Research Article

Development of a standardized combined plant extract containing nutraceutical formulation ameliorating metabolic syndrome components

Baishakhi De[1] · Koushik Bhandari[2] · Prakash Katakam[1] · Tridib Kumar Goswami[3]

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Abstract
Plant bioactives have a great role in combating multifactorial disease conditions like metabolic syndrome (MetS). This research work aims to develop a standardized plant extract combination and formulate it to tablets with nutraceutical potentials. The extract was prepared from the bark powder of *Ficus religiosa*, seed powder of *Syzigium cumini* and leaf powder of *Ocimum bacilicum* chemometrically optimized in the ratio of 1.15:1.15:1.68 (Ashwattha: Jamun: Basil or FR: SC: OB). It is enriched in screened and pharmacologically active plant secondary metabolites. The tablets were prepared by direct compression using single-punch tablet machines. The nutraceutical tablets passed all the prescribed quality control tests with a justified pharmacokinetic profile. Results of animal experimentations have shown the hypoglycemic, hypolipidemic effect and antihypertensive effect of the nutraceutical tablets in relevant animal models. Thus, the nutraceutical formulation that showed effectivity in combating MetS can be opted as an adjunct therapy.

Keywords Metabolic syndrome · Standardized plant extract · Hypoglycemic · Hypolipidemic · Antihypertensive · Nutraceutical formulation · Adjunct therapy

1 Introduction

Metabolic syndrome (MetS) is a very heterogenous complex syndrome with a cluster of events such as glucose intolerance, insulin resistance, abdominal obesity, artherogenic dyslipidemia and hypertension. It is also known by the names of 'Syndome X,' 'insulin resistance syndrome,' 'Reaven's syndrome,' 'metabolic cardiovascular syndrome' [1]. Metabolic syndrome is associated with cardio/cerebrovascular and metabolic risks. This non-communicable disease (NCD) though started in the western world has now become a global health problem. This syndrome triggers the spread of other diseases viz. Type 2 diabetes, coronary diseases, stroke and other disabilities [2]. Inspite of life style interventions (dietary changes, increased physical activity, etc.), many patients with MetS require pharmacological treatment. Sibutramine, Orlistat, metformin, glitazones, rimonabant, calcium antagonists, beta blockers, thiazide diuretics and angiotensin converting enzyme inhibitors are one of the several options but not without side effects [1, 3]. Herbal medicine and the nutraceutical products developed from them with its versatile combinations of pharmacologically active plant secondary metabolites are being used in treatment of several ailments in an evidence based manner. Despite immense potency, lack of proper dosage form, perfect dosimetry, organoleptic unacceptability in crude form, storage, preservation and shelf life issues, the rationale use of herbal medicine gets hindered in many circumstances [4]. Just as

✉ Tridib Kumar Goswami, tkg@agfe.iitkgp.ernet.in | [1]SSS Indira College of Pharmacy, Vishnupuri, Nanded, Maharashtra, India. [2]Advanced Technology Development Centre, Indian Institute of Technology Kharagpur, Kharagpur 721302, India. [3]Department of Agriculture and Food Engineering, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal 721302, India.

combinational dosage forms with synthetic drugs are promoted in treatment of ailments with a multidimensional approach, polyherbal therapy is also more popular than monotherapy [5, 6]. Polyherbalism provides a synergistic therapeutic effect in an evidence-based manner [5]. *Ficus religiosa* (FR) (Fam. *Moraceae*) also known as Peepal tree in hindi, Asvatthah in Sanskrit and sacred fig in Bengali has traditional pharmacological effects of antimicrobial, anti-parasitic, anti-Parkinson's, anti-convulsant, anti-amnesic, anticholinergic, antidiabetic, anti-inflammatory, analgesic, cytotoxic, anti-ulcer, wound healing, antioxidant, anti-asthmatic, reproductive, hepato-, nephro- and dermato-protective. Plant secondary metabolites such as phytosterols, flavonoids, glucosides and furano-coumarin derivatives are the different active compounds present in different parts of this plant [7–11]. *Syzygium cumini* (SC), (Fam. Myrtaceae; also called Jamun or Jambul), is reported to have a wide range of activities viz. antimicrobial, antioxidant, anti-diabetic, neuropsychopharmacological, hepato-gastro-cardio-chemo protective and antihyperlipidemic . Gallic acid, different flavonoids, terpenoids, anthocyanins, isoquercetin, kaempferol, myricetin, alkaloid like jambosine and glycosides like jambolin or antimellin are some of the active compounds present in different plant parts of Jamun [12, 13]. *Ocimum bacilicum* (OB) (Fam. Lamiaceae also called as 'King of herbs' or sweet basil) finds use in traditional and integrative medicine owing to its wide range of pharmacological effects e.g., hypoglycemic, hypolipidemic, cardiac stimulant, immunomodulator, cardioprotective, chemomodulatory and anti-inflammatory. Compounds such as methyl eugenol, methyl chavicol, eugenol, citral, cineole, thymol, camphor, 1,8-cineole and elemicine are found to be present in its different plant parts [14, 15].

Plant secondary metabolites (PSM) have a wide range of pharmacological actions [7–15]. These PSMs have nutraceutical potentialities and being of plant origin are phytonutraceuticals. Often they are used as dietary supplements and PSMs are also used in combinations or as combinatorics to achieve greater nutrotherapeutic effect (use of nutraceuticals as adjunct therapy in the treatment of ailments). This research work reports to the development of a phytonutraceutical combinatoric (PNC) product in the form of oral tablets containing nutrotherapeutic contents prepared as an aqueous powdered extract from the bark powder of *F. religiosa*, seed powder of *Syzigium cumini* and leaf powder of *O. bacilicum* in a chemometrically optimized pharmacologically active ratio with the aid of Design Expert Software 7.0. PNC is enriched in screened and pharmacologically active plant secondary metabolites. Quality evaluations and pharmacokinetic profiling of this nutraceutical product were done along with its evidence based effectivity against MetS.

