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RESEARCH ARTICLE

Hypoglycemic Hypolipidemic Evaluation of a Polyherbal Phytocomposite and the Mechanism of its Synergistic Anti-Diabetic Effect

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ABSTRACT

This research aims to study the safety profile of a Polyherbal phytocomposite (PHC) prepared from the leaf powders of *Ficus benghalensis* (Banyan), *Syzigium cumini* (Jamun) and *Ocimum sanctum* (Tulsi) followed by its *in vivo* hypoglycemic and hypolipidemic studies and comparative evaluation of its effect with its constituent species. PHC did not show any adversities like mortality signs or behavioral anomalies at doses of 7.5g/kg body weight; first death was recorded at 10g/kg. PHC increased body weight to some extent with significant ($p<0.05$) changes in organ weight at doses of 500 and 750mg/kg. There was a significant decrease in plasma protein, ALT and increase in creatinine, AST at $p<0.05$ level at a dose of 750mg/kg. The hemoglobin levels were also significantly increased. PHC showed significant lowering of fasting blood glucose and blood lipid ($p<0.001$) with actions better than metformin. The hypoglycemic-hypolipidemic action of the PHC when compared with its constituent plant species (Banyan, Jamun and Tulsi) and standard drug metformin was found to be significantly better ($p<0.001$) thus suggesting phytosynergism amongst the phytomolecules present within PHC.

Key words: phytocomposite; phytomolecules; hypoglycemic; hypolipidemic; safety profile; phytosynergism

INTRODUCTION

The phyto kingdom being a giant hub of undiscovered phyto-molecules with versatile pharmacology, leading to a great resurgence in the potentials of phytomedicine worldwide. World Health Organization (WHO) has reported that even today 80% of the world's population depends on traditional system of medicine (TSM). TSM recommends the use of either whole plant material or plant extract and in many cases combination of two or more plant products are used for the treatment of one or more disease conditions. In developing countries like India, enriched with wide availability of medicinal plants, there is a tendency of indiscriminate use of such plants or their extracts without being aware of its toxic effects.[1,2] Due to lack of evidence based scientific data on the safety aspects, well defined protocol for chemical characterization, proper dosage monitoring, length of period to use such medicines and sufficient toxicity data to establish safety, the heritage of TSM can't compete with the concurrent allopathic medicinal system. In order to promote the global acceptance of herbal medicine, WHO recommended extensive scientific investigation on the toxic effects of medicinal plants prior to their uses; this was also supported by works of other researchers.[3-6]The Indian subcontinent is bestowed with enriched plant kingdom with diverse pharmacology which in many cases are still unveiled. *Ficus benghalensis* (Banyan tree), *Syzigium cumini* (Jamun) and *Ocimum sanctum* (Tulsi) have documented anti-diabetic potentials. *Ficus benghalensis*, belonging to family *Moraceae*, also known as Indian Banyan and is native to Indian subcontinent. Commonly it is also known as Vata. *Syzygium cumini*, is an evergreen tropical tree in the flowering plant family *Myrtaceae* also called black pulm, is native to the Indian subcontinent and adjoining regions of

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South East Asia. Commonly it is known as jambul, jambolan, or jamun. *Ocimum sanctum*, is an aromatic plant in the family *Lamiaceae*, also known as Holy Basil, is native to Indian subcontinent and cultivated throughout the south east Asian tropics. Commonly it is known as Tulsi or Tulasi. Combination therapy with poly herbals or phytoceuticals has gained popularity in terms of providing multiple and synergistic health benefits.[7,8] Oleanolic acid is found to provide a synergistic effect with first line antidiabetic metformin.[9] Sesame oil forms a synergistic anti diabetic combination with glibenclamide.[10] Fenugreek-tulsi composite was found to control blood gluco-lipid profile of type 2 diabetics. [11] Literature reports the studies of the effect of a composite prepared from the Tulsi leaves (*Ocimum sanctum*), Amla (*Emblica officinalis*), Bitter Gourd (*Momordica charantia*), Gurmur leaves (*Gymnema sylvestre*) and Jamun (*Syzygium cumini*) fruit and its seed on mild diabetic patients. [12] Further research works of Mitra, 2008 on the use of herbal ingredients by the indigenous or tribal populace of Bengal have shown that a small populace in Binpur area is in the habit of consuming 21 tulsi leaves per day to control type 2 diabetes and the rural population in Jhargram area are in practice of taking banyan leaf and bark decoction with a positive impact in controlling blood glucose level. [13]

A phytocomposite (PHC) prepared from the leaf powders of banyan, jamun and tulsi in varying weight ratios is found to show synergistic antioxidant and anti-diabetic actions in various *in vitro* enzyme inhibitory assays that are found to play a role in the pathogenesis of Type 2 diabetes. [8] This research aims to study the safety profile of the PHC and assess its effect on blood biochemistry and cellular evaluation, *in vivo* hypoglycemic and hypolipidemic properties and evaluate its synergistic anti-diabetic effect in comparison to its constituent species (Banyan, Jamun and Tulsi) thus making its therapeutic potentialities evidence based which is new with the relevant PHC formulation.

