Indian Journal of Natural Products and Resources
Vol. 13(3), September 2022, pp. 318-328
DOI: 10.56042/ijnpr.v13i3.54840

Development of cucurbocitrin based nutraceutical formulation: A potential adjuvant herbal therapy in the management of hypertension

Snigdha Bhardwaj[1]*, Sonam Bhatia[2#], Pushpraj S Gupta[2], Shaminder Singh[3] and Sachendra Badal[4]

[1]I.T.S College of Pharmacy, Murad Nagar, Ghaziabad 201206, Uttar Pradesh, India

[2]Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences, SHUATS, Naini, Prayagraj 211007, Uttar Pradesh, India

[3]Regional Centre for Biotechnology, NCR Biotech Science Cluster, 3rd Milestone, Faridabad-Gurugram Expressway, Faridabad 121 001, Haryana, India

[4]Department of Pediatrics, Armed Forces Medical College (AFMC), Wanowrie, Pune 411040, Maharashtra, India

Received 10 September 2021; revised received 02 June 2022; accepted 06 June 2022

Nearly half of the hypertensive patients fail to manage their blood pressure despite pharmacological interventions, which reflects the dire need for adjuvant anti-hypertensive therapies. Nutraceutical formulations are attractive in this regard owing to their efficacy, ease of availability, and moderate pricing. The current investigation involves the formulation of cucurbocitrin nutraceutical tablets by direct compression method and evaluated for its anti-hypertensive effect in the management of hypertension. Cucurbocitrin is a natural compound prepared from *Citrullus lanatus* seeds belonging to the family Cucurbitaceae. The powder blend was examined for pre-compression studies such as angle of repose, bulk density, tapped density, Carr's index, Hausner's ratio, etc. Based on acute-oral toxicity results, two tablet formulations were prepared using a different dose of cucurbocitrin and evaluated for post-compression parameters such as friability, hardness, weight variation, disintegration time, and *in vitro* drug release studies. *In vivo* studies were also performed for both formulations using a 10% Glucose solution-induced hypertension model in albino rats to observe its anti-hypertensive activity. The study indicated that nutraceutical formulation was found to be effective in reducing hypertension in an animal model.

Keywords: Adjuvant therapy, *Citrullus lanatus* (watermelon), Cucurbocitrin, Hypertension, Nutraceutical, Toxicity.

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 36/42, A61K 131/00

Introduction

Hypertension is a condition of chronically elevated blood pressure (BP) (systolic/diastolic BP [SBP/DBP] ≥140/90 mmHg at the brachial artery), which is a major cause in the development and progression of cardiovascular disease. Hypertension is one of the significant hazardous factors for cardiovascular disease. Several clinical trials reported that lowering blood pressure minimizes cardiovascular risk for myocardial infarction, stroke and heart failure by 20-25%, 30-35% and 50% respectively. The estimated lifetime risk of developing hypertension is 90%. Despite many anti-hypertensive medications, which are the only best, prescribed pharmacologic interventions, only about half of the patients can manage their blood pressure in the range[1]. This problem is amplifying with time and in the coming years, as the new 2017 American Stroke Association (AHA) blood pressure guidelines state that an additional 31 million US individuals will need antihypertensive and the patients who are taking antihypertensive drugs need to intensify their current treatment regimens[2]. Hence, the need for the development of adjuvant antihypertensive therapies is trending across the globe, which should be explored scientifically. Since ancient times, a variety of compounds of natural origin have been explored for multiple purposes such as used as an active agent against various disorders like inflammation, microbial infection, hypertension, obesity, psoriasis, arthritis, etc., carriers in advance drug delivery systems, an ingredient in the food industry (thickeners, flavours, sweeteners, etc.), formulation excipient (gelling agent, binding agent, diluents, etc.) and nutraceuticals or food product[3-5]. Many reports citing the beneficial effects of food for the prevention and treatment of cardiovascular diseases are reported[6-9].

*Correspondent author
Email: snigs.16@gmail.com
Mob.: +91-9818887521
#Present address: Vytals Wellbeing India Private Limited, Epitome Tower
DLF Cyber City, Phase 3, Gurugram 122002, Haryana, India
Supplementary figures are available online only

Nutraceuticals can be said as foods or a part of food, are highly focused as these provide health benefits and can be used for the prevention and treatment of such diseases. Some nutraceutical categories such as sterols, polyphenols and spirulina have been investigated for antihypertensive effects[2,10-11]. Guidelines for current hypertension management do not recommend drug therapy for patients with blood pressure in the high-normal range due to side effects of currently available antihypertensive drugs that shrink the treatment margins for these patients. Nutraceuticals, being of natural origin, are free from these types of side effects and could be a potential strategy for the treatment of these specific classes of patients. Therefore, nutraceutical supplementation and their intake could improve blood pressure and must be considered an effective adjuvant therapy for preventing hypertension in a cost-effective manner[12-15].

Citrullus lanatus (watermelon) originated in the Kalahari Desert of Africa[16]. *C. lanatus* is an organic product that is about 93% water, nutrients, sugar, minerals, filaments, carbohydrates, and other critical constituents like cucurbocitrin (reported as a vasodilator) and L-citrulline (an amino acid which diminishes the bioavailability of nitric oxide and has a positive effect on the improvement of vascular tone). Based on reported literature, cucurbocitrin, a constituent present in *C. lanatus* seeds is said to have great potential to control high blood pressure[17]. Cucurbocitrin belongs to the group of glucoside-saponin which has been reported to have dilatory action on smaller arteries as this natural active helps in extending the blood capillaries thereby reducing the circulatory strain levels and lowering blood pressure[18-19]. Also, some studies claimed that watermelon seeds compact with essential constituents which helps in weight loss, improves renal function and reduces cholesterol levels[20-22]. Although, no such data on pre-clinical testing of cucurbocitrin is available to establish its anti-hypertensive activity, the findings are still un-explored. To support the literature and to confirm the truth, the authors performed *in-vivo* studies to investigate the effect of cucurbocitrin in the management of hypertension to establish evidence. The current research works aimed at developing a nutraceutical tablet with cucurbocitrin and evaluate its activity against hypertensive parameters *in-vivo*.

Materials and Methods

Plant sample collection and identification

The seeds of *C. lanatus* were collected from the local market of Ghaziabad Uttar Pradesh, India in November 2018. The sample was submitted to and identified from Raw Materials Herbarium and Museum, CSIR-National Institute of Science Communication and Information Resources, New Delhi (NISCAIR/RHMD/ Consult/2018/3300-01 dt. 10-12-2018).