2 Materials and methods

2.1 Plant materials

Stem bark extract of *F. religiosa* (*voucher specimen:* IITKGP/HB/2019/F1), seeds of *S. cumini* (*voucher specimen:* IITKGP/HB/2019/S1) and leaves of *O. bacilicum* (*voucher specimen:* IITKGP/HB/2019/O1) from the medicinal garden of Agriculture and food engineering department of IIT Kharagpur.

2.2 Chemicals

All chemicals and reagents used for the experimentation were all of analytical grade and were purchased either from Merck (India) and Sigma Aldrich.

2.3 Maintenance and care of animals

After obtaining permission from the animal ethical committee (Registration No: 1722/RO/ERe/S/13/CPCSEA, Approval No: ARTI/CPCSEA/2015/ARTI 09), animals were purchased from local vendors. Healthy, adult male wistar rats weighing 180 ± 5 g were used for the study. Animals were kept in poly-carbonated cages with bedding husk and maintained in lab feed and water ad libitum, as per CPCSEA guidelines.

2.4 Proximate analysis

Proximate analysis of the selected plant parts was carried out according to the Association of Official Analytical Chemists methods (AOAC, 1990) and other standard literatures [16].

2.5 Development of phytonutraceutical combinatorics (PNC)

The ratio of the constituents in PNC has been optimized by mixture design approach of chemometrics with the aid of Design Expert software 7.0 [17, 18]. Ratio of the constituents are considered as inputs and antioxidant potentials (studied by DPPH radical scavenging method) of the combinations are considered as the outputs. Basing on the desirability function, 1.15:1.15:1.68 (Ashwattha: Jamun: Basil or FR: SC: OB) is considered as the optimized ratio.

2.6 Preparation of PNC

The parts of different plants (bark of Ashwattha, seeds of jamun and leaves of Basil) were collected, dried, ground to powder (Bajaj electric grinder) and sieved, and the powder of different plant parts (bark, seed and leaves) are stored in respective air tight containers with proper labeling. Next the respective powdered parts are mixed in the weight ratio of 1.15:1.15:1.68 and macerated in distilled water boiled to 100 °C for 72 h with occasional stirring (both static and dynamic maceration). After subsequent filtration and centrifugation, the extract is concentrated by rotaevaporation, dried in hot air oven at 45 °C in sterile petridishes and the dried powder is scraped and kept in air tight containers in refrigerator. This is the active polynutraceutical combinatorics or PNC which is the active constituent of the oral nutraceutical tablets.

2.7 Chemo-profiling of the secondary metabolites in PNC

Chemo-profiling of secondary metabolites such as alkaloid, saponin, polyphenol, flavonoid, glycoside, triterpenoid and steroids was done as per the literature methods [19–22]. Detection of the different components was done in 10 g of sample powder of the respective plant parts. Total flavonoid content was determined using the Aluminum chloride colorimetric method in terms of standard quercetin equivalents. Total phenolic content was determined by Folin–CioCalteu reagent using gallic acid as the standard equivalents [19]. The total alkaloid content was determined by UV-spectrophotometric method based on the reaction between alkaloid and bromocresol green and the absorbance measured at 470 nm [20]. Saponin estimations were carried out spectrophotometrically, and absorbance was recorded at 544 nm against reagent blank. Diosgenin was used as the standard material [21]. Steroids were determined with 1 mL of the methanolic extracts taken in 10 mL of the volumetric flask; sulfuric acid (4 N, 2 mL) and $FeCl_3$ (0.5% w/v, 2 mL) were added followed by potassium hexacyanoferrate solution (III) (0.5% w/v, 0.5 mL). The mixture was heated in water bath at 70 ± 20 °C for 30 min with occasional shaking and further diluted up to mark with distilled water. The absorbance was measured at 780 nm against reagent blank [21]. Glycoside content was determined spectrophotometrically as per the method given by Kumar Tekeshwar et al., 2011 and absorbance recorded at 515 nm and calculated as aloe-emodin/Rhein [22].

2.8 In vitro studies on the effect of PNC

Antioxidant effect of PNC was studied on the basis of the DPPH free radical scavenging effect as per literature methods [23–25]; *hypoglycemic effect* was studied by *alpha amylase and alpha glucosidase inhibitory assay* procedure [26, 27]; hypolipidemic effect was studied by *pancreatic lipase inhibitory assay* [28–30].

Angiotensin converting enzyme (ACE) activates angiotensin-I into a potent vasoconstrictor called angiotensin-II that influences aldosterone release which increases blood pressure. So, ACE inhibitors are preferred as anti-hypertensives. The antihypertensive effect of PNC was studied by the assay method based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (HHL) catalyzed by the ACE [31–33].

2.9 Acute, sub-chronic and long-term toxicity studies of PNC

Acute toxicity studies were carried out in twelve male wistar rats as per the guidelines of Organization for Economic Cooperation and Development (OECD) guideline 425 and sub-acute toxicity studies for a period of 28 days. In acute toxicity studies, signs of mortality, behavioral and physical abnormalities were observed from the cage side. In sub-chronic toxicity studies, the effects of PNC on body weight of experimental animals, hepatic and renal enzymes were studied [34].