MATERIALS AND METHOD

Plant materials

Good, fresh, disease free mature leaves of *Ficus benghalensis (Voucher Specimen: IITKGP/HB/2014/J1), Ocimum sanctum (Voucher Specimen: IITKGP/HB/2014/J2)* and *Syzigium cumini (Voucher Specimen: IITKGP/HB/2014/J3)* were collected from the natural and manmade forests of IIT Kharagpur and adjoining areas like Prembazar, Gopali, Balarampur etc.

Maintenance of animals

After obtaining the animal ethical committee permission (Clinical Trials number: RKC/IAEC/15/06/01) healthy wistar male rats of 150-200 g were purchased from local vendors and housed in the animal house at Department of Pharmacology, R.G. Kar Medical College, Kolkata. The room conditions were maintained at 26±2° C temperature and 44-56% relative humidity with alternate light and dark cycles of 10 and 14h respectively one week before and during experimentations. Animals were provided with standard rat pellets and water *ad libitum*. Care of animals was taken as per the guidelines of "Principles of laboratory animal's care" (NIH publication no. 82-23, revised 1985).

Preparation of Phytocomposite (PHC)

The PHC prepared from the leaves of banyan, jamun and tulsi showed synergistic anti-diabetic and antioxidant effect as evidenced by works of De et al. 2015.[8] Potentiation of effect was proved by comparing the results of different *in vitro* enzyme inhibitory assays of the extracts of individual species and varying ratios of PHC. [8] The hydroethanolic extract of the PHC banyan: jamun: tulsi (1:1:2) exhibited maximum synergism in terms of anti-diabetic and antioxidant effect.[8] Toxicity studies and *in vivo* animal experimentations are being done with the PHC.

Acute toxicity studies

The study was carried out in male wistar rats as per the guidelines of Organization for Economic Cooperation and Development (OECD) guideline 425. The PHC was administered to nine groups (6 animals in each group) of rats by oral gavage in doses of 1, 2.5, 5, 7.5, 10, 20, 25, 30 g/kg body wt. All animals were observed for general cage side activities and behavioral changes (like autonomic effects viz. salivation, lacrimation, perspiration, piloerection, urinary incontinence and Central Nervous System effects viz. gait, tremor, convulsion, drowsiness), toxicity symptoms (skin and fur condition, subcutaneous swelling, abdominal distension, pupil diameter, dullness & opacity of the eyes, color & consistency of the feces, wetness or soiling of the perineum, condition of teeth, breathing

abnormalities), and mortality signs within first four critical hours of treatment, followed by next 24hr and observed for 14 days following the research protocols of Kulkarni et al. 1992. [14]

Sub chronic toxicity studies

The study protocol was developed as per the literature methodologies. [3, 15, 16] Both male and female wistar rats weighing about 165±10 g were selected for sub chronic test. Animals were divided into one control and four test groups with six animals in each group. The four test groups received doses of 100, 250, 500 and 750 mg/kg body weight respectively; orally once daily for 30 days. From the beginning of experiment till the end, weights of animals were recorded at every 5th day interval to record the weight variations. At the end of treatment, animals were anaesthetized; blood collected by cardiac puncture into two tubes containing EDTA for immediate estimation of hematological parameters and the other tube containing heparin for extracting the plasma and biochemical estimations of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), creatinine, protein content determined by standard enzymatic assay procedures.[16-20] Hemoglobin content was determined by cyanmethaemoglobin method and haematocrit as per literature. [21]

***In vivo* hypoglycemic-hypolipidemic studies of the PHC**

The hypoglycemic-hypolipidemic effects of the PHC have been studied in adult wistar rats (150-175g). Animals were kept in poly carbonated cages with bedding husk and maintained in lab feed and water *ad libitum*, as per CPCSEA guidelines. Dose selection was done on the basis of pilot trial. The five point dose study was conducted and from that study three doses (50 mg/ kg, 100 mg/ kg & 150 mg/ kg) were selected and administered orally in wistar rats. Standard drug Metformin 500 mg/ kg, orally was the selective dose.