Preparation of Cucurbocitrin from *C. lanatus* seeds

The compound Cucurbocitrin was prepared as per the protocol reported by Barksdale[23]. The water-melon seeds (500 g) were taken and reduced to a powder of sufficient texture. This powdered material was then macerated for 12 h with distilled water, which has been rendered slightly alkaline with ammonium hydroxide. The assembly was then heated to a temperature of 60°C followed by the formation of a yellow-brownish infusion. The infusion was filtered several times using a muslin cloth until the liquid became very clear. This liquid was then concentrated over a water bath till it obtained the viscosity of thin syrup. After that, powdered lead subacetate was added to the concentrated liquid with vigorous and constant stirring until a copious, curdy yellowish precipitate was formed. Afterwards, hydrogen sulfide was permitted through the liquid forming vigorous bubbles that help to remove any substance held in precipitated organic matter. In addition, the sulfide also aids in removing the lead from the preparation. This hydrogen sulfide solution was then filtered through the gauze. The obtained solution was concentrated again till it was minimized to one-half its volume and filtered again. In this filtered liquid, equal parts of ethyl ether and dehydrated ethyl alcohol in excess were added resulting in the formation of finely divided creamy white precipitate. The solution was poured off and the precipitated elements were dried in a water bath over a low flame. This dried product was considered to be isolated cucurbocitrin. The prepared compound was further subjected to various physico-chemical analysis.

Pre-formulation studies

Physico-chemical and phytochemical analysis of cucurbocitrin

The prepared cucurbocitrin was examined for colour and odour and found to be creamy-brownish in colour with non-specific odour. The per cent yield, per cent moisture content, and total ash value were found to be 4.2, 6, and 9.75% respectively. The acid insoluble ash and water-insoluble ash were found to be 2.5 and 29.75% respectively. Since, the prepared compound, cucurbocitrin belongs to the glycoside family thus phytochemical screening was performed to confirm the presence of the same in the test compound. Legal's and Baljet's tests for glycosides and hemolysis and foam test

for saponin glycosides were performed to confirm the presence. Several other tests for alkaloids (Dragendroff's test), tannins (Ferric chloride test), flavonoids (alkaline reagent test), and steroids (Salkowski tests) were performed to confirm the absence of all of these constituents in isolated compound[24].

UV-Visible Spectrophotometric analysis of cucurbocitrin

The wavelength of cucurbocitrin was determined by UV Spectroscopy. Samples of cucurbocitrin with concentrations ranging from 1 to 10 µg/mL were scanned in UV-Visible Spectrophotometer (200-800 nm) against reagent blank (0.1 N hydrochloric acid solution (pH 1.2). Samples of different concentrations (10, 20, 30, 40, 50, and 60 µg/mL) were prepared and absorbance was measured at 401 nm (λmax) using UV-Visible Spectrophotometer[25-26].

FT-IR study

FT-IR spectroscopy was carried out for the test compound and physical mixture to observe the presence of main functional moieties in the test compound and also any kind of chemical incompatibility between test compound cucurbocitrin and excipients used in the formulation. The analysis was done with an FT-IR spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan). The FTIR spectra were taken for cucurbocitrin as well as for blend separately. Samples were ground completely with KBr powder in a weight proportion of 1:100 and afterward pellets were made with a pellet press under a pressure of 15 tons for a moment[27].

Pre-compression studies

Various pre-compression parameters have been observed to identify the flow property of the powder blend before tablet compression which is given below[28].

The angle of repose: It was measured through the funnel method. A specific quantity of powder drug was transferred to the funnel preserving the orifice of the funnel blocked through the thumb. When the powder used to be cleared from the funnel then measured its attitude of repose and measured in θ. It is calculated as follows:

$$\theta = \tan\text{-}1\ h/r$$

where θ is the Angle of repose, h is powder maximum height, and r is the radius of the pile

Bulk density: It is calculated as follows:

$$(\rho b) = M/Vb$$

where M is the mass of the sample, Vb is the bulk volume

Tapped density: It was measured by tapping the measuring cylinder containing a known mass of blend for a decided time. Minimum volume (Vt) occupied by powder in the cylinder and initial blend weight (M) were taken to calculate tapped density as follows:

$$\rho t = M/Vt$$

where, ρt is the Tapped density, M is the weight of powder and Vt is the minimum volume occupied after tapping

Carr's index: Carr's compressibility index was calculated as follows:

$$I = (Vt - V0)/ Vt\} \text{ X } 100$$

where, Vt is the Tapped density, V0 is the bulk density

Hausner's ratio: It is a number that is correlated to the flowability of a powder. Lower Hausner's ratio (<1.25) suggests larger glide residences than greater ones (>1.25). It is calculated as follows:

$$\text{Hausner's ratio} = Vt/ V0$$

where Vt is the Tapped density and V0 is the Bulk density.

Formulation of Nutraceutical tablets

Based on acute oral toxicity studies results, two different doses of cucurbocitrin were selected for nutraceutical tablet preparation using the direct compression technique. The excipients such as talc, sodium saccharin, magnesium stearate, and lactose were added to the blend for tablet making[29]. All the ingredients evaluated through pre-compression studies were passed through a 20 mesh sieve and weighed as per the quantities specified in Table 1. All the active and non-active excipients were mixed except glidant and lubricant using geometric mixing for 15 mins. After that talc and magnesium stearate were added to the powder blend followed by compression using a single rotary punching machine

Table 1 — Formulation table of nutraceutical tablet (500 mg)

Ingredients	F1 (%)	F2 (%)
Cucurbocitrin (API)	40	80
Talc	0.1	0.1
Sodium saccharine	0.5	0.5
Magnesium stearate	0.1	0.1
Lactose	57.3	18.3

*Cucurbocitrin 40% represents 200 mg and 80% represents 400 mg amount present in formulation F1& F2 respectively.

to get a 500 mg tablet (MultiTech, India). The prepared nutraceutical tablets were subjected to post-compression evaluation parameters.

Evaluation of nutraceutical tablets

The prepared nutraceutical tablet was subjected to post-compression parameters to observe the tablet properties as mentioned below[30-31].

Weight variation

The weight variantion test was done by weighing 20 tablets individually, calculating the suggested weight, and evaluating a single pill's weight to the average. The weight variation test was done to evaluate the drug content uniformity in the formulated tablets.

Hardness

Hardness is additionally called pill crushing strength. The tablet hardness was measured using a Monsanto hardness tester. The tablets were positioned lengthwise between the top and lower plunger and pressure was utilized with the aid of turning a threaded bolt until the pill fractures and measured hardness of the tablet in Kg.