No observed adverse effect level or NOAEL (mg/kg or mg/kg/day) is the highest dose level that does not produce any significant increase in any adverse effect; though not statistically significant, biologically significant adverse effects are to be accounted for NOAEL calculation. Repeated dose toxicity studies are one of the methods that help to arrive at the NOAEL value [35].

2.10 Formulating the PNC as an oral tablet

Considering the case of organoleptic issues and to maintain a proper effective dosimetry, PNC was formulated to conventional release tablets prepared by direct compression using single-punch tablet machines (Cad mach, Ahmadabad, India) [36]. The steps involved in direct compression include milling of PNC and the excipients (mentioned in Table 1), mixing of PNC with the excipients and finally compression into tablets. Basing on the prepared formula (Table 1), all ingredients were accurately weighed and sieved through #60 standard sieve. In order to mix the ingredients thoroughly, PNC and all the excipients were mixed geometrically in a mortar and pestle for 15 min and blended again thoroughly in a polythene bag. Tablets were compressed on a single-punch tablet machine (Cad mach,

Table 1 Composition for PNC conventional tablets

Ingredient Name	Use	F1	F2	F3	F4	F5	F6	F7	F8	F9
PNC	Active compound	500	500	500	500	500	500	500	500	500
Microcrystalline cellulose	Disintegrant	24	42	60	24	42	60	24	42	60
Lactose	Diluent	68.5	43	13						
Mannitol	Diluent				68.5	43	13			
Starch	Diluent							68.5	43	13
Talc	Lubricant	1.5	3	6	1.5	3	6	1.5	3	6
Magnesium stearate	Lubricant	1.5	3	6	1.5	3	6	1.5	3	6
Colloidal silica	Glidant	1.5	3	6	1.5	3	6	1.5	3	6
PVP	Binder	3	6	9	3	6	9	3	6	9

Ahmadabad, India) using 10 mm punches. Hardness of the tablets was maintained about 4–5 kg/cm^2.

2.11 Evaluation of the tablet

Prior to compression of the PNC tablets, precompressional evaluations (micromeritic studies) were done to study the compressibility and flow properties of the PNC and the excipients powder blend. Flow properties were determined by measuring Angle of Repose (fixed funnel method); Bulk Density (BD) and Tapped Bulk Density (TBD) by Cylinder method; Carr's Compressibility Index using the equation: Carr's Compressibility Index (%) = [(TBD − BD)/TBD] × 100; and Hausner's ratio was determined by the equation: Hausner's Ratio = TBD/LBD (Table 4). Hausner's ratio less than 1.25 indicates good flow while greater than 1.5 indicates poor flow using standard procedures [37].

After compression of the tablets, post-compressional evaluations of PNC tablets were done. PNC tablets were evaluated for hardness using Monsanto hardness tester; friability was determined using Roche Friabilator; the thickness and diameter of the tablets were determined using Vernier calipers; weight variation test was carried out as per official methods with the specification limit that is not more than two of the individual weight deviates from the average weight by 10% and none should deviate by more than twice that percentage [37]. In order to understand the kinetics and mechanism of release of PHC from the formulations, the results of the in vitro release studies were fitted to various kinetic equations such as zero order (cumulative percent drug release vs. time); first order (log cumulative percent drug retained vs. time); Higuchi (cumulative percent released vs. √time); and Peppas (log of cumulative percent drug release vs. log time). The kinetic model that best fits the dissolution data was evaluated by comparing the regression coefficient values (r) obtained in various models. The N values (release exponent) in Peppas model were used to characterize different release mechanisms, where values of $n = 0.5$ indicate Fickian diffusion, values between 0.5 and 1.0 are for non-Fickian diffusion and $n = 1$ indicates zero order [38–41].

2.12 Pharmacokinetics

PNC is a combinatorics of several plant secondary metabolites. For assessment of pharmacokinetic parameters, experimental rats were fed with mini tablets that were placed firmly on the flat end of the gavage needle and inserted directly into stomach by intra gastric gavage. Blood was withdrawn from the tail veins of the rats at 2, 4, 8, 12 h interval and was analyzed for flavonoid content spectrophotometrically [42]. Pharmacokinetic parameters were determined with the help of PK Solver.

2.13 In vivo assessment of multimodal effectivity of PNC oral formulations

To evaluate the multifunctional effectivity of PNC in treating MetS viz hypoglycemic, hypolipidemic effect and antihypertensive effect were studied in two different animal models (Type 2 diabetic rat model and hypertensive rat model). Type 2 diabetic rat models were developed by injecting intravenously streptozotocin (50 mg/kg) dissolved in ice cold citrate buffer, 0.1 M, pH 4.9 along with high fat diet [43, 44]. The high fat diet is composed of: Casein-342 g, Cystine-30 g, Starch-172 g, Sucrose-50 g, Cellulose-50 g, Ground Nut Oil-25 g, Thallow-190 g, Mineral mixture-35 g, Vitamin mixture-10 g with multiple low doses of STZ injection. The diabetic state (fasting blood glucose > 250 mg/dl) was confirmed 3 days after STZ injection by measuring fasting blood glucose (Glucometer).

