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Original article

Evaluation of the acute basic biological effects of herbal formulation to control obesity: a preliminary study

Mukul Kumar,[1,2] Deepika Kaushik,[2] Ravinder Kaushik,[3]* Azhar Khan,[2] Lucia Billeci,[4] Emel Oz,[5]* Charalampos Proestos,[6] Charles Brennan,[7,8] Naushad Ahmad,[9] Tahra Elobeid[10] & Fatih Oz[5]

1 Department of Food Technology and Nutrition, Lovely Professional University, Phagwara Punjab 144411, India
2 Department of Biotechnology, Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan Himachal Pradesh 173229, India
3 University of Petroleum and Energy Stuides-248007, Deharadun, Uttrakhand, India
4 Institute of Clinical Physiology, National Research Council of Italy (CNR-IFC), via Moruzzi 1, 56124 Pisa, Italy
5 Department of Food Engineering, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey
6 Food Chemistry Laboratory, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimipolis Zographou 1577, Athens, Greece
7 School of Science, RMIT University, Melbourne Victoria 3001, Australia
8 Riddet Institute, Palmerston North 4442, New Zealand
9 Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
10 Human Nutrition Department, College of Health Sciences, QU Health, Qatar University, Doha 2713, Qatar

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Summary *Objectives* Obesity is the accumulation of excessive visceral and subcutaneous fat in the body. The herbal formulation can help treat obesity, and our study aims to evaluate the acute basic biological effects of herbal formulation to control obesity.
Methods Antiobesity herbal formulation, prepared by the combination of *Saussurea lappa* root, *Betula utilis* bark, *Aconitum heterophyllum* root, *Bunium periscum* seed, and *Bauhinia variegate* leaf (4:3:1:1:1), was carried out on Wistar male and female rats. Obesity was induced in the rats by feeding the Chow diet, and the effect of herbal formulation on obesity was studied. The effect on obesity was measured by analysing variables like weight, water intake, pellet count, haematological parameters, renal function test, liver function test, and lipid profile test.
Results In the study, it was observed that the female rats treated at the highest dose showed a significantly ($P < 0.005$) higher decrease in weight compared to male rats after 10 days. The food intake, haemoglobin, renal, liver function, and lipid profile showed significant improvement after the intake of herbal formulation.
Conclusions From the above results, it was inferred that herbal formulation increased the activity of metabolism, which is helpful in weight reduction; moreover, it has some beneficial biological effects.

Keywords acute toxicity, haematology parameters, herbal formulation, metabolism, obesity.

Introduction

The evaluation of acute basic biological effects is compulsory for developing a new herbal formulation (Ghosh *et al*., 2019). Acute study aimed at identifying the hazards and risks of any medicine or medicinal formulation (Subha & Geetha, 2017). According to the guidelines of OECD 401, 425, and 423, the use of a drug or formulation without acute toxicity studies and clinical trials is not allowed (Showande *et al*., 2019). In India, 85% of the population still depends upon Ayurvedic medicine, which uses natural herbs extract and formulation prepared in the safest mode to treat several harmful diseases such as obesity, hypertension, diabetes, etc. (Gao *et al*., 2019; Athista *et al*., 2023). Ayurvedic medicine is used to check the drug's effect on the different organs and their side effects in human beings, which could be due to the presence of cell membranes and chemical properties of the toxicants (Meguellati *et al*., 2019). A single dose of a herbal extract showed effective results against the symptoms and absence of toxicity in wound healing (Kpemissi *et al*., 2020). Nowadays, herbal products are more and more used by developing countries as part of their health care system. For example, several plant extracts

*Correspondent: E-mail: ravinder_foodtech2007@rediffmail.com (R.K.); emel.oz@atauni.edu.tr (E.O)

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are used to prepare allopathic and western medicine to treat and cure multiple diseases, *i.e.*, thyroid, diabetes, pain relievers, migraine, and blood pressure medications (Kumar *et al.*, 2020a, 2020b; Kumar *et al.*, 2023).

Obesity accumulates excessive amount of white adipose tissues (WAT) in the body which means imbalance between expenditure and energy storage (Xu *et al.*, 2023). It is also associated with various medical health conditions for every age of human being due to several risk factors such as genetic disorders, a sedentary lifestyle, bad food habits, and lack of physical activity (Romagnolli *et al.*, 2020; WHO. Overweight and Obesity, 2021; Zhang *et al.*, 2023). According to the Indian Council of Medical Research (ICMR), obesity is responsible for several diseases, such as hypertension, heart disease, diabetes, and cancer (McCafferty *et al.*, 2020). Biology, behaviour, and environmental factors stuck the physical activity and diet (Liu *et al.*, 2017). Several herbal medicines are available to treat obesity, such as *Rhizoma coptidis*, *Bergenia ciliate*, *Ren shen*, *Aconitum heterophyllum*, *Radix lithospermi*, *Podophyllum hexandrum*, *Rumex acetosa*, and *Ephedra sinica stapf* which are prepared only from the single herbs (Lim *et al.*, 2016; Kumar *et al.*, 2020a, 2020b). This herbal medicine contains different components such as alkaloids, lignans, saponins, polysaccharides, catechins, epigallocatechin, caffeine, polyphenols, anthocyanins, nobiletin, and different acids (emodin, chrysophanic acid, and amino acids), which help to reduce weight, regulate the metabolism process, reduce the activation of 5-AMP controls lipid synthesis, adipogenic and thermogenesis pathways activated protein kinase alpha (Zou *et al.*, 2015; Panigrahi *et al.*, 2017; Li *et al.*, 2023; Sayed *et al.*, 2023). The findings in Panigrahi *et al.* (Guo *et al.*, 2009) showed that herbal extract helps reduce calorie intake, control the appetite, and boost up. The leaf and root of *Saussurea lappa* helped improve digestion and energy balance due to the presence of huge number of bioactive compounds such as flavonoids, terpenes, alkaloids, and anthraquinones (Kumar & Pundir, 2022), whereas *Aconitum heterophyllum* extract reduced weight due to adrenoreceptor activation (Han *et al.*, 2001; Lemaure *et al.*, 2007). The *Bunium periscum* was responsible for improving the metabolic process through the presence of adiponectin hormones. It also helped in glucose regulation and fatty acid catabolism (Luechtefeld *et al.*, 2016). In another report, it is observed *Bauhinia variegate* contained sitosterol which helps in reducing the level of low-density lipoprotein and cholesterol (Kumar *et al.*, 2022). Therefore, in the present study, the acute basic biological effects of a prepared herbal formulation of selected herb, *i.e.*, *viz Betula utilis*, *Aconitum heterophyllum*, *Bunium periscum*, *Bauhinia variegate* and *Saussurea lappa* extract, was measured and the side effect of these herbs at a high dose in Wistar rats was examined. The herbal formulation was prepared with the combination of different herbs due to presence of huge number of bioactive compounds which show the higher properties in combination as compared with single herb. Therefore, the anti-obesity herbal formulation was developed and found better in comparison to the single herb. Furthermore, this herbal formulation was used to preliminary evaluate the impact on obesity and its related problem through *in vivo* study for long period of time.

Materials and methods

Herbal formulation

The herbal formulation was tuned in a previous study based on *in vitro* analysis parameters such as lipase inhibition, amylase inhibition, and glucose movement from the dialysis membrane to the outer solution and was determined at different time intervals 15, 30, 60, 120, 240, 360, and 480 s. In particular, five herbs were selected: *viz*., *Betula utilis* bark (Bhojpatra), *Aconitum heterophyllum* root (Patish), *Bunium periscum* seed (Kala jeera), *Bauhinia variegate* leaves (Kachnar), and *Saussurea lappa* root (Kushtha or Kuth). Notably, *Saussurea lappa* root shows the maximum antiobesity properties, followed by *Betula utilis* bark, *Aconitum heterophyllum* root, *Bunium periscum* seed, and *Bauhinia variegate* leaves (Lim *et al.*, 2016). These herbs were collected from the Kinnaur region, Himachal Pradesh, India. Ethanol used for the extraction process was procured from Jiangsu Huaxi International (MO, USA). High-speed refrigerated centrifuge (Thermo Scientific, USA), B.D. vacutainer EDTA tubes (Becton, Dickinson and Company, Franklin, UK), microscope (LX-300_LED, Labomed, New Delhi, India), Neubauer's chamber (Marienfeld, New Delhi), Wintrobe's haematocrit tube (Pacific Path Surgi, New Delhi), DiaSys®, Germany kit (Diagnostic System International), creatinine kit, Colorimetrically, urea kit of Excel Diagnostic Pvt Ltd, Hyderabad, autostainer (Model 5020, Leica Biosystems, Wetzlar, Germany) and Homogenisator Potter S (Sartorius AG, Weender Landstrasse, Germany) were the instruments used in the preparation process. All the chemicals were of 'Analytical Reagent' grade. Triple distilled water-washed glassware (class 'A' certified) was used throughout the experiments. Chow diet, high fed diet (Colerangle, 2017), and herbal formulation (powder) were fed to Wistar male and female rats.