Friability

It is determined by using the Roche friabilator, where combined effects of abrasion and shock were observed on tablets utilizing a plastic chamber that revolves at 25 rpm. The tablets were kept on racks/trays and operated for hundred revolutions resulting in continuous fall from the racks. The tablets were dusted and reweighed. Under standard restrictions, friability should be much less than 1%. It is calculated as follows:

$$\% \text{ Friability} = (Wi - Wf) / Wi$$

where Wi is the initial weight of the tablet and Wf is the final weight of a tablet

Disintegration test

The disintegration test was carried out through USP disintegration apparatus with 900 mL distilled water at 37°C. The time required for the complete disintegration of six tablets was recorded.

In-vitro drug release

The dissolution profile of formulated cucurbocitrin tablet was determined using USP dissolution apparatus II incorporating 900 mL of simulated gastric fluid (0.1 N HCl) at 37±0.5 °C with a stirring speed of hundred rpm. Different aliquot samples were withdrawn at 0, 5, 10, 15, and 30 minutes by maintaining sink conditions at each withdrawal. Samples were filtered through Whatman filter paper and absorbance was measured at 401 nm using a UV spectrophotometer.

***In- vivo* studies**

Animals

The animals used for the study were obtained from All India Institutes of Medical Sciences (AIIMS), New Delhi India, and the experiments were conducted at the I.T.S College of Pharmacy, Murad Nagar, Ghaziabad with written consent. A total of 30 Wistar rats of either sex, 100-250 g in weight were considered for investigation and were divided into 5 groups and quarantined in an animal house. Six rats were housed in every cage with a temperature of 24±1°C, humidity (RH) of 65±10%, and a 12/12-h light/dark cycle with the lights on at 7:30 AM. Rats were fed a standardized mouse diet and provided drinking water *ad libitum*. All materials, including feeders, bottles, bedding, and water were autoclaved before use. The animals were deprived of food for 24 h before experimentation but allowed free access to water throughout. The *in-vivo* studies were carried out for 21 days to check antihypertensive activity[32]. The experimental procedures and protocols used in the study were reviewed by the Institutional animal ethics committee (Experiment approval no. I.T.S/05/IAEC/2018) and the care of laboratory animals were taken as per the guidance of CPCSEA, Ministry of forests and environment, Government of India. The experimental design is shown in Table 2.

Acute oral toxicity studies

As per OECD guidelines 425 (Up & Down Procedure), Wistar rats weighing 150-200 g, aged 3-4

Table 2 — Experimental design for *in-vivo* study

Group	Groups	No of animals	Treatment
I	Control	6	No hypertension/ No treatment
II	Negative control (NC)	6	10% Glucose solution for 21 days (No treatment)
III	Standard (STD)	6	Treated with Amlodipine 5 mg/kg/day, orally for 21 days 30 minutes after the treatment with 10% Glucose solution up to 21 days
IV	Test -I (F1)	6	Treated with formulation 1, 30 minutes after the treatment with 10% Glucose solution up to 21 days.
V	Test -2 (F2)	6	Treated with formulation 2, 30 minutes after the treatment with 10% Glucose solution up to 21 days.

weeks were randomly selected. The animals remained kept under standard conditions for one week. An assay was performed at 2000 mg/kg p.o. the only dose. The rats were devoid of food for 3-4 h before dosing but provided with water *ad libitum*. The dose was administered to an animal considering body weight. The animals were kept under observation initially for 30 minutes followed by 4 h. Food was supplied to animals after 1-2 h of dose administration. Based on the survival of the tested animal, another 4 rats were administered with the same dosage under the same conditions. The control group was given the water of 10 mL per kg. Both the groups were keenly observed for any toxic outcome within 6 h and then at regular intervals up to 14 days. The animal survived was determined for any toxic signs onset. The weight of animals was measured and documented. On day 14, the weight of animals was measured followed by histological analysis of collected blood samples[33].

Glucose-induced hypertensive rat model

A study by Hulman and coworkers reported that treatment with simple sugars (carbohydrates) for 1-3 weeks was considered appropriate for early development of sugar-induced hypertension in Wistar rats and associated with increased blood pressure and triglycerides levels without obesity[34]. Another study reported that high glucose concentration demonstrated increased coronary perfusion pressure by increasing free radicals formation and reducing nitric oxide accessibility to target cells giving rise to a state of increased vasomotor tone and ventricular instability resulting in increased blood pressure[35]. Therefore, the 10% glucose model to induce early hypertension was selected for *in-vivo* studies. Glucose-induced hypertension was developed in the experimental animals using 10% glucose in drinking water *ad libitum* up to 3 weeks[36].

Estimation of biochemical parameters

The body weight of individual animals will be taken daily for each group and records were maintained from the starting day of the study till the last dosing before sacrificing the animal. The weight of the animal was also considered for those who couldn't survive till the end. Food intake was measured every day during the animal study. At the end of the study, blood was withdrawn through the Retro-orbital plexus method and serum was separated and sent for biochemical investigation (triglycerides and cholesterol levels). All animals were sacrificed by an overdose of anaesthesia (Halothane, 11.5 or 23 mmol/kg) within 24 h after the final treatment. The blood pressure and heart rate of these groups were measured on weeks 0, 1, 2, and 3 by the non-invasive blood pressure (NIBP) method. The NIBP instrument was used after combining with the Power Lab system to get blood pressure measurements using tail-cuff in rats. The blood pressure was intermittently (for 30 seconds) measured based on periodic occlusion of blood flow in the rat tail.

Histological studies

The heart tissues of rats from each group were preserved in 10% buffered formaldehyde and processed with paraffin wax. For histological examination, very thin sections were taken and staining was done with hematoxylin followed by eosin. The analysis was done using a light microscope. The histological changes in cardiac muscle were studied.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 5 statistical software and results were calculated as Mean±SEM for each group. For Various examinations, One-way analysis of variance (ANOVA), as well as Dunnet's test (P <0.05), were used to calculate significance.

Results

Pre-formulation studies

U.V. Visible Spectrophotometric Analysis of Cucurbocitrin

The calibration curve of the test sample was prepared at 401 nm by using UV spectroscopy. The scanned wavelength maxima (λmax) of the test compound exhibited maximum absorption at 401 nm which was found nearby to the reported value of the similar compound[37].

Phytochemical analysis for cucurbocitrin

Phytochemical tests were performed for an isolated compound for the determination of the phytochemical group present in the same. Being a glycoside, cucurbocitrin showed positive results for both the tests for glycosides and saponin glycosides only, whereas it showed negative for alkaloids, tannins, flavonoids, and steroids. Therefore, confirms the presence of cucurbocitrin which support the reported literature for compounds belonging to the glycoside family[38-39]. The inference of the chemical test performed for cucurbocitrin has been provided in Table 3 and the confirmation test for the presence of glycosides in the isolated compound is shown in Fig. 1.