Animals were divided into five groups with six animals in each group. Group I is normal control which were treated with distilled water (5 ml/kg), Group II is the vehicle-treated negative control group; Group III is the STZ treated diabetic control group, Group IV is PNC treated group at 150 mg/kg doses, Group V was the metformin (150 mg/kg)-treated positive control group. Test substances were administered orally to the experimental

animals by gavage needle. Fasting Blood glucose (FBG) was determined with the help of glucometer. Oral glucose tolerance test (OGTT) was done in rats deprived of food for 12–14 h and subjected to oral glucose challenge at concentration of 2 g/kg b.w.; blood samples were collected from tail vein at 0 (before administration), 60 and 120 min, and the glucose content was analyzed in glucometer. Further homeostasis model assessment or HOMA was determined [45]. For estimating the hypolipidemic activity of the test substances, all animals were sacrificed under deep anesthesia and blood was withdrawn from heart. The biochemical estimations of lipid profile, *i.e.,* total cholesterol, HDL-cholesterol and triglycerides in serum were determined spectrophotometrically using commercial kits [46]. All experimental results were expressed as mean ± SD and analyzed by Student's *t* test (paired or unpaired, as desired) and $P < 0.05$ was considered significant.

2.14 Development of salt-induced hypertensive rat models

Salt-induced hypertensive rat models were developed as per literature [47, 48]. Animals were divided into five groups with six animals in each group. Group I are the normotensive rats administered with water (10 mL/kg/day), Group II are the salt-induced hypertensive rats which were administered with 18% w/v NaCl solution (10 mL/kg/day), Group III is the PNC treated group, and the Group IV is treated with captopril (20 mg/kg/day) that served as positive control. Oral administration was done to experimental animals by oral gavage needle.

2.15 Blood pressure measurement

Systolic and diastolic arterial blood pressure of experimental animals was measured by tail-cuff method [47–49].

2.16 In vivo estimation of concentration of Renin and ACE

After anaesthetizing the rats, blood samples were collected from the inferior venacava, kept for 15–20 min at room temperature and then centrifuged at 3000 rpm; the separated serum stored at − 80 °C and rennin and ACE concentrations measured at ng/mL using ELISA kits [47–49].

2.17 Aortic ring assay

Rats were sacrificed by decapitation, and the thoracic aorta was isolated, cleaned of fat and connective tissue, and all aortas were denuded of endothelium film by gentle mechanical procedure and, finally, cut into rings of about 4–5 mm of width. The rings were tied to stainless steel hooks with silk thread and immersed into 10 mL organ baths of Krebs solution at 37 °C and oxygenated (O_2/CO_2, 95: 5). A basal tension of 2 g was established for all tissues. The temperature was maintained at 37 °C throughout the experiment. The isometric tension generated by the coronary artery was measured using a force–displacement transducer. KCl (120 mM) was administered so as to maximize the contraction of each prepared aorta. Further tissues were pre-incubated with test sample (PNC). Relaxation was expressed as a percentage change from KCl contracted levels i.e., by comparison between maximum vascular contraction before and after addition of test sample (PNC) [47–50].

3 Results

3.1 Proximate analysis

The results of proximate analysis of three selected plant parts have shown that percent moisture content of FR bark, SC seed and OB fresh leaf are 60.4, 16.35, and 84.5, respectively. Carbohydrate content of FR bark and OB fresh leaf was found to be 15.5 and 5.25, respectively; protein contents are FR bark-2.5, SC seed-1.98, OB fresh leaf-4.65; fat contents are FR bark-1.7, SC seed-0.66, OB fresh leaf-1.98; the ash values were found to be 13.5, 2.2 and 1.56 for FR bark, SC seed and OB fresh leaf respectively.

Table 2 Software generated adequacy of model considering DPPH radical scavenging as Response

Source	Sum of squares	df	Mean square	F value	*p* value Prob > *F*	
Mean versus total	14,722.57	1	14,722.57			
Linear versus mean	747.34	2	373.67	11.49	0.0020	
Quadratic versus Linear	305.30	3	101.77	15.54	0.0011	
Sp Cubic versus Quadratic	20.74	1	20.74	4.58	< 0.0695	Suggested
Cubic versus Sp Cub	29.49	2	14.74	33.84	0.0012	Aliased
Residual	2.18	5	0.44			
Total	15,827.62	14	1130.54			

Table 3 Software generated model summary statistics considering DPPH radical scavenging as response

Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	5.70	0.6763	0.6174	0.4494	608.48	
Quadratic	2.56	0.9526	0.9229	0.8731	140.26	
Special cubic	2.13	0.9713	0.9468	0.7620	263.01	Suggested
Cubic	0.66	0.9980	0.9949	0.9494	55.86	Aliased

Table 4 Software generated ANOVA table considering DPPH radical scavenging as response

Source	Sum of squares	df	Mean square	*F* value	*p* value Prob > *F*	
Model	1073.38	6	178.90	39.55	< 0.0001	Significant
Linear mixture	747.34	2	373.67	82.60	< 0.0001	
AB	2.16	1	2.16	0.48	0.5118	
AC	224.59	1	224.59	49.65	0.0002	
BC	87.85	1	87.85	19.42	0.0031	
ABC	20.74	1	20.74	4.58	0.0695	
Residual	31.67	7	4.52			
Lack of fit	29.90	3	9.97	22.52	0.0057	Significant
Pure error	1.77	4	0.44			
Cor total	1105.05	13				

3.2 Chemometrics

Considering RSM, the process order fits to quadratic design model, the adequacy of models (Table 2) and model summary statistics (Table 3) justified by the analysis of variance (Table 4) considering DPPH radical scavenging as the response. The model summary statistics focus on the model maximizing the 'Adjusted R-squared' and the 'predicted R-squared.' The R-squared values of 0.9713 (Table 3) having closeness to unity showed a good fitness between the actual and that obtained from the response model. The 'Pred R-Squared' of 0.7620 is in reasonable agreement with the 'Adj R-Squared' of 0.9468. 'Adeq Precision' measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 18.516 indicates an adequate signal. This model can be used to navigate the design space (Table 3). The Model F value of 39.55 (Table 4) implies the model is significant. There is only a 0.01% chance that a 'Model F Value' this large could occur due to noise. Values of 'Prob > F' less than 0.0500 indicate model terms are significant. In this case, Linear Mixture Components, AC and BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The 'Lack of Fit F value' of 22.52 (Table 4) implies the Lack of Fit is significant. There is only a 0.57% chance that a 'Lack of Fit F value' this large could occur due to noise.

