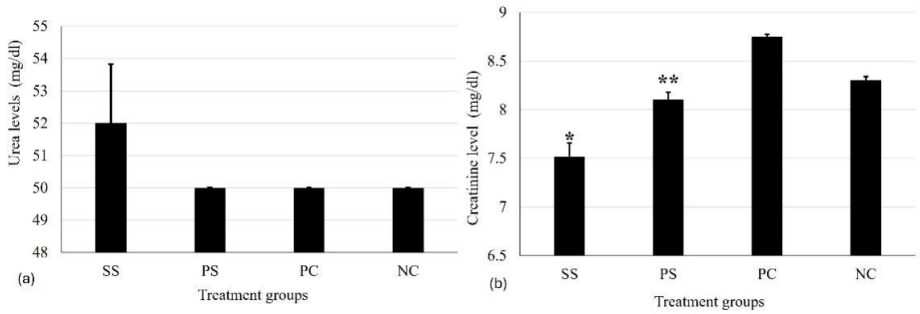

Fig. 3 (a)- Serum urea levels and 3 (b) serum creatinine of experimental groups of rats.

(Note: SS-Seed soaked in water, PS-powdered seeds-soaked water, PC-positive control, NC-negative control) at final bleed. Results are given as Mean ± SE (N=6 rats/group). *Significant compared to NC, **significant compared to PC (Kruskal-Wallis test, $p < 0.05$)).

As shown in Figure 4(a), lymphocyte counts ranged from 68% to 76%, with higher values observed in the fenugreek-treated groups than in the control groups. The mean lymphocyte counts were 75.6 ± 0.93%, 74.0 ± 1.13%, 69.17 ± 0.79%, and 68.25 ± 2.10% for the SS, PS, PC, and NC groups, respectively.

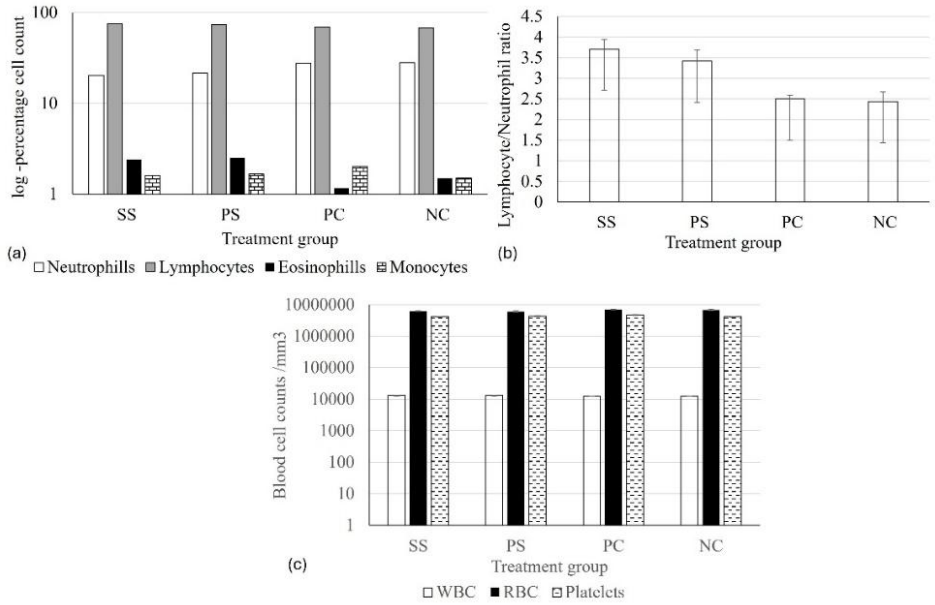

Fig. 4 (a) Differential white blood cell counts, (b) lymphocyte/Neutrophil ratio, and (c) total white blood cell counts, red blood cell counts and platelet counts of experimental groups of rats

(Note: SS-Seed soaked in water, PS-powdered seeds-soaked water, PC-positive control, NC-negative control) at final bleed. Y axis of 4a and 4c were Log10 converted to include a broad range of values. Results are compared as Mean ± SEM (N=6 rats/group) (Kruskal-Wallis test, $p > 0.05$)).