Table 3 — Phytochemical analysis of cucurbocitrin in the sample

Categories	Test reaction	Indication	Results
Glycosides	Legal test	Red colour	Positive
Glycosides	Baljet test	Yellow to orange colour	Positive
Saponin Glycosides	Foam test	Foam formation	Positive
Saponin Glycosides	Hemolysis test	RBCs become ruptured	Positive
Alkaloids	Dragendroff's test	Yellow colour	Negative
Tannins	Ferric chloride test	Brown colour	Negative
Flavonoids	Alkaline reagent test	White colour	Negative
Steroids	Salkowski test	Black colour	Negative

Table 4 — Pre-compression studies of powder blend of nutraceutical tablet formulation

S. No.	Precompression parameters	F1 (Value±SD)	F2 (Value±SD)	Compliance with standards
1	The angle of repose (θ)	23.14±0.11	24.30±0.12	Good flow
2	Bulk density (gm/mL)	0.42±0.13	0.46±0.34	Optimum
3	Tapped density (gm/mL)	0.53±0.17	0.55±0.23	Optimum
4	Carr's index	11.19±0.14	13.07±0.1	Good flow
5	Hausner's Ratio	1.15±0.21	1.31±0.16	Good flow

SD represents standard deviation.

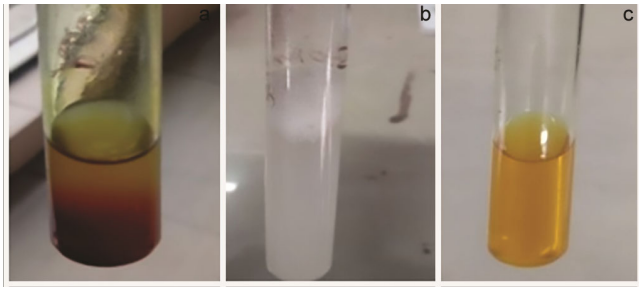

Fig. 1 — Positive chemical tests. a) Legal test; b) Foam test; c) Baljet test.

FT-IR study

FT-IR study of test compound was done for both the test compound and physical mixture. IR spectra of test compounds revealed characteristic peaks which are similar to reported literature[40]. For test compounds, the vibration frequencies at 1194 and 1056 cm^{-1} were due to the presence of ether linkage in glycoside. The vibration frequency at 1637 cm^{-1} demonstrates the presence of an aromatic group in glycosides. Sharp peaks at 3304 and 3420 cm^{-1} were due to the methyl and hydroxyl groups respectively. Stretching frequencies at 3048 and 2963 cm^{-1} are attributed to the alkyl group of glycoside structure and thus confirmed that the test compound retains the functional moieties of glycosides. IR spectroscopy of physical mixture (blend) was performed to observe any kind of interaction between test compound and excipients. The characteristic peaks (%T) were found to be in a range of 1635.34 to 3583.09 showing the presence of various functional groups such as aldehyde, ketone, carboxylic acid, and alcohol groups in both spectra. Therefore, it confirms no significant interactions among the active and excipients used in formulations when compared with the test compound spectra. IR spectra of the test compound and physical mixture are shown in supplementary Fig. 1 and 2 respectively.

Pre-compression studies

The powder blend was evaluated for the following pre-compression parameters to determine powder flow before tablet compression. The results are given in Table 4.

Formulation of nutraceutical tablets

The prepared cucurbocitrin was found to be cream-brownish in colour. Two batches of nutraceutical tablet formulations of cucurbocitrin (F1 & F2) were prepared by direct compression technique. The techniques used were conventional thus eliminating the wetting and drying process. The appearance of the tablet was found to be round in shape, cream-coloured, smooth textured, and odourless.

Evaluation of formulated nutraceutical tablets

Tablets were evaluated for the following post-compression parameters to observe formulation compliance with standards limits. The results are presented in Table 5.

In-vitro release studies

The *in-vitro* per cent drug release of cucurbocitrin from both the nutraceutical tablets (F1 & F2) in 0.1 HCL (pH 1.2) was found to be 88.13±0.34 and

Table 5 — Post-compression studies result of nutraceutical tablet formulation

S. No.	Parameters	F1 (Value±SD)	F2 (Value±SD)	Compliance with standards
1	Hardness (Kg)	4.1±0.1	4.3±0.21	Acceptable
2	Friability (%)	0.56±0.023	0.58±0.12	Acceptable
3	Weight variation	0.488±0.019	0.4928±0.035	Acceptable
4	Disintegration time (min)	12.33±0.172	14.76±0.159	Acceptable

SD represents standard deviation.

95.26±0.56 respectively in 30 mins. The per cent release of cucurbocitrin from nutraceutical tablets was found to be the quick maximum in the F2 formulation it may be due to the higher amount of cucurbocitrin present in the F2 formulation. The per cent release graph is shown in Fig. 2.

In-vivo studies

Acute oral toxicity studies

During acute oral toxicity studies, no death or toxic signs were observed due to treatment given. The result revealed that cucurbocitrin solution could be well tolerated up to a dose of 2000 mg/kg body weight and may be classified as category 5. The test observations indicated that there are no treatment-related alterations due to the high dose level when compared with the control group. Food intake, body weight, and histological analysis demonstrated no abnormalities. Thus, the results revealed tolerability of cucurbocitrin solution administered daily for 14 days up to 2000 mg/kg dose. Based on the inference, 200 and 400 mg/kg of cucurbocitrin were selected for further experiments.

Anti-hypertensive effect on glucose-induced hypertensive rats

The animals included in the study underwent successful 21 days of study design. After the experimental period, the rats showing a minimum of 140 mmHg systolic B.P. and 90 mmHg diastolic B.P. were considered hypertensive[36]. The animals were excluded from the experimentation which did not show any rise in BP, triglyceride, and cholesterol levels. Also, the animal died prematurely were eliminated from the experimental during the collection of behavioural parameters and evaluation of histologic efficacy.

Physical parameters

Glucose solution (10%) was found to relatively increase the body weight in all animal groups as compared to the control group after 21 days. The increased body weight is effectively reduced in the animal group treated with test-2 formulation (400 mg/kg/day) as compared to all other treatment groups. During the study, 10% glucose solution significantly decreased the food intake (P <0.050) relative to the control group. But no

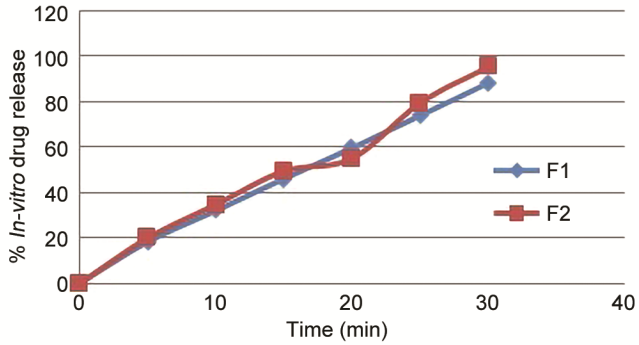

Fig. 2 — The in-vitro drug release profile of prepared cucurbocitrin nutraceutical tablets.