The desirability values for responses are shown in (Fig. 1). The dot on each ramp function graph indicates the optimal level of the parameter. Desirability function value ranges from zero, outside of the limits to one at the goal. The purpose of the program is to maximize the function; seeking of goal begins at a random starting point and proceeds up the steepest slope to a maximum. Basing on the desirability function, a maximal pharmacologic response (antioxidant potential studied by DPPH radical scavenging) was achieved at optimal with 1.15 g of bark powder of Ashwattha, 1.15 g of seed powder of jamun and 1.68 g of leaf powder of Basil.

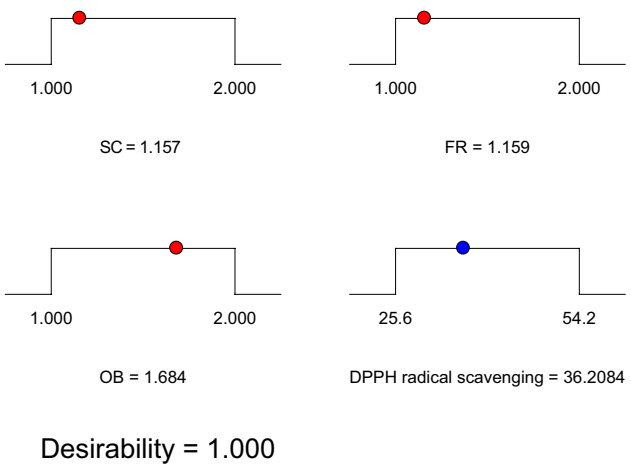

Fig. 1 Desirability ramp function graph

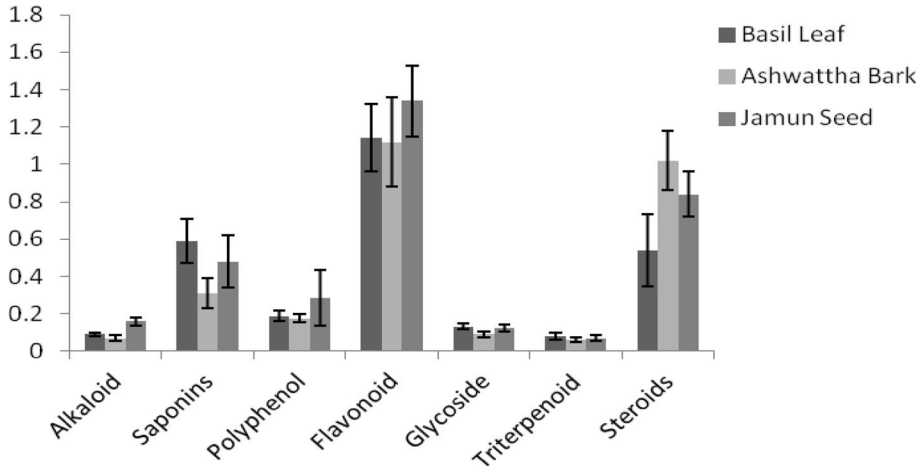

Fig. 2 Comparative chemo-profiling of the constituents in PNC

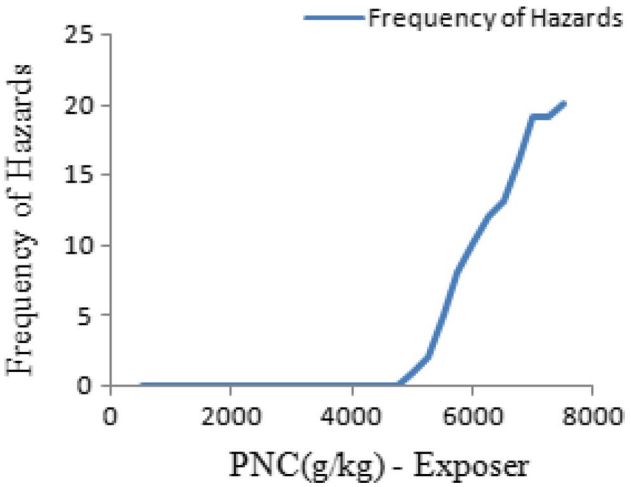

Fig. 3 The no observed adverse effect level (NOAEL) of PNC

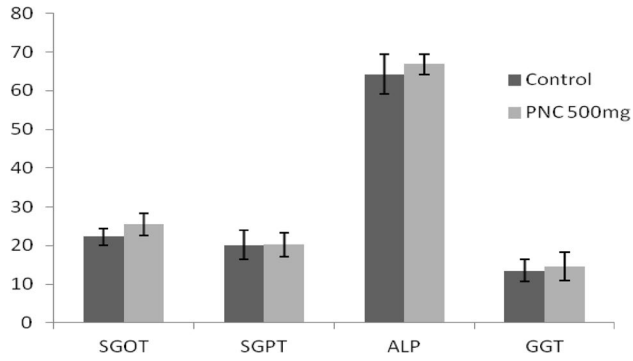

Fig. 4 Effect of PNC on the hepatic enzyme levels

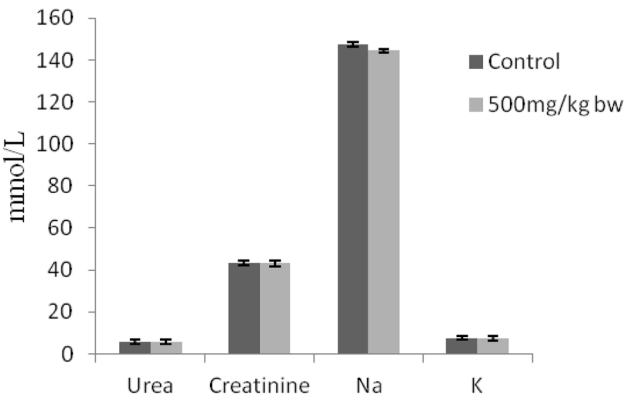

Fig. 5 Effect of PNC on the urea-creatinine level

3.3 Chemo profiling

Chemo-profiling of the constituents in standardized combined plant extract in PNC (Fig. 2) has shown the presence of alkaloids, polyphenols, flavonoids, saponins, glycosides, steroids and triterpenoids.

3.4 Toxicity studies

In acute toxicity studies, no signs of mortality or any behavioral or physical abnormalities were observed among the experimental animals (as observed from cage side) till the dose of 7.5 g/kg b.w. The LD50 value was determined to be 19.5 g/kg b.w. In long-term toxicity studies to achieve the NOAEL value, signs of adversities (changes in hepatic enzyme levels) were observed from 4780 g/Kg exposure to PNC (Fig. 3). So, 4700 g/kg is considered as the cut-off and thus the NOAEL value.