Induction of diabetes

Anti-hyperglycemic activity of PHC was studied in Streptozotocin (Sigma, USA) induced diabetic male wistar rats. Streptozotocin (STZ) was dissolved in ice-cold citrate buffer (0.1 M, pH 4.9) and injected intravenously through the tail vein in rats (except normal control) at the dose of 50 mg/kg. The diabetic state (fasting blood glucose >250 mg/dl) was confirmed 3 days after STZ injection by measuring fasting blood glucose (Glucometer) also corroborated by the literature research works. [22, 23]

Single and multiple dose study

For the purpose of single dose study, on 14th day, blood glucose was estimated in 16 h fasted STZ diabetic rats (blood glucose > 250 mg/dl). The animals were divided into six groups (N=6). Thus animal grouping and drug administration were done as follows:

Group I (Normal Control)- received distilled water 5 ml/kg; Group II (STZ/diabetic Control)- received distilled water 5 ml/kg; Group III (Positive control) – received Metformin 500 mg/kg; Group IV- received 50 mg/kg of PHC; Group V- received 100 mg/kg of PHC and Group VI- received 150 mg/kg of PHC. All drugs were given orally. Thereafter, blood glucose was monitored at 30 min, 60 min, 90 min and 120 min interval after treatment using glucometer. For multiple doses study (28 days), the anti-hyperglycemic activity of test compound PHC was further studied in STZ induced diabetic rats. All the animals were treated with the same dose of PHC and Metformin once daily for 4 weeks. As per experimental protocol, blood glucose was determined 1 h after last dose given as described earlier.

The hypolipidemic activity of the PHC was also studied in STZ induced diabetic rats. The treatment regimen was same as describe earlier and continued once daily for 28 days. Thereafter all animas 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. 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.

Effect of PHC on glycosylated hemoglobin

Estimation of glycosylated hemoglobin (HbA1c) was found to be useful in monitoring the effectivity of a therapy in diabetes. The effect of PHC on glycosylated hemoglobin level was determined by micro chromatographic method using ion exchange resin in a polyphosphate buffer with a pH close to pKa of the carboxyl group of the resin that allows separation of minor components like glycosylated hemoglobin as per literature. [24]

RESULTS

From the cage side observations of animals undergoing acute toxicity testing no such abnormalities were visualized (**Table 1**). In acute toxicity studies with the PHC, 100% death was observed only at a very high dose of 30 g/kg body weight (**Table 2**). No death was observed up to a high dose of 7.5 g/kg b.w. and 7.14, 21.43, 39.29, and 64.29 % death was observed respectively with animals receiving 10, 15, 20, 25 g/kg b.w. of the PHC. The LD_{50} value of the PHC was determined to be 20.25 g/kg b.w.

Table1: Results of cage side observations in acute toxicity studies of the PHC.

Parameters	Observations (0-14 days)
Food/water consumption pattern	Slight Increase in food intake
Cage side activity changes in skin and fur condition	Normal
subcutaneous swelling	Nil
abdominal distension	Nil
pupil diameter	Normal
dullness & opacity of the eyes	Nil
color & consistency of the feces	Nil
condition of teeth	Normal
breathing abnormalities	Nil
other autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence)	Nil
Central Nervous System (gait, tremor, convulsion, drowsiness)	Nil

Table 2: Records of mortality in acute toxicity studies of PHC

Group	No. of Rat	Dose of PHC(g/kg b.w.)	Number of dead rat	% Cumulative of dead rat
1	6	Control*	0	0.0
2	6	2.5	0	0.0
3	6	5.0	0	0.0
4	6	7.5	0	0.0
5	6	10	2	7.14
6	6	15	2	21.43
7	6	20	3	39.29
8	6	25	4	64.29
9	6	30	6	100.00

***Control group each animal received only distilled water**

In sub chronic studies with the PHC, the effect of the PHC on body weight of animals and weight of organs (heart, liver, kidney) are presented in (**Figure 1&2)**; effect on blood biochemical parameters viz. plasma protein, creatinine, AST and ALT and hematological parameters in (**Figure 3 & Table 3)** respectively. There was some increase in body weight of PHC treated experimental animals in comparison to the control group (**Figure 1**) and significant changes ($p<0.05$) in the weight of organs (heart, liver, kidney) was seen in experimental groups treated with only higher doses of 500 and 750 mg/kg b.w. (**Figure 2**) though macroscopic observations did not show any changes in color and physical deformities in the organs. (**Figure 4)** shows the 5-point dose response curve. Effect of the single dose (50mg/kg) of the PHC on fasting blood glucose and multiple dose of PHC (50, 100 and 150 mg/kg) on blood gluco-lipid profile along with its synergistic hypoglycemic-hypolipidemic effect in comparison to the individual plant species (FB, SC, OS)are presented in (**Figure 5&6)** respectively. The molecular characterization of PHC is presented in (**Figure 7)**.