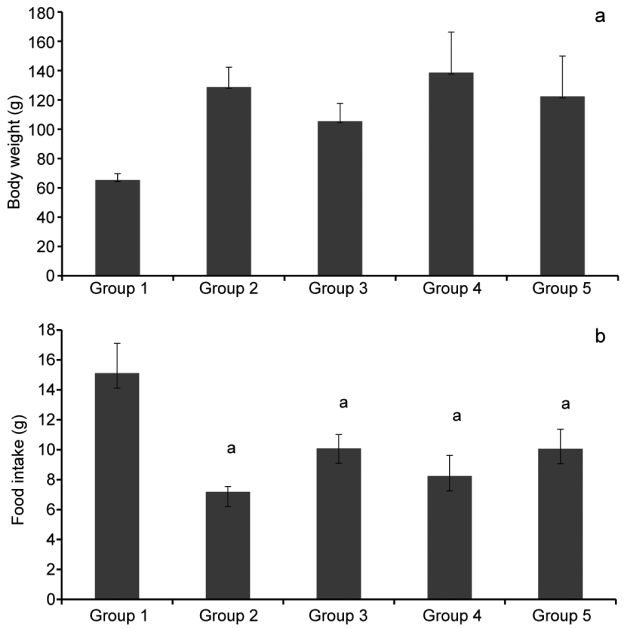

Fig. 3 — Physical parameters level in the rats after 21 days. a) 10% glucose solution increased the body weight relative to the control group. The increased body weight is reduced by the test drug, cucurbocitrin (400 mg/kg/day); b) 10% glucose solution significantly decreased the food intake (P <0.050) relative to the control group. But no treatment groups significantly increased the food intake as compared to the negative control group (10% glucose solution group).

treatment groups significantly increased the food intake as compared to the negative control group (10% glucose solution). The physical parameters are graphically represented in Fig. 3a and Fig. 3b respectively.

Triglycerides and cholesterol levels

In all glucose-induced hypertensive animals, serum triglyceride levels significantly reduced (P <0.05) in treatment groups when compared to the control group. The level of triglyceride in animals was significantly reduced by standard drug (amlodipine, 5 mg/kg/day), Test-1 (cucurbocitrin, 200 mg/kg/day), and Test-2 (cucurbocitrin, 400 mg/kg/day) after the treatment of 10% glucose solution as shown in Fig. 4a. Similarly, a significant difference was observed in the serum cholesterol level among all the treatment groups when compared to the control group as shown in Fig. 4b.

Systolic blood pressure, diastolic blood pressure, heart rate

The systolic blood pressure, diastolic blood pressure, and heart rate were significantly increased (P <0.05) by administration of 10% glucose solution for 21 days. After administration of amlodipine (5 mg/kg/day) and Cucurbocitrin (200 and 400 mg/kg/day) with simultaneous administration of 10% glucose solution, the systolic blood pressure, diastolic blood pressure was significantly declined (P <0.05) in STD, Test-1, and Test-2 groups as shown in Fig. 5a and Fig. 5b respectively. The heart rate was significantly declined (P <0.05) with standard drug amlodipine (5 mg/kg/day) but no significant reduction in heart rate was found in groups treated with cucurbocitrin nutraceutical tablet-based treatments groups (200 and 400 mg/kg/day) as shown in Fig. 6.

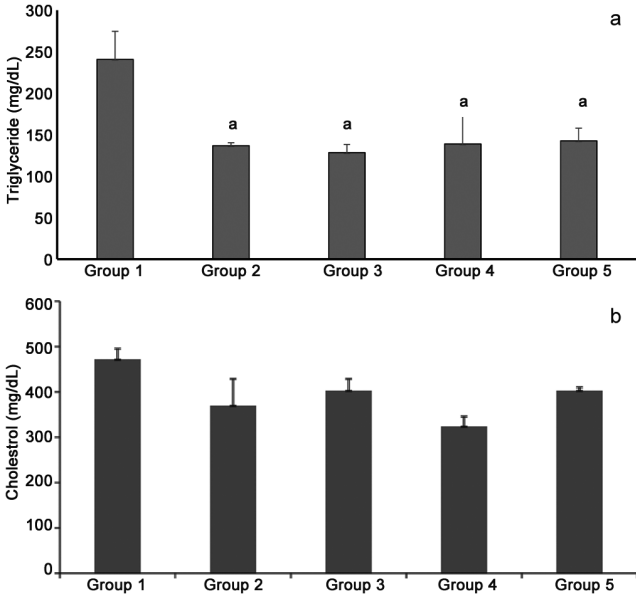

Fig. 4 — a) The serum triglyceride levels. In the negative control group, the serum triglyceride level significantly reduced (P <0.05) relative to the control. This reduced level of triglyceride was not significantly recovered by Amlodipine (5 mg/kg/day) and cucurbocitrin (test, 200 mg/kg/day and 400 mg/kg/day) after the administration of 10% glucose solution; b) The serum cholesterol levels. There was a difference in the serum cholesterol level among all the groups.

Histological examination

For evaluation, all slides were put on auto-focusing mode and virtually scanned by using a virtual slide scanner Nanozoomer (Shizuoka, Japan) with 40X magnification (0.23 µm/pixel). The histological investigation of animal tissue is shown in Fig. 7. The cross-section of the control group has shown normal histology and showing the branching and anatomizing

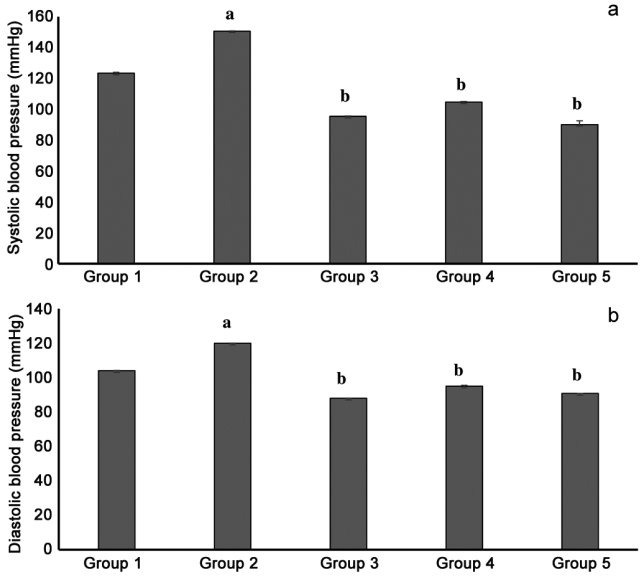

Fig. 5 — a) Systolic blood pressure; b) Diastolic blood pressure. Blood pressure was significantly increased (P <0.05) by the administration of 10% glucose solution for 21 days. After the administration of Amlodipine (STD, 5 mg/kg/day) and cucurbocitrin-based nutraceutical test formulations (Test-1, 200 mg/kg/day and Test-2, 400 mg/kg/day) followed by the administration of 10% glucose solution, the systolic and diastolic blood pressure levels significantly declined in rats (P <0.05).