Sample

In the present work, twelve male and twelve female Wistar rats were used to check the acute basic biological effects of the selected formulation against obesity, as shown in Fig. 1. Two high doses of herbal

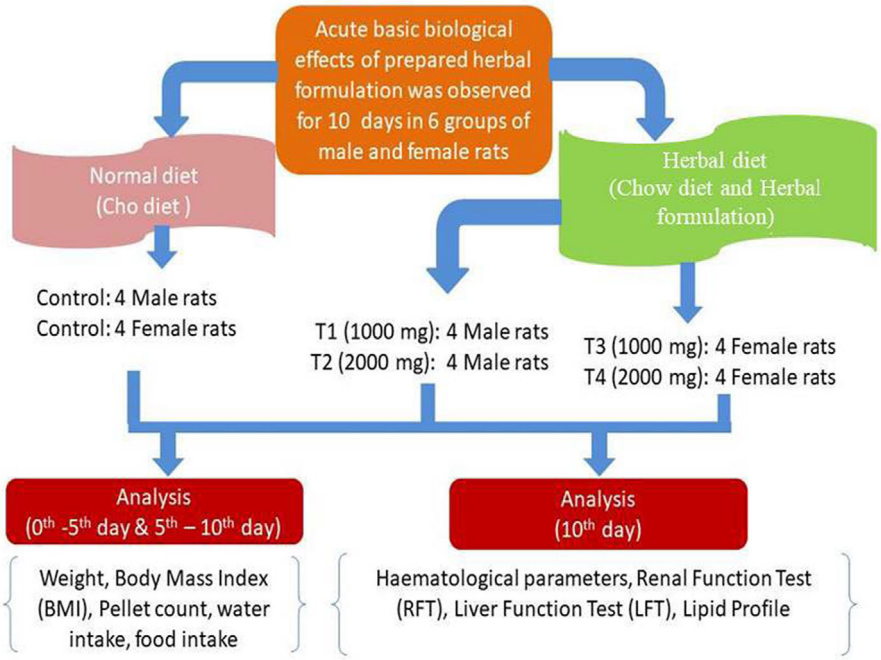

Figure 1 Graphical representation of acute basic biological effects of prepared herbal formulation on male and female Wistar rats.

formulations, 1000 and 2000 mg, were given to Wistar rats for ten days to observe the effect of the prepared herbal formulation according to OECD guidelines 420 and 425 (Subha & Geetha, 2017).

Ethical clearance

The study was approved by the Committee for Control and Supervision of Experiments on Animals (CPCSEA) (Regd. no. 470/01/a/CPCSEA, dt. Dec 5, 2018).

Bodyweight

The weight of rats was measured using a weighing machine (Mettler Toledo, Mumbai, India). The weight was measured on 0-day and after 10 days of herbal administration. The percentage change was determined.

Water intake

Choco nose no-drip water bottle was used to measure the water intake. A measured volume of clean potable water was filled in the morning and consumed the next day.

Pellet weight

The pellet weight of the rat was measured using weighing balance. The pellets were collected daily and weighed. The mean weight after 10 days was considered as pellet weight.

Body mass index

Anthropometrical parameters were measured using body weight (g) and body length (Warden & Fisler, 2008). BMI was calculated weekly as:

$$\mathrm{BMI} = (\mathrm{Bodyweight\ (g)})/(\mathrm{Length\ (cm^2)}).$$

Food intake

A chow diet and a high-fed diet were given to the Wistar rat to provide the energy, help to perform the body function, and increase the body weight. Diet contained vitamin and minerals mix (250 mg), cholesterol (10 mg), d-l-methionine (3 mg), yeast (1 mg), salt (1 mg), and MCD (365 mg). The high-fed diet consisted of everyday supplementation of milk powder (250 mg), ghee (310 mg), and refined brown sugar powder. The intake for a single male rat was 20–25 g per day, while for the female rat, it was 15–20 g per day. The calculated amount of feed was placed in rat cases in the morning, and the amount of feed consumed was calculated after 24 h (Colerangle, 2017).

Acute basic biological effects of prepared herbal formulation

The acute basic biological effects study was done for 10 days on four male and four female Wistar rats at

different ranges of dose (1000 mg or 2000 mg) with the normal food intake to see the effects on haematological parameters, renal functional test (RFT), liver function test (LFT), and lipid profiling (Aniagu *et al.*, 2005).

Collecting of blood serum
Wistar rats were unconscious under chloroform anaesthesia. Then, the blood of rats was taken out from the vein with the help of a capillary after the treatment, as shown in Fig. 1. The serum was separated from the plasma by centrifugation at 3000 rpm for 10 min. Serum and plasma were collected in a non-anticoagulant and EDTA (Ethylene Diamine Tetra Acetic acid) vacuum blood collection tube (Sairam *et al.*, 2014).

Haematological parameters
The haemoglobin, total leucocyte counts (TLC), red blood cell (RBC), mean red blood cell volume (MCV), MCHC (%), platelet count, and blood glucose estimation were carried out using the (Aniagu *et al.*, 2005; Sairam *et al.*, 2014).

Renal function test
The enzymatic method with dry-slide measured serum creatinine and urine was applied (Yıldırım *et al.*, 2018). The sarcosine oxidase, enzymes creatinine amidohydrolase, and creatine amidinohydrolase were used to prepare the reagent layer. Then, hydrogen peroxide was converted by using the peroxidase enzyme. It was detected by dye which is proportional to the creatinine concentration. 20-fold diluted urine before the analysis and undiluted serum were analysed on the slide. Then, samples were immediately frozen at 2–8 °C. Finally, the sample was inserted into a DxC 800 analyser to measure the serum creatinine and urine level (Beckman Coulter, Inc; Brea, CA).

Liver function test
The colorimetric method was used to determine the glutamate oxaloacetate transaminase (GOT, AST), glutamate pyruvate transaminase (GPT, ALT), alkaline phosphatase (ALP), serum proteins, albumin, total bilirubin, bilirubin (direct), bilirubin (indirect), globulin, and A/G ratio (Human Gesellschaft fur Biochemica and Diagnostica MBH, Germany) (Aniagu *et al.*, 2005).

Lipid profile

Measuring of total cholesterol level. DiaSys®, Germany kit (Diagnostic System International, USA), was used to measure the total cholesterol level with the help of the enzymatic colorimetric CHOD-PAP method (Toffaletti & McDonnell, 2008). An amount of 10 µL serum was added to 1000 µL of reagent, then mixed and incubated for 10 min at 20–25 °C. The sample absorption was analysed at 500 nm using a spectrophotometer. The total cholesterol level was measured with the formula:

$$\text{Total cholesterol level} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Total cholesterol level of standard}$$

Low-density lipoprotein (LDL-C) Level. DiaSys®, Germany kit (Diagnostic System International, USA), was used to measure the LDL-C level. 3 µL of blood serum was added to 280 µL reagent 1 and mixed (Toffaletti & McDonnell, 2008). After that, incubation was done at 37 °C for 5 min, and absorbance was measured at 600/700 nm (bichromatic measurement) as A1. After that, 70 µL of reagent 2 was added and mixed properly. Finally, it was incubated at 37 °C for 5 min, and at 600/700 nm, the absorbance was measured as A2. The following formula was then applied:

$$\Delta A = [(A2-A1)\ \text{Sample}]-[(A2-A1)\ \text{blank}]$$

The LDL cholesterol level was calculated as:

$$\text{LDL}-\text{C} = \frac{\Delta A\ \text{sample}}{\Delta A\ \text{calibrator}} \times \text{Conc. Calibrator}$$

High-density lipoprotein cholesterol (HDL-C) Level. The LDL and VLDL methods were used to measure HDL-C using the DiaSys®, Germany kit (Diagnostic System International, USA; Toffaletti & McDonnell, 2008). 500 µL of blood serum was added to 1000 µL of HDL reagent and mixed. After that, incubation was done at room temperature for 10 min. Then, the centrifugation was done at 10,000 rpm for 2 min, and the supernatant was separated from the precipitant. Subsequently, 100 µL of supernatant was added to 1000 µL cholesterol reagent. Then, the mixing was done and incubated at 37 °C for 5 min. Absorbance was measured at 500 nm. The formula used to calculate the HDL-cholesterol level is:

$$\text{HDL}-\text{C} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Complete blood count. The blood samples were also analysed to measure haemoglobin, total leucocyte count, and different leucocyte count using Coulter LH 780 analyser (Beckman Coulter, USA) (Taira *et al.*, 2017).