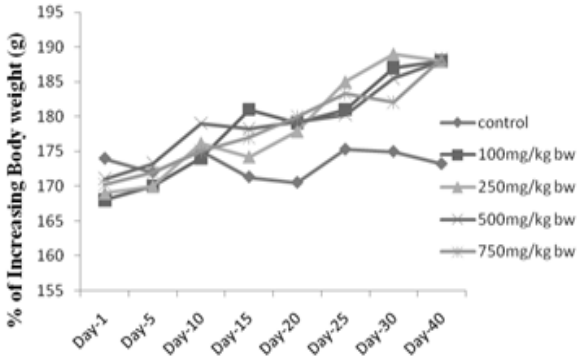

Figure 1: Mean percentage increase in body weight of the PHC treated gropus in comparison to the control group as that observed in 40 days of sub chronic toxicity study. Control, 100 mg/kg body weight, 250 mg/kg body weight , 500 mg/kg body weight and 750 mg/kg body weight.

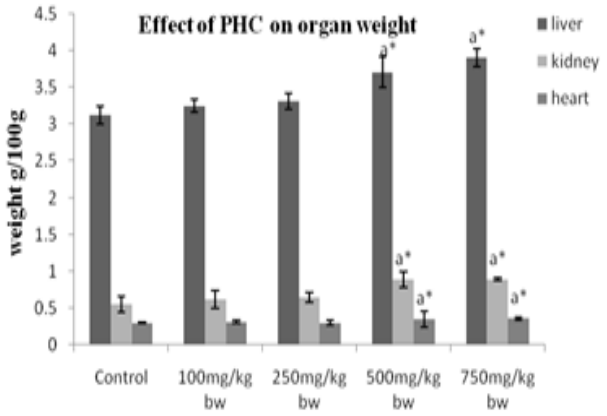

Figure 2: Effect of PHC on organ weight in control and treated rats in sub chronic toxicity studies. All data expressed as Mean ± SD, (n = 6), *significantly different from control at $p<0.05$; Control group received 0.5 ml distil water.

Table 3: Effect on the hematology in control and PHC treated experimental animals during sub chronic studies

Parameter	Control	100mg/ kg bw	250mg/ kg bw	500mg/ kg bw	750mg/ kg bw
Hemoglobin (mg/dl)	12.10± 0.2	13.90 ± 0.11*	13.6 ± 1.20*	12.1 ± 0.20*	10.29 ± 0.05*
RBC (10^6/mm^3)	9.12±0. 02	9.45 ± 0.02	9.01 ± 0.02	8.98 ± 0.06	7.91 ± 0.13
WBC (10^3/mm^3)	12.40± 0.02	10.60 ± 1.65	11.90 ± 0.03	12.8 ± 0.12	11.62 ± 0.43
PCV %	39.5 ± 2.10	40.05 ± 1.12	35.85 ± 1.50	32.55± 0.20*	31.55± 0.20*

****All results are expressed as mean ± SD, (n = 6), *significantly different from control at p<0.05; control group was provided with 0.5ml of distilled water.**

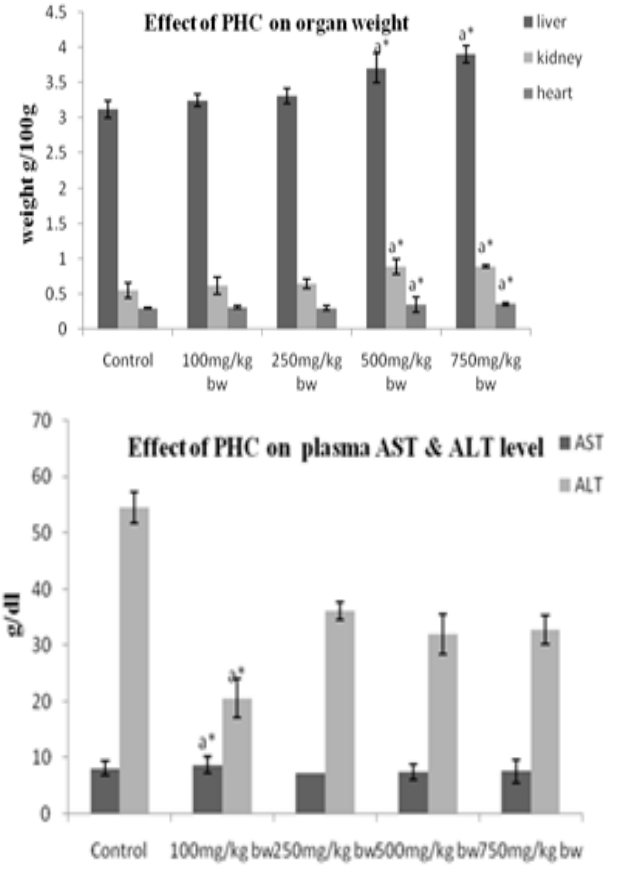

Figure 3: Effect of PHC on plasma protein, creatinine (left) and AST, ALT (right) in control and treated experimental groups in 40 days of sub chronic tests. All data expressed as mean ± SD, (n = 6), * significantly different from control at p<0.05; Control group received 0.5 ml distil water