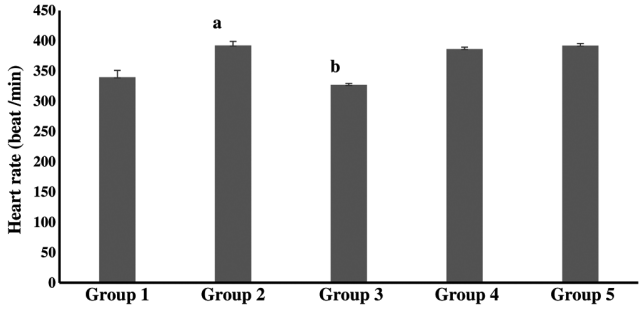

Fig. 6 — Heart rate. The Heart rate significantly declined (P <0.05) with the Amlodipine (STD, 5 mg/kg/day) as the standard drug but, the treatments groups of cucurbocitrin (test, 200 mg/kg/day and 400 mg/kg/day) have no significant effect on Heart rate after the administration of 10% glucose solution.

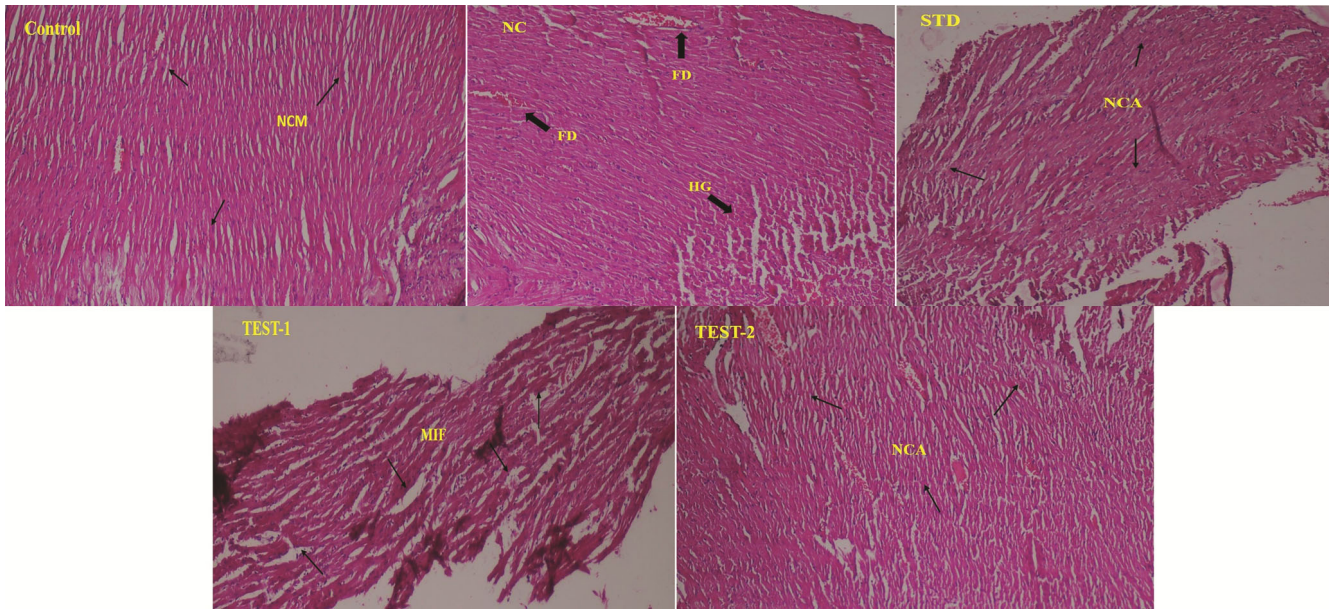

Fig. 7 — Histopathological examination of the Heart, a) Control group: cross-section showed normal histology and showing the branching and anatomizing of normal cardiac myocytes (NCM) with central placed oval nuclei (depicted by arrow), b) Negative control (NC) group: Cross-section demonstrated wavy fibers with focal damage (FD) to some fibers, peripherally placed dark nucleus, hemorrhage (HG) between muscle fibers, c) Standard (STD) group: Group treated with standard drug exhibited normal cardiac architecture (NCA) on day 21, d) Test-1 group: the animal group treated with cucurbocitrin nutraceutical tablet (200 mg/kg/day) (Test-1) showed minimal damage and mild infiltration (MIF) to cardiac architecture with normal myocytes on day 21, e) Test-2 group: the animal group treated with cucurbocitrin nutraceutical tablet (400 mg/kg/day) (Test-2) showed significant effect, almost normal branching and anatomizing of cardiac muscle with normal myocytes post-treatment on day 21.

of normal cardiac myocytes with central placed oval nuclei. After administration of 10% glucose solution cross-section showed wavy fibres with focal damage to some fibres, peripherally placed dark nucleus, and haemorrhage between muscle fibres in the negative control group. Histopathological examination of rat heart of standard group treated with the standard drug having the normal cardiac architecture. In the test-1 group, after the treatment with F1 formulation (200 mg/kg/day), the cross-section showed mild damage and infiltration to cardiac architecture with normal myocytes. In the test-2 group, after treating with F2 (400 mg/kg/day), the cross-section demonstrated the normal branching and anatomizing of cardiac muscle with normal myocytes. The histology revealed that an anti-hypertensive effect was observed in a dose-dependent manner, thus animal group treated with nutraceutical formulation (400 mg/kg/day) showed significant recovery of cardiac architecture as compared to the group treated with nutraceutical formulation (200 mg/kg/day) on day 21.