In sub-chronic toxicity studies with 500 mg of PNC (PNC nutracetical tablets were formulated with this dose) no adverse effects were observed on hepatic enzymes (Serum Glutamate Oxalate Transaminase or SGOT, Serum Glutamate Pyruvate Transaminase or SGPT, Alkaline Phosphatase or ALP, Gamma glutamyl transaminase or GGT) and urea-creatinine (renal effect) levels (Figs. 4 and 5).

Table 5 Precompressional evaluation of PNC tablets

Formulation code	Loose bulk density (gm/ml)*	Tapped bulk density (gm/ml)*	Hausner's ratio *	Carr's index (%)*	Angle of repose (θ°)*
F1	0.780 ± 0.001	0.918 ± 0.001	1.159 ± 0.001	12.956 ± 0.001	23.43 ± 0.639
F2	0.693 ± 0.006	0.782 ± 0.007	1.161 ± 0.003	13.793 ± 0.005	24.39 ± 0.029
F3	0.670 ± 0.007	0.799 ± 0.008	1.145 ± 0.006	12.648 ± 0.002	23.25 ± 0.345
F4	0.786 ± 0.001	0.889 ± 0.001	1.129 ± 0.001	11.497 ± 0.001	25.38 ± 0.350
F5	0.695 ± 0.008	0.788 ± 0.001	1.135 ± 0.005	11.854 ± 0.001	24.36 ± 0.432
F6	0.790 ± 0.001	0.885 ± 0.005	1.149 ± 0.001	12.899 ± 0.007	25.78 ± 0.273
F7	0.760 ± 0.005	0.878 ± 0.001	1.158 ± 0.003	13.794 ± 0.001	25.38 ± 0.375
F8	0.753 ± 0.001	0.889 ± 0.001	1.201 ± 0.001	17.323 ± 0.001	23.59 ± 0.201
F9	0.763 ± 0.001	0.911 ± 0.002	1.165 ± 0.001	14.396 ± 0.001	22.38 ± 0.462

*All experiments done in triplicate ad the data expressed in ±SD

Table 6 Post-compressional evaluation of PNC tablets

Formulation code	Dimension		Hardness (kg/cm^2)	Friability (%)	Weight variation (%)	Drug content (%w/w)
	Diameter (mm)	Thickness (mm)				
F1	7.49 ± 0.005	4.52 ± 0.004	4.26 ± 0.22	0.28 ± 0.03	224.66 ± 0.57	100.89 ± 0.73
F2	7.48 ± 0.004	4.55 ± 0.005	4.33 ± 0.25	0.35 ± 0.07	226.16 ± 0.28	99.67 ± 0.26
F3	7.49 ± 0.005	4.54 ± 0.004	4.27 ± 0.24	0.29 ± 0.03	222.56 ± 0.47	101.78 ± 0.53
F4	7.49 ± 0.005	4.55 ± 0.005	4.31 ± 0.21	0.33 ± 0.05	225.23 ± 0.44	99.59 ± 0.29
F5	7.49 ± 0.004	4.55 ± 0.005	4.28 ± 0.25	0.35 ± 0.05	225.52 ± 0.45	101.56 ± 0.45
F6	7.49 ± 0.004	4.52 ± 0.004	4.31 ± 0.26	0.34 ± 0.06	226.49 ± 0.41	100.67 ± 0.71
F7	7.48 ± 0.005	4.54 ± 0.004	4.29 ± 0.24	0.29 ± 0.05	226.53 ± 0.51	101.01 ± 0.54
F8	7.48 ± 0.005	4.55 ± 0.005	4.31 ± 0.23	0.33 ± 0.07	224.61 ± 0.32	99.87 ± 0.65
F9	7.48 ± 0.004	4.55 ± 0.005	4.25 ± 0.25	0.34 ± 0.07	226.62 ± 0.35	99.81 ± 0.29