A single dose of intravenous STZ injection (50mg/kg) elevated FBG level by nearly 213.9% compared to normal fasting blood glucose in rats. Moreover, FBG levels in all groups (expect group I) were same before drug treatment. FBG (mg/dl) determined after 30 min showed that, STZ elevated FBG level by 219.8% (p<0.0001) in comparison to normal fasting blood glucose in rats. Metformin lowered FBG levels by 8.9% and PHC at the doses of 50, 100 and 150 mg/kg exhibited 5.4%, 6.8% and 8.1% reduction respectively than STZ control within 30 min, but all results were statistically not significant. At 60 min interval, FBG which was elevated by about 216.6% (p<0.001) showed 22.1% lowering (p<0.01) by metformin and 15.7%, 19.6% and 21.8% lowering by PHC at doses 50, 100, 150 mg/kg respectively with statistical significance of p<0.01 for 50mg/kg PHC and p<0.001 for 100 and 150mg/kg. Here the actions of PHC were found to be better than metformin. After 90 min FBG which was elevated by about 244% (p<0.001), was lowered by 26.8%, 29.2% and 30.4% with 50, 100, 150 mg/kg of PHC respectively at p<0.01 level of significance, the actions of PHC being similar to metformin (30.8% reduction, p<0.001) in comparison to STZ control.

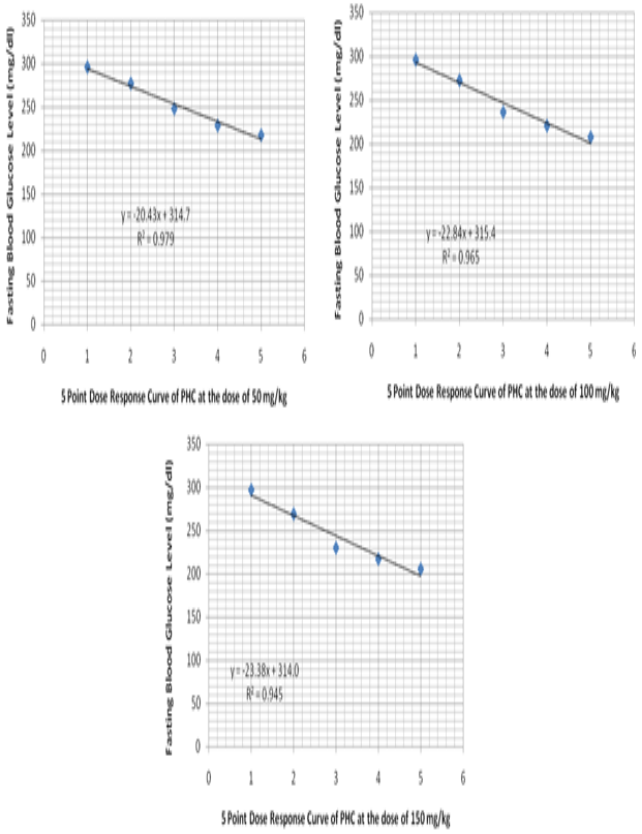

Figure 4: 5-point dose response curve for dose selection of PHC for hypoglycemic-hypolipidemic studies

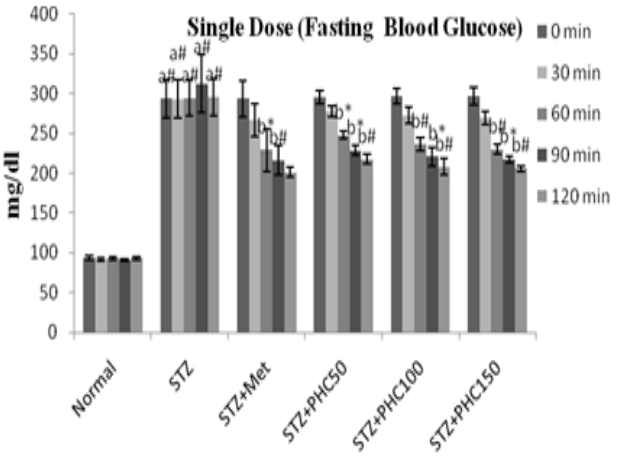

Figure 5: Effect of Single dose (50mg/kg) of PHC on the fasting blood glucose level at different time intervals. All data are expressed in mean ± SD; N=6; 'a' means compared to normal and 'b' means compared to STZ control; * means significant at p<0.01 and # means significant at p<0.001

After 2hr, FBG which was elevated by 217.9% with STZ (p<0.001), was reduced by 31.8% with metformin (p<0.001) and PHC showed 26.2%, 29.5% and 30.2% reductions with 50, 100, 150

mg/kg of doses respectively with a statistical significance of p<0.001.