Discussion

This study aimed to formulate cucurbocitrin based-nutraceutical tablet for the management of hypertension. Cucurbocitrin, a nutraceutical was first prepared from watermelon seeds. The compound showed a positive result for the test for glycosides and saponin, hence it confirmed the presence of saponin glycoside while it showed negative results for the test of alkaloids, steroids, tannins, and flavonoids. In IR spectroscopy, the characteristic peaks %T were found to be in a range of 1635.34 to 3583.09 which shows the presence of various functional groups such as C-O (aldehyde, ketone), COOH (carboxylic acid), O-H (alcohol) groups in the pure compound which remained prominent in IR spectra of blend and hence confirms the presence of these groups in pure compounds. Both the tablet formulations (F1 and F2) were observed for pre-compression parameters such as angle of repose (23.14±0.11 and 24.30±0.12), bulk density (0.42±0.13 and 0.46±0.34), tapped density (0.53±0.17 and 0.55±0.23), Carr's index (11.19±0.14 and 13.07±0.1), and Hausner's ratio (1.15±0.21 and 1.31±0.16) respectively. Based on the above observations, F1 formulation was shown to have good powder characteristics. Post formulation parameters for both the tablets (F1 and F2) exhibited hardness were 4.1±0.1 and 4.3±0.21 respectively, friability was 0.56±0.023 and 0.58±0.120 respectively, weight variation was 0.488±0.019 and 0.4928±0.035 respectively and disintegration time were 12.33±0.172

14.76±0.159 minutes respectively. The release profiles of both the formulations (F1 and F2) were found within the limits of the standard and were found to be 88.13±0.34 and 95.26±0.56 release in 30 minutes respectively. For an *in-vivo* study, 10% glucose in drinking water is equivalent to a diet (approx. 50% of calories value) glucose was found significant in the production of glucose-induced hypertension in rats. 10% glucose in drinking water caused concentration and duration-related increase in blood pressure, body weight, triglycerides, and cholesterol levels but a decrease in food intake. The administration of cucurbocitrin-loaded nutraceutical tablets (in doses i.e., 200 and 400 mg/kg/day) also resulted in a significant reduction in triglycerides and total cholesterol by 35.2% and 31.1% respectively in treatment groups as compared to the normal group. The cucurbocitrin-based nutraceutical formulation was also found to reduce systolic and diastolic blood pressure in hypertensive rats. In support of this, earlier reported pieces of literature by Massa *et al.*[41], Figueroa *et al.*[42], & Yuan *et al.*[43], investigated the effect of watermelon extract and its components in reducing blood pressure thereby preventing hypertension *in-vivo*[41-43]. The histological analysis demonstrated that nutraceutical tablets, administered to rats with induced hypertension (10% glucose solution) were found to protect the histological abnormalities in heart cells and wavy fibres with focal damage and showed a positive consequence on body weight, triglyceride, and cholesterol level, B.P. and heart rate. The systolic and diastolic blood pressure was found to be significantly decreased in test groups (Test-1 and Test-2) after administration of nutraceutical tablets in which cucurbocitrin (200 and 400 mg/kg/day) treatment were given after inducing hypertension by 10% glucose solution. From the study, results suggested that the test-2 formulation (400 mg/kg/day) was found to be more effective as compared to the test-1 formulation (200 mg/kg/day). The finding may hold clinical relevance for cucurbocitrin-based nutraceuticals as adjuvant therapy in hypertension cases. Thus, the study emphasizes that a moderate dose of cucurbocitrin prevents hypertension by improving blood vessel tension. Therefore, the current study presents new experimental evidence for developing this novel nutraceutical formulation as anti-hypertensive therapy.

Conclusion

The current study emphasizes on the effectiveness of natural compound, cucurbocitrin based-nutraceutical tablets being used as a supplementary treatment in hypertension. Also, being of natural origin, this compound has lesser or no side effects as compared to synthetic drugs available. The cucurbocitrin-based nutraceutical tablets showed a dilatory effect on the cardiac muscle and blood capillaries and may offer promising results in the management of hypertension and hence, the natural active may be developed for prophylaxis as either as supplement to promote heart health or as adjuvant therapy to reduce the dose and side effects of commercial synthetic anti-hypertensives. However, this natural compound with potential health benefits is not much explored in terms of clinical effectiveness, therefore, this study only supports the reported fact that cucurbocitrin significantly reduced hypertension *in-vivo* but further research is necessary to investigate other potential effect of cucurbocitrin on cardiac muscles and lipid profiles to establish its safety and efficacy in humans.

Conflict of interest

The authors declare no conflict of interest.

References

1 Elliott W J and Black H R, Prehypertension, *Nat Clin Pract Cardiovasc Med*, 2007, **4**(10), 538-548.
2 Braithwaite M C, Tyagi C, Tomar L K, Kumar P, Choonara Y E, *et al.*, Nutraceutical-based therapeutics and formulation strategies augmenting their efficiency to complement modern medicine: An overview, *J Funct Foods*, 2014, **6**(1), 82-99.
3 Yadav J and Bhardwaj S, A review on plants in malignancy with counteractive action, *J Pharm Sci Innov*, 2019, **8**, 3-10.
4 Bhardwaj S and Bhatia S, Development and characterization of niosomal gel system using *Lallementia royaleana Benth*. mucilage for the treatment of rheumatoid arthritis, *Iran J Pharm Sci*, 2020, **19**(3), 465-482.
5 Bhardwaj S, Gaur P K and Tiwari A, Development of topical nanoemulgel using combined therapy for treating psoriasis, *Assay Drug Dev Technol*, 2022, **20**(1), 42-54.
6 Pem D and Jeewon R, Fruit and vegetable intake: Benefits and progress of nutrition education interventions- narrative review article, *Iran J Public Health,* 2015, **44**(10), 1309-1321.
7 Cory H, Passarelli S, Szeto J, Tamez M and Mattei J, The role of polyphenols in human health and food systems: A mini-review, *Front Nutr*, 2018, **5**, 87.
8 Hasler C M, Functional foods: Benefits, concerns and challenges-a position paper from the American council on science and health, *J Nutr, 2002,* **132**(9), 3772-3781.
9 Das L, Bhaumik E, Raychaudhuri U and Chakraborty R, Role of nutraceuticals in human health, *J Food Sci Technol*, 2012, **49**(2), 173-183.
10 Patil C S, Current trends and future prospective of nutraceuticals in health promotion, *Bioinfo Pharm Biotechnol*, 2011, **1**(1), 1-7.
11 Dureja H, Kaushik D and Kumar V, Developments in nutraceuticals, *Indian J Pharmacol*, 2003, **35**, 363-372.

12 Eskin N A M and Tamir S, *Dictionary of nutraceuticals and functional foods*, (CRC Press, Boca Raton, USA), 2006, 507.

13 Nasri H, Baradaran A, Shirzad H and Rafieian-Kopaei M, New concepts in nutraceuticals as alternative for pharmaceuticals, *Int J Prev Med*, 2014, **5**(12), 1487-1499.