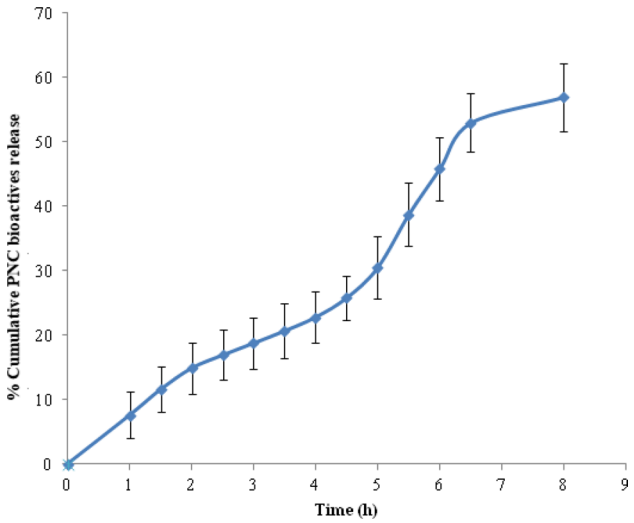

Fig. 6 Dissolution profile of PNC tablets

3.5 Pre- and post-compressional studies

The PNC have been formulated to oral nutraceutical tablet. The micromeritic studies of PNC and excipient blend (as per Table 1) have shown good flow properties and compressibility (Table 5) and results of post-compressional evaluation (Table 6) show that PNC nutraceutical tablet passes the quality control tests.

PNC tablets are conventional release tablets; the dissolution profile of PNC tablets (Fig. 6) on being fitted to different kinetic equations (zero order, first order, Higuchi and Peppas) is found to follow first-order release kinetics. The release of PNC is by erosion mechanism with also a non-Fickian diffusion pattern.

3.6 Pharmacokinetic studies

Considering the pharmacokinetic parameters, as studied by non-compartmental analysis of plasma data after extravascular input by Linear Trapezoidal method, $AUC_{0\text{-}t}$ (µg/ml × h) is 493.80, $AUC_{0\text{-}inf\_obs}$ (µg/ml × h) is 582.20,

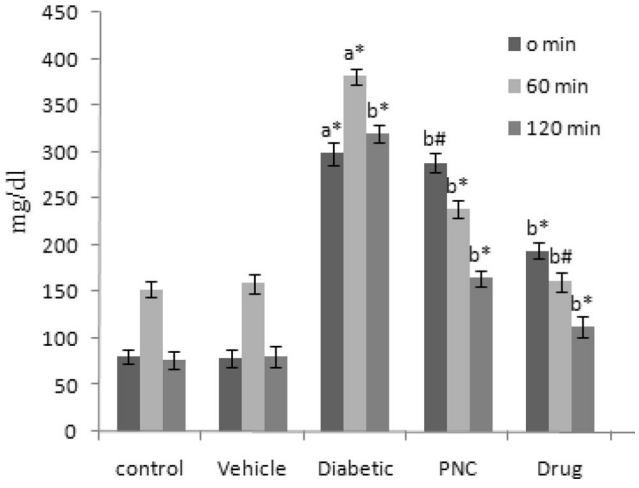

Fig. 7 Blood glucose lowering effect of PNC in oral glucose tolerance test

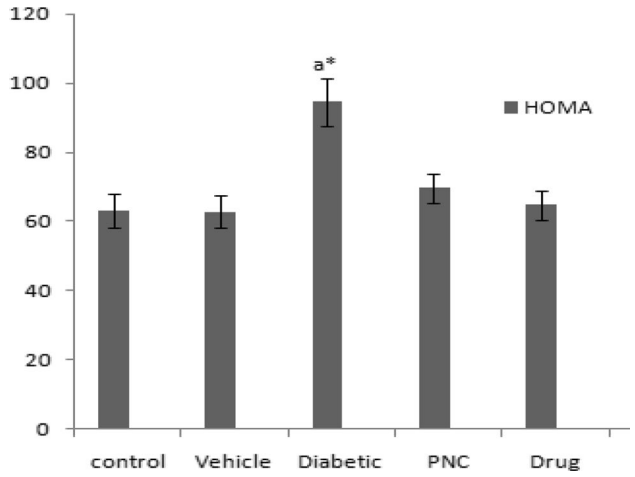

Fig. 8 Effect of PNC on homeostasis model assessment

$AUMC_{0\text{-inf\_obs}}$ (μg/ml × h^2) is 7294.62, $MRT_{0\text{-inf\_obs}}$ (h) is 12.53, C_{max} (μg/ml) is 48.43, T_{max} (h) is 10.

3.7 Hypoglycemic, hypolipidemic and antihypertensive effect

The hypoglycemic effect of PNC was observed in its significant lowering of blood glucose level in oral glucose tolerance test (Fig. 7) and HOMA (Fig. 8) in comparison to diabetic group. Further PNC showed a significant lowering of blood lipid profile (Table 7) with respect to diabetic group.

Further the antihypertensive effect of PNC was substantiated by its lowering of blood pressure, diminution in rennin values and enhancement in ACE level and also increasing the % relaxation in comparison to salt-induced hypertensive group (Table 8).