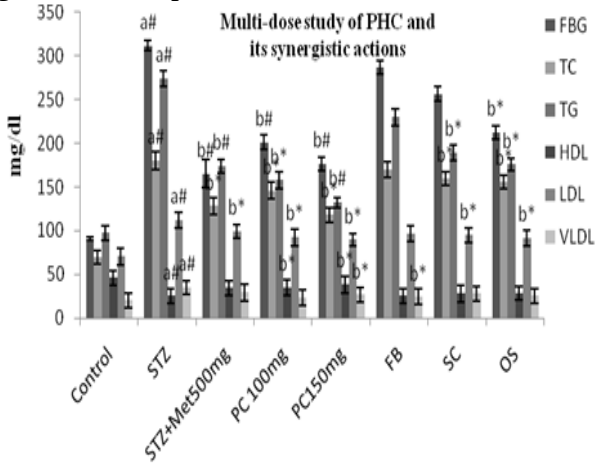

Figure 6: Synergistic hypoglycemic-hypolipidemic effect of the PHC in comparison to the individual plant species (FB- Banyan, SC- Jamun, and OS- Tulsi). Here N=6; All data are expressed in mean ± SD; 'a' means compared to normal and 'b' means compared to STZ control; * means significant at p<0.01 and # means significant at p<0.001.

In case of multiple-dose study, after 4 week of STZ injection FBG level which was elevated by 244.58% (p<0.001) in diabetes induced groups in comparison to normal control was reduced by 8.03% on treating with banyan (FB), 17.59% on treatment with jamun (SC); however in both cases results are not statistically significant. Tulsi lowered the elevated FBG level by 31.85% (p<0.01). 100 mg/kg b.w. of PHC lowered the elevated FBG level by 35.42% and 150 mg/kg b.w. by 43.45% at p<0.001 level of significance in both cases. Metformin (500 mg/kg b.w.) lowered FBG by 88.98% (p<0.001). Thus at same dose of 100 mg/kg PHC is found to exhibit better control on FBG level than the three individual plant species and at higher dose of 150 mg/kg it is found to show better anti-diabetic effect than the standard drug metformin. Considering the hypolipidemic action, the total cholesterol level (TC) was elevated by 158.54% (p<0.001) in diabetic control group in comparison to normal control. After treatment with banyan, it was reduced by 5.55% in comparison to diabetic control, by 11.33% on treatment with jamun (p<0.01) and by 13.6% with tulsi (p<0.01). PHC at 100 mg/kg lowered TC by 19.03% (p<0.01) and at 150 mg/kg by 34.39% (p<0.01) while metformin at 500 mg/kg lowered by 40.53% (p<0.01). Triglyceride level (TG) which rose by 181.83% (p<0.001) in comparison to normal control was lowered by 16.1% after treating with banyan, reductions with jamun by 30.76% (p<0.01) and with tulsi by 35.81% (p<0.01). PHC at 100 and 150 mg/kg doses reduced TG level by 42.36 % (p<0.01) and 51.67 % (p<0.001) respectively while metformin at 500 mg/kg reduced by 36.64% (p<0.001). The "good cholesterol" HDL level which was lowered by 44.96% (p<0.001) in comparison to normal group did not show any elevation on treatment with banyan; jamun and tulsi elevated HDL level by 8.63% and 12.55% respectively and with metformin (500 mg/kg) by 35.92% but results were not statistically significant. PHC at 100 and 150 mg/kg elevated HDL level by 37.65% and 51.61% respectively at p<0.01 level of significance in both cases. The "bad cholesterol" LDL which was elevated by 59.4% (p<0.001) was lowered by 13.76%, 15.28% (p<0.01) and 18.05% (p<0.01) by banyan, jamun and tulsi respectively while PHC lowers by 17.43% (100mg/kg) and 19.84% (150mg/kg) and metformin (500mg/kg) by 11.35% all being statistically significant (p<0.01). The VLDL level which was increased by 72.77% (p<0.001), on treatment with banyan was reduced by 30.66% (p<0.01), jamun and tulsi by 19.48% and 26.25% respectively, PHC (100 and 150 mg/kg) by 24.64% and 32.38% and metformin by 16.05%. However statistical significance was observed only in case of PHC at 150 mg/kg doses (p<0.01).The effect of PHC on HbA1c and total Hb on normal control and experimental animals has been shown in (**Table 4**). In comparison to normal control, Hb level is significantly decreased (p<0.01) and HbA1c is significantly increased (p<0.01) in diabetic control group which is significantly restored (**Table 4**) in the positive control treated with metformin (500 mg/kg); restoration of Hb and HbA1c levels at p<0.001 was observed in the group treated with PHC (100 and 150 mg/kg b.w.), HbA1c levels were restored at p<0.01 level with PHC at 150 mg/kg (Table 4), Tulsi alone also showed recovery at p<0.01 but no statistical significance was observed in case of Banyan or Jamun.