Table 1 Change in weight and BMI (body mass index) of male and female rats from 0 to 10th day of treatment

Treatment	% change in weight		% change in BMI	
	0–5th day	5–10th day	0–5th days	5–10th days
Control Male	2.53 ± 0.30^{Da}	1.67 ± 0.42^{Bb}	5.05 ± 0.27^{Bb}	5.79 ± 0.43^{Ba}
Control Female	2.09 ± 1.03^{Ca}	2.68 ± 1.33^{Aa}	4.05 ± 0.43^{Ab}	11.20 ± 0.19^{Aa}
T1 Male (1000 mg)	1.38 ± 0.70^{Ba}	2.95 ± 0.70^{Db}	4.19 ± 0.79^{Ca}	6.21 ± 1.67^{Db}
T2 Male (2000 mg)	1.70 ± 0.61^{CDa}	4.45 ± 0.24^{Eb}	5.48 ± 0.81^{CDa}	7.61 ± 1.08^{Db}
T3 Female (1000 mg)	0.44 ± 1.11^{Aa}	1.97 ± 0.39^{Cb}	3.31 ± 0.006^{Cb}	4.48 ± 0.18^{Ca}
T4 Female (2000 mg)	1.02 ± 1.45^{Ba}	2.96 ± 0.12^{Db}	3.64 ± 0.19^{Cb}	4.95 ± 0.201^{Ca}

Data are presented as mean ± SD ($n = 3$).
$^{A–D}$Mean values with uppercase superscripts within a column and $^{a–b}$lowercase superscript for the row are significantly ($P < 0.05$) from each other.

Statistical analysis

Means, standard error mean (SEM), linear regression analysis, and 95% confidence intervals were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA). Data were subjected to a single-way analysis of variance (ANOVA) to calculate the critical difference (CD) value of all the features from the beginning to the end of treatment. A value of $P < 0.05$ was considered significant (Kaushik *et al.*, 2015).

Results

Weight

It was observed that from day 0 to day 10 of the study, T2 male rats administered with a 2000 mg dose showed significant ($P < 0.05$) changes in weight (1.70%–4.45%) as compared with T1 male rats administered with 1000 mg (1.38%–2.95%) as shown in Table 1. Similarly, T4 female rats administered with 2000 mg (1.02%–2.96%) showed a significant ($P < 0.05$) change in weight as compared with T3 female rats administered with 1000 mg dose (0.44%–1.97%) up to the 10th day of treatment as shown in Table 1. In particular, male rats (T1 and T2) showed a significantly ($P < 0.05$) higher percentage change in weight as compared to female rats (T3 and T4) and control rats (male and female) up to the 10th day of the study.

Body mass index (BMI)

T1 (4.19%–6.21%) and T2 (5.48%–7.61%) male rats and T3 (3.31%–4.48%) and T4 (3.64%–4.95%) female rats showed significantly ($P < 0.05$) higher percentage change in BMI as compared to control rats (male and female) up to the 10th day of the treatment. Male rats (T1 and T2) showed non-significantly ($P < 0.05$) differences in BMI as compared to female rats (T3 and T4) up to the 10th day of the treatment (Table 1).

Food intake

The percentage increase in food intake is represented in Table 2. The chow diet provides the 8.2% of protein, 26.62% of carbohydrate, 74% of fat, and 828 Kcal, and calculation was done on the basis of Indian Food Composition Tables, 2017 (Longvah *et al.*, 2017). T1 male

Table 2 Dietary behaviour of male and female rats during observation of acute basic biological effects of prepared herbal formulation

Treatment	% increase in pellet count		% increase in water intake (day)		% increase in food intake (day)	
	0–5th day	5–10th day	0–5th day	5–10th day	0–5th day	5–10th day
Control male	8.97 ± 3.40^{Cb}	7.00 ± 1.42^{Ca}	2.77 ± 0.17^{Aa}	3.43 ± 0.61^{Ba}	11.42 ± 4.71^{Cb}	4.04 ± 2.24^{Ca}
Control female	10.5 ± 3.58^{Db}	8.00 ± 1.38^{Da}	5.94 ± 3.41^{Cb}	2.03 ± 0.80^{Aa}	7.13 ± 2.25^{Ab}	2.44 ± 0.60^{Ba}
T1 male (1000 mg)	5.10 ± 0.23^{Aa}	5.00 ± 0.21^{Aa}	4.19 ± 0.77^{Ba}	2.58 ± 0.75^{Aa}	6.02 ± 1.20^{Aa}	5.10 ± 2.78^{Ca}
T2 male (2000 mg)	7.08 ± 2.31^{Ba}	6.00 ± 1.11^{Ba}	4.66 ± 0.57^{Ba}	3.76 ± 0.84^{Ba}	7.10 ± 1.68^{Aa}	5.44 ± 3.77^{Da}
T3 female (1000 mg)	8.97 ± 3.40^{Cb}	5.45 ± 0.29^{Aa}	7.58 ± 2.04^{Da}	2.27 ± 1.00^{Aa}	8.95 ± 1.98^{Bb}	1.74 ± 0.44^{Aa}
T4 female (2000 mg)	11.2 ± 1.87^{Db}	6.05 ± 1.38^{Ba}	8.02 ± 0.19^{Db}	2.60 ± 0.89^{Aa}	9.27 ± 0.24^{Bb}	2.97 ± 0.93^{Ba}

Data are presented as mean ± SD ($n = 3$).
$^{A–D}$Uppercase superscript with mean value in column and $^{a–b}$lowercase superscript within row are significantly ($P < 0.05$) from each other.

Table 3 Haematological parameters of male and female rats

Treatment	Haemoglobin (gm dL^{-1})	Total Leucocyte count (/cumm)	Red blood cells (RBC; 10^2/L)	Mean red blood cell volume (MCV; fl)	Mean corpuscular haemoglobin (MCH; pg)	MCHC (%)	Platelet count (lacs cmm^{-1})	Blood glucose (random; mg dL^{-1})
Normal diet								
Control male	15.50 ± 0.40^{b}	3925 ± 875^{a}	6.06 ± 0.18^{b}	84.95 ± 0.35^{b}	25.05 ± 0.55^{a}	30.05 ± 0.05^{b}	2.50 ± 0.35^{c}	98.80 ± 9.70^{d}
Control female	15.15 ± 0.15^{b}	4300 ± 35.00^{a}	6.04 ± 0.07^{b}	81.70 ± 3.50^{ab}	24.80 ± 0.20^{a}	30.65 ± 0.65^{b}	2.53 ± 0.18^{c}	99.85 ± 7.15^{d}
Herbal treatment								
T1 male (1000 mg)	14.65 ± 1.65^{b}	3675 ± 475^{a}	5.90 ± 0.62^{b}	83.50 ± 1.10^{ab}	24.50 ± 1.00^{a}	29.65 ± 0.15^{ab}	2.17 ± 0.07^{c}	92.10 ± 12.00^{c}
T2 male (2000 mg)	15.25 ± 0.85^{b}	4600 ± 90.00^{b}	6.30 ± 0.54^{b}	79.40 ± 4.6^{a}	25.25 ± 0.25^{a}	30.55 ± 0.85^{b}	1.35 ± 0.10^{ab}	78.05 ± 4.55^{a}
T3 female (1000 mg)	13.90 ± 0.10^{b}	3200 ± 50.00^{a}	5.50 ± 0.02^{a}	84.50 ± 0.50^{ab}	25.05 ± 0.55^{a}	29.85 ± 0.15^{ab}	1.66 ± 0.08^{b}	81.45 ± 2.15^{b}
T4 female (2000 mg)	11.90 ± 0.40^{a}	5065 ± 85.00^{c}	4.78 ± 0.11^{a}	81.90 ± 0.40^{ab}	25.05 ± 0.55^{a}	28.85 ± 0.85^{a}	1.01 ± 0.06^{a}	77.70 ± 1.20^{a}

Data are presented as mean ± SD ($n = 3$). Mean values with lower superscript (a–d) within column are significantly ($P < 0.05$) from each other.

rats and T4 female rats showed a non-significantly ($P < 0.05$) difference in percentage food intake increase as compared to control rats (male and female) up to the 10th day of the treatment. T2 male rats and T3 female rats showed ($P < 0.05$) a significantly higher percentage change in food intake increase as compared to control rats (male and female) up to the 10th day of the treatment.