4 Discussion

MetS is a combination of risk factors of metabolic origin viz. insulin resistance, hyperinsulinemia, impaired glucose tolerance, type 2 diabetes, obesity, elevated blood pressure and dyslipidemia all coupled together. MetS can be treated in both pharmacologic and non pharmacologic manner. Integrative medicine focuses to combine complementary and alternative medicine practices with conventional medicine. Different pharmacological treatment approaches for MetS though available but always not free of side effects and in many cases long-term safety is not guaranteed. Non-pharmacological treatment approaches is often preferred owing to cost-effectivity, for those who are intolerant to pharmacological therapy and non-pharmacological approaches are often an adjunct to conventional therapy. Nutraceuticals are mostly of plant origin (hence phytonutraceuticals) and can be applied as adjunct therapy in combating several ailments. Effectivity of phytochemicals as preventive aid for MetS is also supported by corroborative research

Table 7 Hypolipidemic effect of PNC

Parameter	Day	Group 1 (normal control)	Group 2 (vehicle treated)	Group 3 (diabetic control)	Group 4 (Intake of PNC in doses 150 mg/kg)	Group 5 (positive control-received metformin, 150 mg/kg)
TC	28	72.4 ± 8.4	72.9 ± 7.6	168.4 ± 15.6*[a]	111. 9 ± 9.3*[b]	108.8 ± 9.7*[b]
TG	28	90.2 ± 12.4	88.2 ± 11.8	243.8 ± 13.9*[a]	125.6 ± 5.4*[b]	141.5 ± 6.8#[b]
HDL	28	44.1 ± 5.6	46.2 ± 6.2	16.9 ± 5.4 *[a]	34.5 ± 9.4*[b]	33.6 ± 7.5
LDL	28	68.7 ± 7.6	65.2 ± 8.1	114.7 ± 8.4*[a]	85.7 ± 6.8*[b]	86.7 ± 7.2*[b]
VLDL	28	16.8 ± 4.8	15.7 ± 3.9	42.6 ± 8.2*[a]	25.8 ± 6.2*[b]	36.3 ± 8.4

*means significant at $p<0.05$ and #means significant at $p<0.005$

[a]means compared to control group

[b]means compared to treatment group

Table 8 Effect of PNC on salt-induced hypertensive rats

Groups	NT (water 10 ml/kg/day)	SIH (HS 10 ml/kg/day)	PNC treated	Drug treated
Systolic BP(mm of Hg)	121.2 ± 7.5	206.5 ± 15.3	145.6 ± 10.2	128.4 ± 9.5
Diastolic(mm of Hg)	80.8 ± 5.2	154.3 ± 8.6	106.7 ± 7.9	91.3 ± 9.2
Renin (ng/ml)	3.94 ± 0.52	1.17 ± 0.27	3.54 ± 0.51	4.02 ± 0.41
ACE (ng/ml)	17.54 ± 1.54	23.62 ± 2.16	18.96 ± 1.39	17.54 ± 1.32
Aortic ring assay	Near to 94% relaxation	Sustained contraction with 5275 ± 564 at 120 mM KCl was considered max	78.54 ± 5.54% relaxation	92.64 ± 3.4% relaxation

evidences [51–53]. The experimental results have shown that PNC has a hypoglycemic (Figs. 7, 8), hypolipidemic (Table 7) and antihypertensive effect (Table 8). PNC is a combinatoric nutraceutical product of bark of Ashwattha, seeds of jamun and leaves of Basil. PNC thus consists of a huge library of secondary metabolites viz. polyphenols, flavonoids, saponins, alkaloids and steroids etc. (Figure 2). Apart from antioxidant potentials by scavenging free radicals, alkaloids aid in glycemic control by influencing glucose transport, affecting digestion and absorption of carbohydrate by inhibiting alpha amylase and alpha glucosidase, inhibiting dipeptidyl peptidase-4 so as to inhibit the antidiabetic gut hormone Glucagon like peptide-1, stimulating insulin secretion, regeneration of pancreatic beta cells, etc. Glycosides mostly promote glycogen synthesis and trigger insulin secretion [54]. The hypoglycemic effect of dietary polyphenols and flavonoids are mostly due to alpha amylase and alpha glucosidase inhibitory effect, stimulation of insulin secretion and thereby reducing hepatic glucose output, enhancing insulin dependent glucose uptake, activating 5´ adenosine monophosphate activated protein kinase (AMPK) etc. [55]. Flavonoids not only powerful antioxidants but stimulates insulin secretion, has insulinomimetic actions, exerts hypolipidemic effect by suppressing HMG-CoA (Hexa methyl glucose-coenzyme A) etc. [54, 55]. Saponins enhance insulin secretion, regeneration of pancreatic beta cells, free radical scavenging etc. [54]. Available research evidences have also documented the antihypertensive potentials of phenolics and flavonoids. Apart from being a powerful antioxidant, hypoglycemic and hypolipidemic, flavonoids are found to decrease formation of artherosclerotic plaques, reduce arterial stiffness,and exert a vasodilatory effect. Polyphenolic compounds with their antioxidant potentials are found to decrease blood pressure by increasing endothelial nitric oxide bioavailability; suppression of mRNA expression of NADOH oxidase and improvement in endothelium dependent vasodialation in aorta [56]. Since PNC is a combinatorics of several plant secondary metabolites, its multimodal pharmacological effect is due to the cumulative synergistic presence of the secondary metabolites.

5 Conclusion

The standardized combined plant extract in PNC formulation had shown significant effectivity in combating MetS. Our research evidences have shown its hypoglycemic, hypolipidemic and antihypertensive effect. The phytonutraceutical combinatoric product in the form of oral tablet is expected to facilitate its applicability. However extensive human studies are warranted in this regard.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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