The SS group exhibited a marginally higher lymphocyte count than the NC group (75.6% vs. 68.25%; Kruskal–Wallis test, $p = 0.05$), whereas the difference between the PS and NC groups was not statistically significant (74.0% vs. 68.25%; Kruskal–Wallis test, $p = 0.06$). No significant differences were observed in neutrophil, monocyte, or eosinophil counts among the fenugreek-treated and control groups (Kruskal–Wallis test, $p > 0.05$; Figure 4a). As illustrated in Figure 4(b), the SS group exhibited a significantly higher lymphocyte/neutrophil ratio than the NC group (3.70 vs. 2.43; Kruskal–Wallis test, $p<0.05$), whereas no significant differences were detected among the remaining treatment groups. Moreover, total white blood cell (WBC), red blood cell (RBC) and platelet counts did not differ significantly between the SS and PS groups (Kruskal–Wallis test, $p > 0.05$) (Figure 4(c)).

4 Discussion

The current study assessed the glycemic, lipidemic and immunomodulatory potential of the seeds of fenugreek (*Trigonella foenum-graecum*) using Wistar rats as an *in vivo* model to identify the most efficient method of fenugreek administration out of the two methods adopted; seed-soaked extract and the seed-powder soaked extract. Phytochemical analysis indicated the presence of reducing sugars, tannins, saponins and flavonoids in all fenugreek preparations made with different soaking times and fenugreek form. Studies focused on analyzing the presence of phytochemicals in seeds soaked in water and fenugreek powder in water are scarce.

Several studies conducted with laboratory rat models have demonstrated that saponins present in fenugreek contribute to the hypocholesterolemic effect of the seed by binding bile acids in the intestine, thereby reducing cholesterol absorption and enhancing fecal excretion of bile acids and sterols. These mechanisms lead to reductions in serum cholesterol concentrations, although the effects on triglyceride levels have been inconsistent across studies (Sauvaire et al., 1991, Stark and Madar, 1993). Trigonelline, an alkaloid, is thought to reduce glycosuria in diabetes (Srinivasan 2006). The present study confirmed the presence of saponins in the aqueous fenugreek extracts, which was the antilipidemic agent according to Srinivasan (2006). The extract prepared using seeds-soaked (SS) in water overnight qualitatively demonstrated the highest concentration of saponins among all the extracts. There were no marked differences in the presence of phytochemicals among three aqueous extract types, i.e. fenugreek seeds soaked in water overnight, seeds soaked for 48 hours (in a shaker) and powder soaked for 48 hours (in a shaker). This indicates that the rigorous mixing is not an essential step to enhance phytochemical extraction from fenugreek seed or its powder. Moreover, the results verify that increase in extraction time also does not necessarily increase the number of phytochemicals in the aqueous extract as shown by the relative intensities of phytochemicals in seed-soaked extract from overnight versus 48 hours soaking, suggesting that the traditional method of fenugreek consumption from overnight soaking seems more effective.

As reviewed by Roberts (2011), fenugreek exhibits hypoglycemic activity in both animal and human studies. The review attributes these effects primarily to fenugreek seed preparations, including whole seed powder (Sharma *et al*. 1996), aqueous seed extracts (Vijayakumar *et al*. 2005), and soluble dietary fiber fractions rich in galactomannan (Hannan *et al*. 2007), which have been shown to delay carbohydrate digestion and glucose absorption and to enhance insulin action. Studies directly comparing aqueous extracts obtained from soaked fenugreek seeds (SS) with aqueous preparations of soaked fenugreek seed powder (PS) are scarce. Nevertheless, the reduction in blood glucose observed in the present study is consistent with the hypoglycemic effects reported in previous rat studies using fenugreek seed preparations. For example, Rao *et al*. (1996) demonstrated beneficial metabolic effects following dietary supplementation with whole fenugreek seed powder, whereas Nagamma *et al*. (2019) reported improved biochemical parameters in rats administered fenugreek seed extract. Although the forms of fenugreek used in these studies differ from the soaked seed and soaked seed powder preparations evaluated in the present investigation, the findings collectively support the glucose-lowering potential of fenugreek-derived products. Following the oral administration of the respective fenugreek treatments, both the SS and PS groups reported a decrement in the glucose concentrations when compared with the control groups PC and NC. The present study highlights that consuming the water in which fenugreek seeds were soaked overnight, as practiced traditionally by people in Sri Lanka was more potent than using powdered seeds in terms of anti-glycemic effect.