Water intake

T1 male rats (4.19%–2.58%), female rats T3 (7.58%–2.27%), T4 (8.02%–2.60%), and control female rats showed a non-significant ($P < 0.05$) percentage change in water intake as compared to T2 male rats (4.66%–3.76%) and control male rats as shown in Table 2.

Pellet count

T1 male rats (5.10%–5.0%) and T2 male rats (7.08%–6.0%) showed a non-significant ($P < 0.05$) percentage change in pellet count as compared to female rats T3 (8.97%–5.45%) and T4 (11.2%–6.05%) up to the 10th day of the treatment as shown in Table 2. Similarly, male rats (T1 and T2) and female rats (T3 and T4) showed a significantly ($P < 0.05$) higher percentage pellet count as compared to control rat's male (8.97%–7.00%) and female rats (10.5%–8.00%) up to the 10th day of the treatment.

Haematological parameters of male and female rats

Haemoglobin

The results of the haemoglobin level are represented in Table 3. Male rats ($P < 0.05$) T1 (14.65 gm dL^{-1}) and T2 (15.25 gm dL^{-1}) and female rats T3 (11.90 gm dL^{-1}) showed a non-significant ($P > 0.05$) difference in haemoglobin level as compared to control male and female rats (15.50 and 15.15 gm dL^{-1}) with a normal diet up to the 10th day of the treatment. However, male rats T1 and T2 (14.65 and 15.25 gm dL^{-1}) and female rat T3 (13.90 gm dL^{-1}) showed a significantly ($P < 0.05$) increase haemoglobin level as compared to female rat T4 (11.90 gm dL^{-1}) up to the 10th day of the treatment.

Total leucocyte count

T1 male rats (3675/cumm) showed a non-significant ($P > 0.05$) difference compared to control male rats (3925/cumm) in total leucocyte count as compared to control male rats, whereas T2 male rats (4600/cumm) showed a significant ($P < 0.05$) increase in total leucocyte count as compared to control male rats (3925/cumm) up to the 10th day of the treatment as shown in Table 3. However, female rats T3 (3200/cumm) showed a non-significant ($P < 0.05$) reduction in total leucocyte count as compared to control female rats (4300/cumm) whereas T4 female rats (5065/cumm) showed a significant ($P < 0.05$) increase on total leucocyte count up to the 10th day of the treatment as shown in Table 3.

Red blood cells

Male rats T1 and T2 (5.90 and 6.30 10^2/L) showed significant ($P < 0.05$) increase in red blood cells as compared to female rats T3 and T4 (5.50 and 4.78 10^2/L), as shown in Table 3, whereas male rats (T1 and T2) showed a non-significant ($P < 0.05$) difference in red blood cells as compared to control male rats (6.06 10^2/L). The female rats T3 and T4 (5.50 and 4.78 10^2/L, respectively) showed significant ($P < 0.05$) decrease in

red blood cells as compared to control female rats (6.04 10^2/L).

Mean red blood cell volume (MCV)
Control male rats (84.95 fl) showed significant ($P < 0.05$) increase in MCV as compared to male rats T1 (83.50 fl) and T2 (79.40 fl), female T3 (84.50 fl) and T4 (81.90 fl), and controlled female rats (81.70 fl) as shown in Table 3. Except for control male rats, all the groups showed non-significant ($P < 0.05$) differences.

Mean corpuscular haemoglobin (MCH)
Results of the mean corpuscular haemoglobin are represented in Table 3. All the male rats T1 and T2 (24.50 and 25.25 pg) and female rats T3 and T4 (25.05 and 25.05 pg) showed a non-significant ($P < 0.05$) difference in mean corpuscular haemoglobin (MCH) level. Mean corpuscular haemoglobin was increased in female rats than in male rats.

Mean corpuscular haemoglobin concentration (MCHC)
The male rats T1 and T2 (29.65% and 30.55%) showed a non-significant ($P < 0.05$) difference in control male rats (30.05%) and control female rats (30.65%) and T3 female rats (29.85%) as shown in Table 3. The T4 female rats (28.85%) showed a significant ($P < 0.05$) reduction in MCHC as compared to control female and male rats, as shown in Table 3. It was observed that males showed no changes in MCHC, whereas in females, MCHC reduced significantly.

Platelet count
In the male and female rats, platelet counts decreased with an increase in herbal formulation amount. In T1 male rats (2.17 lacs cmm^{-1}), platelet count was non-significantly different ($P < 0.05$) compared to control male and female rats. However, T2 rats (1.35 lacs cmm^{-1}) showed a significant difference between T1 male rats and controlled female and male rats, as shown in Table 3. T3 and T4 female rats (1.66 and 1.01 lacs cmm^{-1}) showed a significant ($P < 0.05$) difference between each other and also in comparison to control female and male rats (2.50 and 2.53 lacs cmm^{-1}). A higher decrease in platelet count was observed in female rats in comparison to male rats (Table 3).

Blood glucose
All treated rats (male and female) showed significant ($P < 0.05$) reduction in the blood glucose in comparison to control male and female rats, as shown in Table 3. T1 male rats at (92.10 mg dL^{-1}) and T3 female rats (81.45 mg dL^{-1}) treated with the lower dose of herbal formulation (1000 mg) showed significant ($P < 0.05$) rise in the blood glucose in comparison to T2 male rats and T4 female rats treated with the higher dose (2000 mg; 78.05 and 77.70 mg dL^{-1}).

Table 4 Renal function test of male and female rats

Treatment	Renal function test		
	Blood urea (mg dL^{-1})	Serum creatinine (mg dL^{-1})	Serum uric acid (mg dL^{-1})
Normal diet			
Control male	47.50 ± 2.70[a]	0.73 ± 0.01[a]	1.34 ± 0.02[a]
Control female	55.75 ± 1.15[b]	0.69 ± 0.04[a]	1.96 ± 0.08[b]
Herbal treatment			
T1 male (1000 mg)	65.05 ± 1.45[c]	0.70 ± 0.02[a]	1.95 ± 0.01[b]
T2 male (2000 mg)	48.15 ± 1.05[a]	0.72 ± 0.01[a]	2.08 ± 0.33[c]
T3 female (1000 mg)	66.70 ± 3.96[b]	0.79 ± 0.01[b]	1.62 ± 0.12[a]
T4 female (2000 mg)	43.65 ± 0.65[a]	0.71 ± 0.01[a]	1.66 ± 0.01[a]

Data are presented as mean ± SD ($n = 3$).
[a–c]Mean values within column with lower superscript are significantly ($P < 0.05$) from each other.

Renal function test (RFT) of male and female rats

Blood urea level
In Table 4, it can be observed that a higher dose of herbal formulation showed effective results in the blood urea level. Up to the 10th day of treatment, T1 male rats (65.05 mg dL^{-1}) showed significant ($P < 0.05$) increase in blood urea levels as compared to control male rats (47.50 mg dL^{-1}), whereas T2 male rats (48.15 mg dL^{-1}) showed a non-significant ($P < 0.05$) reduction in blood urea level as compared to control male rats (47.50 mg dL^{-1}). T3 female rats (66.70 mg dL^{-1}) showed non-significant ($P < 0.05$) rise in blood urea levels as compared to control female rats (55.75 mg dL^{-1}), whereas T4 female rats (43.65 mg dL^{-1}) showed significant ($P < 0.05$) decrease in blood urea level as compared to control female rats (55.75 mg dL^{-1}) up to the 10th day of the treatment.