Table 4: Effect of PHC on the levels Hb and HbA1c in control and experimental rats

Groups	Hb (g/dl)	HbA1c (% Hb)
Normal control	14.91±1.23	6.99±0.27
STZ (Diabetic Control)	6.92±0.53 ^{a*}	14.59±1.11 ^{a*}
STZ + Metformin 500 mg (Positive Control)	13.98±0.74 ^{b*}	7.87±0.24 $^{b\#}$
PHC 100 mg/kg bw	11.21±0.49 $^{b\#}$	10.72±0.95 $^{b\#}$
PHC 150 mg/kg bw	14.72±1.01 $^{b\#}$	7.09±0.51 ^{b*}
FB	7.29±1.05	12.43±0.47
SC	9.02±0.37	11.91±0.21
OS	10.43±0.91 ^{b*}	11.49±1.05 ^{b*}

***Synergistic effect of the PHC in comparison to the individual plant species (FB- Banyan, SC- Jamun, OS- Tulsi). Here N=6;**

All data are expressed in mean ± SD; 'a' means compared to normal and 'b' means compared to STZ control; * means significant at p<0.01 level and # means significant at p<0.001 level.

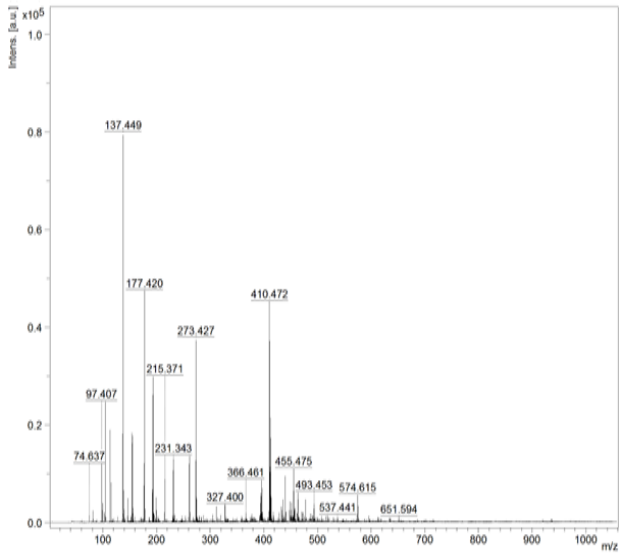

Figure 7: MALDI-ToF spectra of the phytocomposite. Basing on the available MALDI-ToF spectra compounds with good abundance were detected in the molecular range of 137-537g/mol. Some of the identifiable compounds in the phytocomposite include eugenol, ursolic acid, quercetin, gallic acid, lupeol

DISCUSSION AND CONCLUSION

PHC showed an appetite stimulating effect in the treated animals which attributed to their increased body weight in comparison to the control group. A significant decrease ($p<0.05$) in plasma protein and significant increase ($p<0.05$) in creatinine levels were observed only at higher doses of 500 and 750 mg/kg b.w. (**Figure 3**). Decrease in plasma protein level and increase in plasma creatinine (**Figure 3**) at high doses suggests that PHC can impair the normal functionality of kidney and effect the renal filtration mechanism (elevation in creatinine level) at such high doses which is also corroborated the research works of Ogbonnia et al. 2009. [3] AST (also called SGOT) and ALT are found in low concentrations in plasma and widely distributed in body tissues viz. liver, kidney, heart, brain etc. However ALT is considered mostly the marker for proper functionality of liver and AST for the heart. Any damage to these organs releases the enzymes into the blood and their levels are elevated.[3] Significant increase in AST levels were observed in animals treated with very high doses of 750mg/kg b.w. and significant decrease in ALT was observed in all treated groups (**Figure 3**). Decrease in ALT levels shows some reducing effect of PHC on cardiovascular risk factors. Thus PHC in normal to moderately high doses do not cause any hepato-renal threat but at very high doses can impair their functionality. There is significant increase ($p<0.05$) in hemoglobin content (may be due to increased iron absorption) and increase in WBC count (**Table 3**), a positive impact on the experimental animals.