The results validate the findings of past studies that fenugreek contains a great ability in decreasing serum lipid levels in mammals (Roberts 2011). Further analysis shows that the cholesterol concentration of the NC group, which received its normal diet only (no cholesterol-inducing diet) showed an increase in the levels after a period of 28 days. Previous studies suggest that individuals' diet, age and low physical activity are causative factors for hyperlipidemia (Ma and Shieh 2006) which may explain why the control groups had slightly elevated levels at the final bleed compared to the pre-bleed. Following the administration of seeds-soaked water, there was a marked decrease in the total serum cholesterol level. Overall, the present study proves that the tested fenugreek extract possessed the ability to lower both the serum glucose and cholesterol levels.

Complying with the previous studies, overall observations did not show any signs of toxicity in the treated rat groups by the end of the treatment period. Slightly elevated serum AST levels of the SS group compared to both control groups were still in their normal range for Wistar rats (AST 50-150 U/L). On the other hand, the serum ALT levels of the PS showed a marked rise in the levels when compared with the control groups, without an explanation, thus, further analysis on toxicity effects of the powdered extract is required. Overall, the usage of the seeds-soaked fenugreek extract at the dose used in this study is safe in terms of hepatotoxicity in rats.

Values of creatinine and urea did not differ significantly between fenugreek treated groups and control groups, and these results were consistent with previous reports (Saber and Ezz 2011) particularly with regard to urea levels. Serum urea levels were

nearly similar across all experimental groups, which may indicate that the administered dose of fenugreek was insufficient to exert any effect on nephrotoxicity. However, both the SS and PS treatment groups exhibited slightly lower serum creatinine levels following treatment. Overall, oral administration of fenugreek at the concentration used in this study did not produce any adverse toxicological effects in rats, therefore, fenugreek may be considered safe for further investigation in clinical trials and drug development research.

Previous studies indicated that upon fenugreek treatment in whole fenugreek seed powder, total and differential counts of WBC were unaltered (Rao *et al*. 1996). Significant increase in lymphocyte count of the rats in the SS group compared to the negative control may be due to the presence of some immuno-potentiating substances in these extracts. Moreover, a higher lymphocyte to neutrophil ratio (LNR) in the fenugreek-treated groups may indicate a positive immune modulation relative to the control groups even though the values were insignificant.

Assessment of hematological parameters such as total white blood cell counts, red blood cells and platelets may also be indicators of deleterious effects of plant extracts (Yuet-Ping *et al*. 2013). A previous study conducted on human subjects has reported that chronic inflammation is a key feature of atherosclerosis, while WBC count is a marker of inflammation that is widely available in clinical practice (Kim *et al*. 2017). A high WBC count regardless of the subtype is associated with non-calcified plaque formation as well as significant coronary artery stenosis in asymptomatic individuals. Moreover, WBC counts, especially monocytes, were independent risk factors of cardiovascular diseases (CVDs). Thus, WBC could be a readily available and informative marker for CVDs in asymptomatic individuals (Kim *et al*. 2017). In the present study, total WBC, RBC and platelet counts remained comparable among all groups despite the increased lymphocyte counts observed in the treated rats. Accordingly, further studies are required to validate the observed effects of fenugreek treatment on blood cell counts in rats.

Even though, the present study evaluated total and differential blood cell counts to assess the potential immunomodulatory effects of fenugreek, these hematological parameters alone provide only a partial indication of immune function. Therefore, future studies should involve assessing key pro-inflammatory and anti-inflammatory cytokines in serum, together with other immune biomarkers, to obtain a more comprehensive understanding of the immunomodulatory mechanisms and overall immune status associated with fenugreek supplementation. Such studies would help elucidating the cellular and molecular pathways underlying the observed hematological responses and strengthen the evidence for the immunomodulatory potential of fenugreek.

5 Conclusions

This comparative study validated that the traditional practice of soaking fenugreek seeds overnight in water is more effective than consuming them in other forms such as

powdered seeds soaked in water. The present study revealed a significant potency of the traditional method of fenugreek consumption in Sri Lanka for glycemic and lipidemic control, in a Wistar rat model, with potential implications for extrapolation to human subjects. Further, the qualitative analysis of the prepared fenugreek extracts indicated that the aqueous extract of whole seed fenugreek soaked overnight is rich in most of the tested phytochemicals including reducing sugars, tannins, saponins and flavonoids, yet a quantitative analysis is necessary before recommending its use with a given dosage. The toxicity evaluation in this study indicates that the traditional extract preparation method is safe within the tested conditions and supports its application in future research.

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