Serum creatinine
All the male rats (T1 and T2) and T4 female rats showed non-significant ($P < 0.05$) differences from each other in terms of serum creatinine content, whereas T3 female rats showed significant ($P < 0.05$) differences in serum creatinine content. However, male rats T1 and T2 (0.70 and 0.72 mg dL^{-1}) showed a non-significant ($P < 0.05$) reduction in serum creatinine content as compared to control male rats (0.73 mg dL^{-1}), whereas T3 female rats showed significant ($P < 0.05$) increase in serum creatinine content as compared to control female rats (0.69 mg dL^{-1}) and T4 female rats (0.71 mg dL^{-1}) showed non-significant ($P < 0.05$) increase in serum creatinine content as

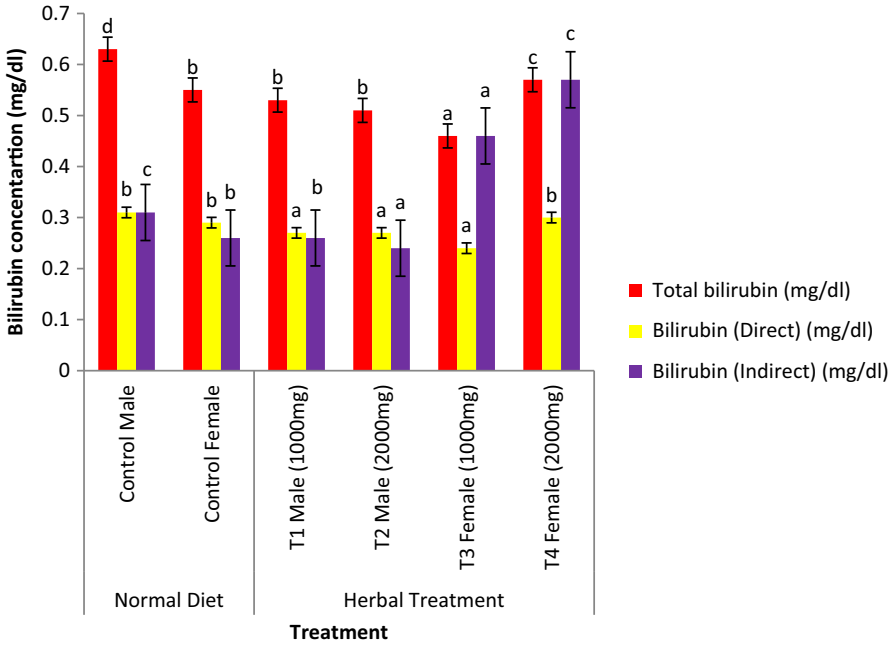

Figure 2 Representation of bilirubin level of male and female rats during acute basic biological effects of prepared herbal formulation. Data are presented as mean ± SD (n = 3). Mean values with lower superscript (a–d) within column are significantly ($P < 0.05$) from each other.

compared to control female rat up to 10th day of the treatment as shown in Table 4.

Serum uric acid

At high dose, 1000 and 2000 mg, herbal formulation showed an effective result of serum uric acid in the control rats (male and female), male rats (T1 and T2), and female rats (T3 and T4). T1 and T2 male rats (1.95 and 2.08 mg dL^{-1}) showed significant ($P < 0.05$) change in serum uric acid levels as compared to control male rats (1.34 mg dL^{-1}), whereas T3 and T4 female rats (1.62 and 1.66 mg dL^{-1}) showed a significant ($P < 0.05$) minimum change in serum uric acid levels as compared to control female rats (1.96 mg dL^{-1}) up to the 10th day of treatment as shown in Table 4.

Liver function test of male and female rats

Total bilirubin

T1 and T2 male rats (0.53 and 0.51 mg dL^{-1}) showed a non-significant ($P < 0.05$) reduction in total bilirubin level as compared to control male rats (0.63 mg dL^{-1}), whereas female rats T3 (0.46 mg dL^{-1}) showed significant ($P < 0.05$) decrease in total bilirubin level as compared to control female rats (0.55 mg dL^{-1}). T4 female rats (0.57 mg dL^{-1}) showed significant ($P < 0.05$) increase in total bilirubin levels as compared to control female rats (0.55 mg dL^{-1}), as shown in Fig. 2.

Direct bilirubin level

Results of the direct bilirubin level are represented in Fig. 2. All the rats (T1, T2, and T3) showed non-significant ($P < 0.05$) differences between each other in terms of direct bilirubin level up to the 10th day of treatment. However, T1 and T2 male rats (0.27 and 0.27 mg dL^{-1}) showed significant ($P < 0.05$) minimum change in direct bilirubin level as compared with control male rats (0.31 mg dL^{-1}). T3 female rats (0.24 mg dL^{-1}) showed a significant ($P < 0.05$) minimum change in direct bilirubin level as compared to control female rats (0.295 mg dL^{-1}). T4 female rats (0.30 mg dL^{-1}) showed non-significantly ($P < 0.05$) rise direct bilirubin level as compared to control female rats (0.30 mg dL^{-1}).

Indirect bilirubin level

T1 and T2 male rats (0.26 and 0.24 mg dL^{-1}) showed significant ($P < 0.05$) reduction in indirect bilirubin level as compared to control male rats (0.31 mg dL^{-1}), whereas female rats T3 and T4 (0.46 and 0.57 mg dL^{-1}) showed non-significant ($P < 0.05$) increase in indirect bilirubin level as compared to control female rats (0.26 mg dL^{-1}) as shown in Fig. 2.

Serum glutamic oxaloacetic transaminase (SGOT)

T2 male rats (119.10 U L^{-1}) showed significantly ($P < 0.05$) higher SGOT level as compared to control male rats (103.65 U L^{-1}), while T1 male rats (106.35 U L^{-1}) showed non-significantly ($P < 0.05$)

Table 5 Liver function test of male and female rats

Treatment	SGOT (U L^{-1})	SGPT (U L^{-1})	Serum alkaline phosphatase (U L^{-1})	Serum protein (gms%)	Albumin (gms%)	Globulin (gms%)
Normal diet						
Control male	103.65 ± 8.15[a]	33.30 ± 3.60[a]	264.15 ± 42.50[b]	8.35 ± 0.50[c]	3.85 ± 0.10[a]	4.50 ± 0.40[b]
Control female	87.65 ± 15.25[a]	39.00 ± 3.70[a]	276.70 ± 34.20[b]	8.65 ± 0.20[c]	4.30 ± 0.30[b]	4.35 ± 0.20[b]
Herbal treatment						
T1 male (1000 mg)	106.35 ± 1.15[a]	35.45 ± 0.10[a]	514.75 ± 44.40[d]	8.45 ± 0.10[c]	4.35 ± 0.20[b]	4.10 ± 0.20[b]
T2 male (2000 mg)	119.10 ± 10.40[b]	48.00 ± 3.20[b]	381.30 ± 28.90[c]	7.75 ± 0.20[a]	4.45 ± 0.40[b]	3.30 ± 0.50[a]
T3 female (1000 mg)	88.40 ± 3.60[a]	32.60 ± 5.30[a]	237.40 ± 23.60[a]	9.40 ± 0.20[d]	4.65 ± 0.20[b]	4.75 ± 0.30[b]
T4 female (2000 mg)	91.95 ± 1.85[a]	35.00 ± 0.20[a]	117.90 ± 0.40[a]	8.05 ± 0.10[b]	3.65 ± 0.20[a]	4.15 ± 0.05[b]

Data are presented as mean ± SD ($n = 3$).
[a–d]Means with the lower superscript within column are significantly ($P < 0.05$) from each other.

change in SGOT level as compared to control male rats. The female rats T3 and T4 (88.40 and 91.95 U L^{-1}) showed a non-significantly ($P < 0.05$) change in SGOT level as compared to control female rats (87.65 U L^{-1}), as shown in Table 5.

Serum glutamic pyruvic transaminase (SGPT)
The present study reported that male rats treated with herbal extract showed a high SGOT level compared to female rats. T1 male rats (35.45 U L^{-1}) showed non-significant ($P < 0.05$) SGPT as compared to control male rats (33.30 U L^{-1}), whereas T2 male rats (48.00 U L^{-1}) showed non-significant ($P < 0.05$) higher difference in SGPT as compared with control male rats as shown in Table 5. T3 and T4 female rats (32.60 and 35.00 U L^{-1}) showed a non-significant ($P < 0.05$) lower SGPT level as compared to control female rats (39.00 U L^{-1}), as shown in Table 5.

Serum alkaline phosphatase level
All the herbal treatment rats (male T1, T2, and female T3, T4) showed significant ($P < 0.05$) differences from each other in terms of serum alkaline phosphatase level. However, male rats T1 and T2 (514.75 and 381.30 U L^{-1}) showed significant ($P < 0.05$) change in serum alkaline phosphatase level as compared to control male rats (264.15 U L^{-1}), whereas T3 and T4 female rats (237.40 and 117.90 U L^{-1}) showed significant ($P < 0.05$) lower change in serum alkaline phosphatase level as compared with female control rats (276.70 U L^{-1}) as shown in Table 5.

Serum protein content
T1 male rats (8.45 gms%) showed a non-significantly ($P < 0.05$) change in serum protein content as compared to control male rats (8.35 gms%), whereas T2 male rats (7.75 gms%) showed a significant ($P < 0.05$) change in serum protein content as compared with control male rats (8.35 gms%). Similarly, female rats T3 (9.40 gms%) showed significant ($P < 0.05$) higher serum protein content as compared to control female rats (8.65 gms%). In comparison, T4 female rats (8.05 gms%) showed significantly ($P < 0.05$) change in serum protein content as compared to control female rats (8.65 gms%). The present study reported that the female rats treated with herbal extract showed higher serum protein levels than male rats.