Basing on the results of sub chronic and *in vivo* studies with the PHC it was found that, apart from significant hypoglycemic and hypolipidemic effect, PHC improves appetite, reduces cardiovascular risk factors, improves hemoglobin content by enhancing iron absorption which are other potentialities of this poly herbal product. For evaluating the hypoglycemic-hypolipidemic effect of the PHC both single and multi-dose study was conducted (**Figure 5-6**). The poly herbal product PHC is a combination of the leaf powders of banyan: jamun: tulsi (1:1:2). [8] All these three plants have evidence based anti-diabetic potentials.[25-31] To evaluate the synergistic anti-diabetic potentials of PHC in comparison to its constituent species (banyan, jamun and tulsi) hypoglycemic hypolipidemic actions of these individual species as well as the PHC were determined simultaneously (**Figure 6**). From the experimental results it was found that PHC showed enhanced hypoglycemic-hypolipidemic actions in comparison to metformin. The anti-diabetic effect of the PHC was found to be better (**Figure 6**) than its individual constituent species (Banyan, Jamun, Tulsi) suggesting the probability of "phytosynergism" amongst several phyto molecules in the PHC. PHC is prepared from the leaf powders of Banyan: Jamun: Tulsi mixed in the weight ratio of 1:1:2.[8] Some of the suggested anti-diabetic mechanisms of tulsi include stimulatory effects on physiological pathways of insulin, decrease in serum concentration of cortisol and glucose, reducing the actions of carbohydrate metabolizing enzymes viz. glucokinase, hexokinase, phosphofructokinase, increase in insulin and peptide levels, glucose tolerance etc.[32-39] Jamun and Banyan were found to inhibit insulinase activity,[40] Jamun was found to be hypoglycemic (increase in insulin levels) rather than hyperglycemic. [41] All these three plant species have antioxidant potentials, cholesterol lowering and hypolipidemic action; tulsi increases hemoglobin content and acts as cardio protective by reduction of glutathione, superoxide dismutase and LDH levels.[42-48] Detailed chemo profiling and molecular characterization of PHC carried out by De et al. 2015 have shown significant presence of poly

phenols, flavonoids, triterpenoids. [8] The MALDI-ToF (Matrix Assisted Laser Desorption Ionization- Time of Flight) spectra (**Figure 7**) of the PHC have shown the presence of biomolecules like eugenol, ursolic acid, quercetin, gallic acid, lupeol etc. Phytomolecules with a common pharmacological action but of different origin in combinations are found to exhibit synergism.[8] Hypoglycemic effects of poly phenols are mostly due to inhibition of alpha-amylase, alpha-glucosidase, inhibition of glycosidases, and glucose transporters.[49] Flavonoids exhibit their anti-diabetic effect by promoting insulin secretion, proliferation of pancreatic β cells, enhancing peripheral glucose utilizations, reducing insulin resistance and oxidative stress.[50] Amongst the molecules detected in PHC, Quercetin, a bioflavonoid is a potent antioxidant and exerts anti-diabetic effect by inhibiting alpha glucosidase, scavenges oxygen radical, inhibits xanthine oxidase, and inhibits lipid per-oxidation *in vitro*. [49] Eugenol, a phenyl propanoid is a powerful antioxidant and potent anti-diabetic that influences glucose transporter subtype 4 (GLUT-4) and phosphoinositide-3-kinase (PI3K).[51] Gallic acid, another phenolic compound is also an excellent antioxidant and shows anti-diabetic potentiality by enhancing peripheral glucose utilizations by direct insulin like or insulin mimetic effect.[52] Tri-terpenoids have potent antioxidant, hypoglycemic and hypolipidemic properties. They exert anti-diabetic effect by inhibiting the target molecules viz. alpha amylase, alpha glucosidase, aldose reductase, protein tyrosine phosphatase 1B (PTB-1B), glycogen phosphorylase etc.[53] Ursolic acid, a triterpenoid is a inhibitor of PTB1B, stimulates GLUT4 to enhance peripheral glucose utilizations, influences the PI3K/AKT pathway, enhances lipolysis and inhibits adipogenesis.[53] Research works of De et al. 2015 have shown that PHC is found to inhibit some enzymes having roles in the pathogenesis of type 2 diabetes viz. alpha amylase, alpha glucosidase, aldose reductase, dipeptidyl peptidase 4 with significant antioxidant activities which is one of the suggested mechanisms of anti-diabetic potentials of this novel poly herbal product.[8] Experimental evidences have shown that PHC exhibited synergistic anti-diabetic effect in *in vitro* studies than its three constituent plant species (banyan, jamun and tulsi) and the identified bioactive compounds may have contributed to synergism. Constituent plant species have retained their individual pharmacologic actions with a dominance of the effect of tulsi since its amount has been doubled.[8]

From the experimental results it have been found that the new poly herbal product PHC prepared from three common Indian medicinal plants possess a good safety profile and is likely to impair hepato-renal functionality at very high doses. As observed from sub chronic studies, PHC improves hemoglobin content and appetite and may exert cardio protective effect. Since PHC is enriched in polyphenol, flavonoids and tri-terpenoids their hypoglycemic-hypolipidemic action is influenced by the effect of these chemical groups along with the biomolecules detected. The *in vitro* mechanistic studies of PHC as a whole show the persistence of the effects of these chemical groups. However detailed mechanistic studies including involvement of cell signaling pathways are warranted to explore the synergistic activities of this product so as to consider it as an effective anti-diabetic therapeutic entity.

CONFLICT OF INTEREST: All authors declare the conflict of interest is nil.

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