14 Prabu S L, Suriya Prakash T N K, Kumar D C, Kumar S S and Ragavendran T, Nutraceuticals: A review, *Elixir Pharm*, 2012, **46**, 8372-8377.

15 Keservani R K, Kesharwani R K, Vyas N, Jain S, Raghuvanshi R, *et al.*, Nutraceutical and functional food as future food: A review, *Der Pharm Lett*, 2010, **2**(1), 106-116.

16 Erhirhie E O and Ekene N E, Medicinal values on *Citrullus lanatus* (Watermelon), *Int J Res Pharm Biomed Sci*, 2013, **4**(4), 1305-1312.

17 Gul S, Rashid Z and Sarwer G, *Citrullus lanatus* (watermelon) as diuretic agent: An *in-vivo* investigation on mice, *Am J Drug Deliv Ther*, 2013, **4**, 89-92.

18 Smirk F H, Pathogenesis of essential hypertension, *Br Med J*, 1949, **1**(4609), 791-799.

19 Azeem MW, Nadeem M and Rao S, Analytical characterization of pure and blended watermelon (*Citrullus lanatus*) oil: Impact of blending on oxidative stability, *Pak J Anal Environ Chem*, 2015, **16**(1), 52-58.

20 Oseni O A and Okoye V I, Studies of phytochemical and antioxidant properties of the fruit of watermelon (*Citrullus lanatus*). (Thunb.), *J Pharm Biomed Sci*, 2013, **27**(27), 508-514.

21 Akhuetie J O, Oyeyemi M O and Oloye A A, Studies on crude powder and ethanolic extract of *Citrullus lanatus* seeds: phytochemical analysis, effects on haemogram and some reproductive characteristics of male albino rats, *Niger Vet J*, 2018, **39**(2), 133-142.

22 Shahein M R, Atwaa E S H, El-Zahar K M, Elmaadawy A A, Hijazy H H A, *et al.,* Remedial action of yoghurt enriched with watermelon seed milk on renal injured hyperuricemic rats, *Fermentation*, 2022, **8**(41), 1-16.

23 Barksdale I S, Process for the preparation of Cucurbocitrin, *US Pat 1626321*, 26 April 1927.

24 Evans W C, *Trease and Evans Pharmacognosy*, 15th edn, (W B Sauders Company Ltd, London), 2002, 137-139 & 230-240.

25 Garg R K and Singh I, UV Spectrophotometric method development and validation for quantitative estimation of Nizatidine, *J Innov Pharm Biol Sci*, 2015, **2**(3), 333-336.

26 Pramod K, Ansari S H and Ali J, Development and validation of UV spectrophotometric method for the quantitative estimation of eugenol, *Asian J Pharm Anal*, 2013, **3**(2), 58-61.

27 Ingebrigtson D N and Smith A L, Infrared analysis of solids by potassium bromide pellet technique, *Anal Chem*, 1954, **26**(11), 1765-1768.

28 Lachman L, Lieberman H A and Kanig J L, *The theory and practice of industrial pharmacy*, 3rd edn, (Varghese Publishing House, Mumbai), 2009, 296-303.

29 Gallo L, Ramirez-Rig M V, Pina V, Palma S, Allemandi M, *et al.*, *Valeriana officinalis* dry plant extract for direct compression: Preparation and characterization, *Sci Pharm*, 2012, **80**(4), 1013-1026.

30 Chauhan B, Current concepts and prospects of herbal nutraceutical: A review, *J Adv Pharm Technol Res*, 2013, **4**(1), 4-8.

31 Patricio J P, Santos C and Cerdeira R, *In-vitro* dissolution profile of two commercially available iron preparations, *Drugs R D*, 2012, **12**(1), 35-40.

32 Alamgeer A, Akhtar M S, Jabeen Q, Akram M A, Khan H U, *et al.*, Antihypertensive activity of aqueous methanol extract of *Berberis orthobotrys* Bien Ex- Aitch in rats, *Trop J Pharm Res*, 2013, **12**(3), 393-399.

33 Saleem U, Amin S, Ahmad B, Azeem H, Anwar F, *et al.,* Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb. roots in albino mice as per OECD 425 TG, *Toxicol Rep*, 2017, **4**, 580-585.

34 Hulman S and Falkner B, The effect of excess dietary sucrose on growth, blood pressure, and metabolism in developing Sprague-Dawley rats, *Pediatr Res*, 1994, **36**(1 Pt 1), 95-101.

35 D'Amico M, Marfella R, Nappo F, Di Filippo C, De Angelis L, *et al*., High glucose induces ventricular instability and increases vasomotor tone in rats, *Diabetologia*, 2001, **44**(4), 464-470.

36 Tom E N, Demougeot C, Mtopi O B, Dimo T, Djomeni P D, *et al.*, The aqueous extract of *Terminalia superba* (Combretaceae) prevents glucose-induced hypertension in rats, *J Ethnopharmacol*, 2011, **133**(2), 828-833.

37 Ndikau M, Noah N M, Andala D M and Masika E, Green synthesis and characterization of silver nanoparticles using *Citrullus lanatus* fruit rind extract, *Int J Anal Chem*, 2017, **2017**, 8108504.

38 Simeon E O and Clement M A, Phytochemical screening and physiologic functions of metals in seed and peel of *Citrullus lanatus* (watermelon), *Int J Green Herb Chem*, 2017, **6**(1), 35-46.

39 Bayero A S, Datti Y, Usman L A, Asmau A A, Nura T, *et al.*, Phytochemical composition and antioxidant activity of the indigenous watermelon seeds obtained kano state, Nigeria, *Int J Food Sci Nutr*, 2019, **4**(5), 184-187.

40 Lopusiewicz L, Antioxidant, antibacterial properties and the light barrier assessment of raw and purified melanins isolated from *Citrullus lanatus* (watermelon) seeds, *Herba Pol*, 2018, **64**(2), 25-36.

41 Massa N M, Silva A S, Toscano L T, Silva J D, Persuhn D C, *et al.*, Watermelon extract reduces blood pressure but does not change the sympathovagal balance in prehypertensive and hypertensive subjects, *Blood Press*, 2016, **25**(4), 244-248.

42 Figueroa A, Sanchez-Gonzalez M A, Wong A and Arjmandi B H, Watermelon extract supplementation reduces ankle blood pressure and carotid augmentation index in obese adults with prehypertension or hypertension, *Am J Hypertens*, 2012, **25**(6), 640-643.

43 Yuan R Q, Qian L, Yun W J, Cui X H, Lv G X, *et al.*, Cucurbitacins extracted from *Cucumis melo* L. (CuEC) exert a hypotensive effect via regulating vascular tone, *Hypertens Res*, 2019, **42**(8), 1152-1161.