Albumin level
Male rats (T1 and T2) and female rats T3 showed non-significant ($P < 0.05$) differences between each other in terms of albumin level. In T1 and T2, male rats (4.34 and 4.45 gms%) observed a significant ($P < 0.05$) change in albumin levels as compared to control male rats (3.85 gms%). T3 female rats (4.65 gms%) showed non-significant ($P > 0.05$) differences in albumin level as compared to control female rats (4.30 gms%), whereas T4 female rats (3.65 gms%) also showed significant ($P < 0.05$) change in albumin level as compared to control female rats (4.30 gms%) as shown in Table 5.

Globulin level
All the rats (control male and female, male T1 and female T3 and T4) showed non-significant ($P < 0.05$) difference between each other in terms of globulin level, whereas T2 male rat (3.30 gms%) showed a significant ($P < 0.05$) change in globulin level as compared with control male rats (4.50 gms%). However, T1 male rats and T4 female rats (4.10 and 4.15 gms%) showed non-significant ($P < 0.05$) change in globulin levels as compared to control male and female rats (4.50 and 4.35 gms%). In T3 female rats (4.75 gms%), it was observed non-significant ($P < 0.05$) change in globulin level as compared to control female rats (4.35 gms%).

Lipid profile of male and female rats during toxicity

Serum cholesterol
T1 male rats (75.60 mg dL^{-1}) showed a non-significantly ($P < 0.05$) increase in serum cholesterol

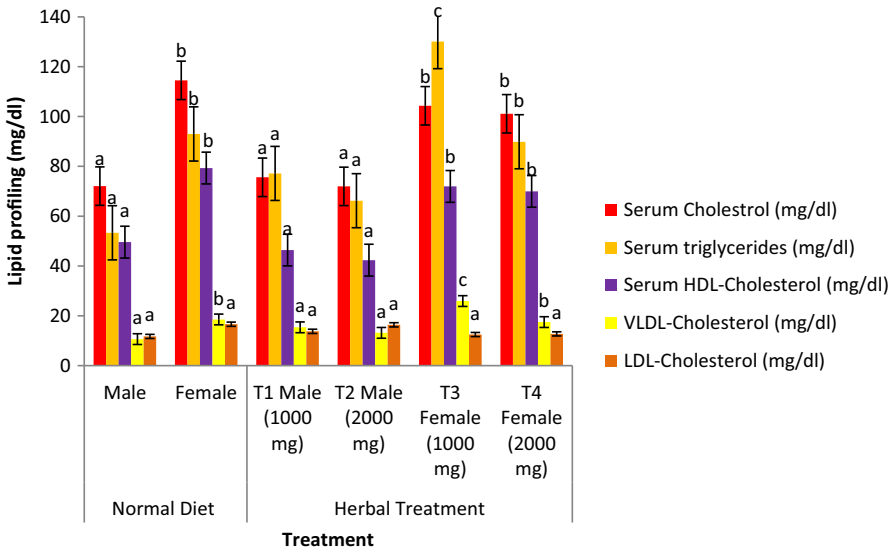

Figure 3 Lipid profiling of male and female rats during acute basic biological effects of prepared herbal formulation. Data are presented as mean ± SD ($n = 3$). [a–c]Mean values with lower superscript within column are significantly ($P < 0.05$) from each other.

level as compared to control male rats (72.05 mg dL^{-1}), whereas T2 male rats (71.95 mg dL^{-1}) showed non-significant ($P < 0.05$) change in serum cholesterol as compared to control male rats (72.05 mg dL^{-1}) as shown in Fig. 3. Similarly, T3 and T4 female rats (104.30 and 101.10 mg dL^{-1}) showed non-significant ($P < 0.05$) differences in serum cholesterol levels as compared to control female rats (115.50 mg dL^{-1}), as shown in Fig. 3.

Serum triglycerides

Results of the serum triglycerides are represented in Fig. 3. Male T1 and T2 rats (77.15 and 66.20 mg dL^{-1}) showed non-significantly ($P < 0.05$) increase in serum triglycerides level as compared to control male rats (53.33 mg dL^{-1}), whereas T3 female rats (130.05 mg dL^{-1}) showed significantly ($P < 0.05$) change in serum triglycerides level as compared to control female rats (93.00 mg dL^{-1}). T4 female rats (89.85 mg dL^{-1}) showed non-significant ($P < 0.05$) decrease in serum triglycerides level as compared to control female rats.

Serum high-density lipoprotein (HDL) cholesterol

Results of the serum HDL cholesterol are represented in Fig. 3. T1 and T2 male rats (46.40 and 42.35 mg dL^{-1}) showed a non-significant ($P < 0.05$) change in serum HDL cholesterol level as compared to control male rats (49.60 mg dL^{-1}), whereas T3 and T4 female rats (71.95 and 65.95 mg dL^{-1}) showed non-significant ($P < 0.05$) decrease in serum HDL cholesterol level as compared to control female rats (93.00 mg dL^{-1}).

Very low-density Lipoprotein (VLDL) cholesterol

T1 and T2 male rats (15.40 and 13.20 mg dL^{-1}) showed non-significant ($P < 0.05$) increase in VLDL cholesterol level as compared to control male rats (10.65 mg dL^{-1}), whereas T3 female rats (71.95 mg dL^{-1}) showed significant ($P < 0.05$) increase in VLDL cholesterol level as compared to control female rats (18.55 mg dL^{-1}). In comparison, T4 female rats (69.95 mg dL^{-1}) showed a non-significant ($P < 0.05$) change in VLDL cholesterol level as compared to control female rats, as shown in Fig. 3.

Low-density lipoprotein (LDL) cholesterol

Male rats T1 and T2 (13.80 and 16.40 mg dL^{-1}) showed non-significant ($P < 0.05$) increase in LDL cholesterol level as compared to control male rats (11.75 mg dL^{-1}), whereas female rats T3 and T4 (12.50 and 12.75 mg dL^{-1}) also showed non-significant ($P < 0.05$) lower change in LDL cholesterol level as compared to control female rats (16.65 mg dL^{-1}) as shown in Fig. 3.

Cholesterol (CHL)/high-density lipoprotein (HDL) ratio

All the rats (control male and female, male T1 and female T3 and T4) showed a non-significant ($P < 0.05$) difference between each other in terms of CHL/HDL ratio. In contrast, male rats' T2 (1.70) showed a significant ($P < 0.05$) increase in the CHL/HDL ratio as compared to control male rats (1.46), as shown in Table 6. However, T1 male rats (1.65) showed a non-significant ($P < 0.05$) change in CHL/HDL ratio as compared to control male rats (1.46),

and T3 and T4 female rats (1.45 and 1.43) showed non-significant ($P < 0.05$) difference in CHL/HDL ratio as compared to control female rats (1.44), as shown in Table 6. In the present study, female rats showed higher CHL/HDL than male rats.

Low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ratio

T1 and T2 male rats (0.31 and 0.39) showed a significantly ($P < 0.05$) increase in LDL/HDL ratio as compared to control male rats (0.24), whereas T3 and T4 female rats (0.09 and 0.17) showed non-significant ($P < 0.05$) lower change in LDL/HDL ratio as compared to control female rats (0.20) as shown in Table 6. In the present study, female rats showed higher LDL/HDL than male rats.

Discussion

In developing and non-developing countries, the demand for medicinal plants or herbal products is frequently increasing to cure several life-threatening diseases. Humans worldwide believe herbal approaches are non-toxic and with zero side effects compared with allopathy and homeopathy medicines. However, in medicinal herbs, the toxicological and pharmacological properties should be evaluated to check the herb's effectiveness against severe diseases and safe consumption for patients (Musa *et al*., 2022). Literature suggests that herbal formulations can be beneficial in obesity treatment which is too common issue nowadays. Hence, the present study evaluated the combination of different herbs such as *Betula utilis* bark, *Aconitum heterophyllum* root, *Bunium periscum* seed, *Bauhinia variegate* leaves and *Saussurea lappa* root that are effective in the treatment of obesity. In particular, the acute basic biological effects were evaluated at two high doses, 1000 and 2000 mg. In this study, various parameters are investigated such as haematological parameters, renal function parameters, liver function parameters, and lipid profiling. Along with this other parameters were also measured in the animals such as food intake, body weight, pellet count, water intake, and clinical signs.

Table 6 CHL and LDL ratio of male and female rats

CHL/LDL ratio		
Treatment	CHL/HDL ratio	LDL/HDL ratio
Normal diet		
Male	1.46 ± 0.06[a]	0.24 ± 0.07[a]
Female	1.44 ± 0.01[a]	0.20 ± 0.07[a]
Herbal treatment		
T1 male (1000 mg)	1.65 ± 0.17[a]	0.31 ± 0.13[b]
T2 male (2000 mg)	1.70 ± 0.13[b]	0.39 ± 0.12[b]
T3 female (1000 mg)	1.45 ± 0.04[a]	0.09 ± 0.07[a]
T4 female (2000 mg)	1.43 ± 0.01[a]	0.17 ± 0.005[a]

Data are presented as mean ± SD ($n = 3$).
[a–b]Mean values with the lower superscript within row are significantly ($P < 0.05$) from each other.

In this research, it is investigated that at maximum dose (2000 mg) of prepared herbal formulation no deaths were observed in treated rats. Throughout the study, the body weight, food intake, pellet count, and water intake were not altered in the experimental group of male and female rats compared to control male and female rats. A significant ($P < 0.005$) higher weight changes were observed in female rats as compared with male rats. Similarly, the food and water intake were significantly ($P < 0.005$) reduced in every animal group at higher doses, which helps to maintain the body's energy level, calories, and control appetite due to high soluble fibre components. It also helps to maintain the regulation of blood sugar level and storage of carbs (Lim *et al*., 2016). The findings of this study are in line with previous research. An increased metabolic process previously measured by adiponectin hormones and an improvement in glucose regulation and fatty acid catabolism (Subha & Geetha, 2017). Bendtsen *et al*. (2013) reported the pellet count of rats was significantly ($P < 0.005$) increased with a high-fed diet, which was responsible for the production of insoluble calcium fatty acid and affected the metabolism process. Polydipsia problems are caused in rats due to the intake of a NaCl-rich diet, which is also responsible for increased water intake (Stricker & Hoffmann, 2007). In a previous study, it was reported that the food intake was increased due to a decrease in adiponectin level (Chien *et al*., 2016). In particular, *Aconitum heterophyllum* contains the protease protein inhibitor, which helps control hunger and maintain energy balance (Saleem *et al*., 2013; Olubobokun *et al*., 2014). It has also been reported that *Bauhinia variegate* determines a significant ($P < 0.005$) change in food intake due to improved transportation of a neurotransmitter (serotonin) in the hypothalamus and thus helping in controlling food-seeking behaviour (Balamurugan & Muralidharan, 2010). In another study, it was observed that the anti-oxidant compounds in *Ipomoea batatas* extract helped to decrease food intake by regulating the activity of 5' AMP-activated protein kinase (Kalra & Unnikrishnan, 2012). In the present study, it was observed that hyperglycaemia-induced muscle wastage was prevented through polyherbal formulation, which also significantly improves the body weight (Petchi *et al*., 2014).

In the current study, haematological parameters such as haemoglobin, total leucocyte count, red blood cells, mean red blood cell volume, mean corpuscular haemoglobin, MCHC, platelet count, and blood sugar were studied, and it was observed that female rats

showed more effective improvement as compared with male rats. The haemoglobin level was significantly ($P < 0.05$) increased in male rats as compared to female rats, as already reported in controls (14.5 gm dL^{-1} males and 13.08 gm dL^{-1} females; Ashour *et al*., 2007). On the other hand, it was reported that during the herbal treatment in male rats that the haemoglobin level was 14.5 gm dL^{-1} (Ojezele *et al*., 2017). Farooq *et al*. (2015) showed that mistletoe extract helped reduce haemoglobin level in rats. The low level of haemoglobin in female rats might be due to the deficiency of iron content. Even though abundant amount of tannin content present in herbal formulation also reduce the level of iron (Pandeya *et al*., 2021). Another specific mechanism to degrade the level of haemoglobin was H_2O_2 which was responsible for the inactivation of enzyme by contacting with iron (Ndefo *et al*., 2021). In the present study, it was observed the total leucocyte count was significantly ($P < 0.05$) increased in female rats as compared to male rats while fed the higher doses (2000 mg). This is in line with a previous study reporting that the total leucocyte count was 7000/cumm in treated male rats, whereas it was significantly elevated in female rats ($P < 0.05$; 5570/cumm; WHO. Overweight and Obesity, 2021). Similarly, in the present study, the RBC level was significantly increased in male and female rats. It has been reported that the RBC level was significantly ($P < 0.05$) increased in male rats (7.6 10^2/L), whereas female rats showed 6.5 10^2/L RBC content. In this study, female rats showed a higher mean red blood cell volume than male rats. Several supporting data were available (Ashour *et al*., 2007). For example, Mugahi *et al*. (2003) reported that the mean red blood cell volume level in male and female rats was 50.2 and 56.3 fl, respectively. The specific reason behind the reduction of red blood cell in male rats due to the disturbances occurs in osmoregulatory system of the blood cells. However, oxidative injury occurs on the cell membrane and blood cell lysis also decreases the level of red blood cell volume (Mohammed *et al*., 2021). Similarly, in present study it is observed female rats showed significantly ($P < 0.05$) reduction in the level of MCH and MCHC due to the deficiency of iron content which is known as microcytic (Gajbhiye *et al*., 2021). In another study, it is reported the level of MCH in male rats (17.9 pg) was significantly ($P < 0.05$) increased as compared with female rats (18.1 pg) in another study (Imafidon & Okunrobo, 2012). MCHC levels in female rats (32.20%) were significantly elevated ($P < 0.05$) as compared with male rats (34.5%) (Ashour *et al*., 2007). The blood sugar level was increased in control male and female rats in the present research compared to treated animals of both sexes. It has been reported that the male rats (4.59 mg dL^{-1}) showed a significant ($P < 0.05$) decrease in blood sugar level as compared with female rats (5.05 mg dL^{-1}; Brøns *et al*., 2009). The finding of Chinnappan *et al*. (2019) reported that female rats (41.7 mg dL^{-1}) showed significantly elevated blood sugar level ($P < 0.05$) in blood urea as compared to male rats (42.8 mg dL^{-1}). The prepared herbal formulation helps in lowering down the fasting blood sugar, glycated haemoglobin, and postprandial blood sugar (Suvarna *et al*., 2021). It also shows the hypoglycaemic activity which trigger by the pancreatic secretion of insulin from beta-cell (Sharma & Goyal, 2017). Polyherbal formulations contains several bioactive compounds such as phenolic, tannin, saponins, flavonoids, and steroids compounds which might be targeting the bone marrow cells that responsible for higher red blood cell, platelet count, haemoglobin, mean red blood cell volume, and mean corpuscular haemoglobin due to lower content of oxygen in body tissues (Sheth *et al*., 2021). Also, there is formation of erythropoietin hormones through kidneys which stimulate bone marrow to produce higher amount of red blood cells. It also helps to transport the glucose in peripheral tissue and reduce the level of glucose in blood due to increase in plasma insulin levels (Petchi *et al*., 2014). Polyphenols and flavonoids are triggering the level of neutrophils, whereas alkaloids increase the immunobioactivities leading to enhancement of antibody circulation (Yahaya *et al*., 2018). Even though polyherbal formulation target pathway such as enhance the glucose uptake through preventing hepatic glucose production and reduce the absorption of carbohydrates (Zarvandi *et al*., 2017).

In the present research, renal function tests such as blood urea level, serum creatine level, and serum uric acid level were measured in the treated group of both sex animals. In the treated groups, it was observed that the serum creatinine levels were significantly ($P < 0.05$) decreased in male rats as compared to female rats. Results are in line with those of those who reported that male rats (0.70 mg dL^{-1}) showed significant ($P < 0.05$) decrease in serum creatinine level as compared to (0.90 mg dL^{-1}) female rats (Chinnappan *et al*., 2019). Moreover, in the present work, it was observed that treated male rats showed a significant ($P < 0.05$) increase in serum uric acid level compared to female rats. It has been suggested that the *Biota orientalis* extract contains high amounts of quercetin and rutin, which help to reduce the serum uric acid levels in male and female rats (Zhu *et al*., 2004). The elevation occurs in the creatinine and urea level due to the hepatoprotective which is the specific mechanism of individual herb present in the herbal formulation. However, it is considered a major marker for the renal dysfunction and liver (Raj & Reddy, 2015). Polyherbal formulations responsible to reduce the level of creatinine while improving the filtration capacity of kidneys, also help in reformation of kidney cells, and protecting of kidney internal structure (Chinnappan *et al*., 2019). It also reduces the reactive oxygen species and

oxidative damage in both kidney and liver cells. However, bioactive compounds of polyherbal formulation were ameliorating the endoplasmic reticulum stabilisation process which led to increase protein synthesis and also showed the anti-hyperlipidaemic effect (Ahmad *et al*., 2020). Compounds present in polyherbal formulation help in blocking of interaction of oxidative stress, fibrosis pathways, and inflammatory pathways to exhibit the proper nephroprotective activity (Reddy *et al*., 2019).

The total bilirubin level (direct and indirect), SGOT, SGPT, serum alkaline phosphatase levels, albumin, and globulin were evaluated in liver function tests. The male rats showed significantly elevated ($P < 0.05$) total bilirubin level (direct and indirect) as compared to female rats. It has been reported that Wistar female rats (0.58 and 0.55 mg dL^{-1}) showed significant ($P < 0.05$) decrease in total bilirubin level (direct and indirect) as compared to male rats (2 and 0.59 mg dL^{-1}) (Panahandeh *et al*., 2017). In the present study, it has been observed that herbal extract at a higher dose (2000 mg) elevated the level of SGOT in female rats compared to male rats. In a previous study, it was reported that the intake of *Amaranthus paniculatus* extract was responsible for reducing the level of SGOT (73 U L^{-1}) and SGPT (65.7 U L^{-1}) in the treated female group (Nawale *et al*., 2017). Similarly, it has been shown that no treated female Wistar rats (31.2 U L^{-1}) showed a significant ($P < 0.05$) decrease in SGOT level as compared to male rats (40.90 U L^{-1}) (Ghule *et al*., 2009). However, with oralist intake, the level of SGOT was reduced in male rats (34.01 U L^{-1}) compared to female rats (36.10 U L^{-1}). The current study also observed that the herbal extract determined significantly elevated ($P < 0.05$) serum alkaline phosphatase levels in female rats compared to male rats. It has previously been observed that aqueous extract of *Psidium guajava* leaves determined a significant ($P < 0.05$) increase in albumin and globulin level in females as compared to male rats (Uboh *et al*., 2010). The *Phyllanthus niruri* extract showed a significant ($P < 0.005$) increase in reduction in albumin level in male rats at 3.07 gms%. Similarly, *Hippobromus pauciflorus* extract at two different doses, 100 and 200 mg, showed a significant ($P < 0.05$) decrease in globulin level in female rats at 51.80 mg kg^{-1} as compared to male rats at 54.40 mg kg^{-1} (Pendota *et al*., 2010). In liver function test total bilirubin level (direct and indirect), SGOT, SGPT, serum alkaline phosphatase levels, albumin, and globulin are elevated due to the hepatoprotective mechanism of individual herbs of prepared herbal formulation (Raj & Reddy, 2015). The decrease in serum alkaline phosphatase levels is responsible for the reduction in the protein synthesis level. Therefore, the level of globulin, albumin, and the plasma protein also decrease due to the presence of huge amount of bioactive compounds present in individual herbs. The adverse effect was observed on the albumin secretion due to increase in protein catabolism in treated animals (Murugan & Pari, 2007). Similarly, the serum enzyme activity also crucial for the functioning of body if there was changes occur that is responsible for the liver and kidney damage. Even though the activity of the body increased due to metabolic and circulatory alterations occur when the tissues are damaged (Ma, 2004). Polyherbal formulation contains the andrographolide in steam and leaves which show the anti-hepatotoxic activity and also reduces the level of CCL4-induced hepatic enzymes (Khan *et al*., 2015). Flavone and flavonoid concentrates present in polyherbal formulation give hepatoprotective property which maintain the level of serum glutamate pyruvate transaminase, alkaline phosphatase, serum glutamate oxaloacetate transaminase, and direct and indirect bilirubin (Gupta *et al*., 2013).

Regarding the lipid profiling, the results showed that the male rats presented significantly elevated ($P < 0.05$) serum cholesterol, serum triglycerides, serum HDL cholesterol, and VLDL cholesterol level as compared to female rats. Results are well in accordance with different findings suggesting that male rats showed significant ($P < 0.05$) increase in serum cholesterol level (15.83 mg dL^{-1}), serum triglycerides level (82.70 mg dL^{-1}), serum HDL cholesterol level (50.28 mg dL^{-1}), and serum HDL cholesterol level (16.54 mg dL^{-1}) as compared to female rats (118.30, 78.15, 58.20, 43.33, and 11.64 mg dL^{-1} respectively) by intake of herbal extract such as *Phyllantus niruri*, *Achyranthes aspera* Linn, *Platycodon grandiflorum*, and *Panax ginseng* (Drexel, 2006; Barter *et al*., 2007; Ihedioha *et al*., 2013; Singh *et al*., 2016). Igbokwe *et al*. (2009) also reported higher serum HDL cholesterol in male rats (46.35 mg dL^{-1}) as compared to female rats (45.20 mg dL^{-1}) while decreasing the level of hepatic content. The lipid level was reduced in treated animal due to presence of alkaloids, glycoside, and flavonoids components in prepared herbal formulation (Kumar *et al*., 2023). The serum cholesterol level was decreased which directly triggers the activity of lecithin-cholesterol acyl transferase due to the presence of saponin in polyherbal formulation (Bharathi *et al*., 2011). The serum cholesterol level was reduced due to the increase in activity of lipoprotein lipase and presence of cholesterol-7-hydroxylase, hepatic 3-methyglutaryl-COA reductase, and fatty acid synthetase (Singh *et al*., 2016; Bharathi *et al*., 2011). Polyherbal formulation shows the hypolipidemic properties and antioxidant properties which decreases the low-density lipoprotein, total cholesterol, very low-density lipoprotein, and increase the high-density lipoproteins (Parasuraman *et al*., 2010).

Limitations of this study are the small number of animals, the short duration of the treatment, and the absence of a histological evaluation. Notably, in this preliminary study, the herbal doses and the duration of treatment were selected according to the OECD guidelines to observe the potential effect against obesity of the prepared herbal formulation. The selected doses were fixed for the non-toxic substances where limit tests occur to check the upper dose level and their effect on the body function (Botham, 2002). The present study's data are considered preliminary for further analysis, including histopathological assessment and the evaluation of other physiological parameters like cardiovascular changes. In particular, an investigation involving 12 weeks of treatment with the prepared herbal formulation is ongoing.

Conclusions

To treat obesity, the acute basic biological effects of the herbal formulation were analysed for 10 days at two high doses, 1000 and 2000 mg, respectively. In study, it is concluded that significant ($P < 0.005$) weight reduction from 1.97% to 4.45% was observed within 10 days. A significant ($P < 0.005$) higher weight reduction was observed in female rats due to the suppression of appetite as compared with male rats. It was also observed in research that the metabolism process of rats was improved, and consequently, several metabolic parameters such as blood urea and uric acid were increased. In this study, it was observed that prepared herbal formulation helps in the breakdown of fat by activating macrophages which reduce adiposities and prevents cholesterol deposition in the vascular system. Therefore, the cholesterol level was increased in blood and serum. In herbal formulation feed rats, the liver function parameters were significantly ($P < 0.05$) normal as compared with control rats. Similarly, it is reported in study that MCHC did not change in male rats whereas significant ($P < 0.05$) reduction was observed in female rats. An additional benefit of the herbal formulation was also observed in reducing blood glucose levels. After this research, it was suggested that prepared herbal formulation showed significantly ($P < 0.05$) low acute basic biological effects and could effectively reduce obesity and diabetes. Larger group and a longer treatment time are needed to evaluate the possible emergence of side effects.

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Author contributions

Mukul Kumar: Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); resources (equal); software (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **Deepika Kaushik:** Conceptualization (equal); software (equal); visualization (equal); writing – review and editing (equal). **Ravinder Kaushik:** Conceptualization (equal); formal analysis (equal); software (equal); supervision (equal); visualization (equal). **Azhar Khan:** Methodology (equal); validation (equal); visualization (equal). **Lucia Billeci:** Formal analysis (equal); resources (equal). **Emel Oz:** Formal analysis (equal); methodology (equal); software (equal). **Charalampos Proestos:** Data curation (equal); validation (equal). **Charles Brennan:** Conceptualization (equal); visualization (equal). **Naushad Ahmad:** Formal analysis (equal); software (equal). **Tahra Elobeid:** Data curation (equal); validation (equal). **Fatih Oz:** Formal analysis (equal); resources (equal); software (equal); validation (equal).

Conflicts of interest

The authors declare no conflict of interest.

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Institutional review board statement

The approval of animal ethical committee clearance for the experiments on these rats was approved from the Committee for Control and Supervision of Experiments on Animals (CPCSEA) of Shoolini University, Solan, Himachal Pradesh, India (Regd. no. 470/01/a/CPCSEA, dt. 5th Dec 2018).

Data availability statement

The data that support the findings of this study are available from the authors upon reasonable request.

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