J Young Pharm, 2022; 14(4) : 387-393
A multifaceted peer reviewed journal in the field of Pharmacy
www.jyoungpharm.org | www.phcog.net

Original Article

Comparative Study of Developed Formulation and Market Formulation for Antidiabetic Potential in Alloxan Induced Diabetic Wistar Rats

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ABSTRACT

Background: The present study was designed with the aim of minimizing the incorporation of the drugs in a polyherbal antidiabetic formulation so the load of the drugs to the patient can be avoided and also helps in standardization. **Materials and Methods:** Molecular docking-based screening of potential herbal leads of *M. charantia*, (Drug A), *G. sylvestre*, (Drug B) and *W. somnifera*, (Drug C) were performed with intent to prioritize the ratio of herbal extracts. Lyophilised hydro-alcohol (50%) extracts of above three drugs were combined in different ratios as HA: Hydroalcoholic extracts combination A, HB: Hydroalcoholic extracts combination B and HC: Hydroalcoholic extracts combination C. The study of 21 days, carried out on alloxan-induced diabetic Wistar rats to compare its anti-diabetic effect with the marketed herbal formulation (MHF). The biochemical parameters studied were serum glucose level, lipid profile and liver glycogen content along with the body weight measurement. **Results:** The proposed Hydroalcoholic extracts combination B (HB, 1000 mg/kg per oral) possess antihyperglycemic effect with improvement in the abnormal lipid profile as observed in the diabetic experimental animals. Moreover, the formulation also reported check on the loss of body weight when compared with untreated diabetic rats. The observations were found to be at par with standard drug, metformin (500 mg/kg). **Conclusion:** Our findings suggest the proposed formulation have antihyperglycemic and anti-hyperlipidimic potentials. Furthermore, its effect on chronic diabetes and its complications is needed to be explored. Based on the docking results it has been clearly indicated that the antidiabetic effect of the used herbal extract is because of the presence of Withanolide-A and Charantin.

Keywords: Diabetes, Polyherbal formulation, Metformin, Docking, Charantin, Withanolide.

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DOI: 10.5530/jyp.2022.14.78

INTRODUCTION

Diabetes mellitus is a heterogeneous primary disorder of carbohydrates metabolism with multiple etiologic factors, generally involves absolute or relative insulin deficiency or insulin resistance or both, result in hyperglycemia. The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia, pruritis and unexpected weight loss, etc. along with hyperglycemia and abnormalities in serum lipids.[1] Diabetes is associated with micro and macrovascular complications, which are major causes of morbidity and death in diabetic subjects.[2] Diabetes has even become more common with COVID-19 patients these days and associated with unacceptable outcomes.[3] Natural products are gaining demand for the management of life style disorders including management of diabetes, due to side effects associated with the use of synthetic oral hypoglycemic agents.[4,5] Large number of botanicals are used in the treatment of diabetes e.g. *Momordica charantia, Eugenia jambolana, Cuminum cyminum, Salvadora persica, Catharanthus roseus, Azadirachta indica, Allium cepa, Allium sativum, Gymnema sylvestre, Garcinia indica* etc.[6] There are many number of polyherbal formulation those are available in the market containing combination of more than ten herbs. Internationally the use of medical herbs has limited up to three herbs in 90% of the market formulation and only 10% of the market formulations contain more than 3 herbs, keeping in view the difficulty in analyzing a complex form for the active ingredient present in it.[7,8] Therefore, in present study three drugs have been selected for the development of the poly herbal formulation. The drugs have been selected on the basis of their scientific literature available for anti-hyperglycemic potentials as well as free radical scavenging activity. Hence, *M. charantia* (Karela), *G. sylvestre* (Gurmar) and *Withania somnifera* (Aswagandha) were selected for the study.

M. charantia, commonly known as "bitter gourd" or "bitter melon" is a climber from Cucurbitaceae family. The fruits of the plant are an edible part which has been reported to possess antihyperglycemic, cholesterol lowering, antioxidant and many other activities.[9,10]

Gurmar, a woody climber from Asclepiadaceae family, has been profound place and well documented in Ayurvedic text as an ingredient of different formulations to treat metabolic disorders. The plant is very well established antihyperglycemic agent and traditionally used for the management of diabetes. It is known as a "Gur-mar" because of its taste. The plant is reported for various biological activities but mainly claims for its effectiveness in the treatment of diabetes.[11,12] The leaves extract (alcoholic) is reported to reduce hyperglycaemia in diabetic rabbits and humans. The leaves also have cholesterol-lowering effect that is generally associated with the diabetes.[13,14]

Aswagandha also known as Winter Cherry (Andallu and Radhika, 2000), is a green shrub (family Solanaceae) throughout the dry parts of India, Baluchistan, Pakistan, Afghanistan, Sri Lanka, Congo, South Africa,

Egypt, Morocco and Jordan. In India, it is widely grown in the provinces of Madhya Pradesh, Uttar Pradesh, plains of Punjab and northwestern parts of India like Gujarat and Rajasthan. It has reported to have anti-depressant, anti-stress, immuno-modulator, anti-oxidant activities etc.[15-17] Docking simulation is a trending approach to predict the strength of association between a ligand and a specific macromolecular target at molecular level require identifying the most potent lead molecule present in a bioactive herbal extract. The objective of the proposed study was designed to obtain the effective anti-diabetic herbal combination with minimum number of ingredients so that standardization of the formulation can be achieved. The docking simulation is done to select the most potent combination from the available herbs as we as to make the combination more competent and to establish the probable mechanism of action.

MATERIALS AND METHODS

Molecular Docking Studies

A ligand library of 22 herbal leads from plants, *M. charantia*, karela (Drug A), *G. sylvestre*, gurmar (Drug B) and *W. somnifera*, ashwagandha (Drug C) were prepared by exploring the literatures from various sources. The herbal leads from the above discussed plants used for preparing the ligand library are supposed to execute the anti-diabetic potential.[18,19] These plants were reported for the occurrence of alkaloids, steroids, glycosides, terpenoids etc. Thus, 22 ligands of the plant belonging to the diverse chemical classes were included in the ligand library with intent to identify the most prominent lead molecule responsible for the generation of anti-diabetic effect in humans and establishing the most probable mechanism involved in the anti-diabetic activity of that particular active constituent of the concerned plants.[20-23] Macromolecular target proteins α-Glucosidase, dipeptidyl peptidase IV (DPP4), G-protein-coupled receptor 40 (GPR40), Protein Tyrosine Phosphatase 1B (PTP1B), and sodium-glucose co transporter 22 (SGCT2) were found to be actively involved in the pathophysiology of diabetes. Therefore, the prepared ligand library was computationally screened against these therapeutic targets to identify the most potential anti-diabetic agent from the used library.[24,25]

Plant material and its extraction

M. charantia, karela fruit (Drug A), *G. sylvestre*, gurmar leaves (Drug B) and *W. somnifera*, ashwagandha roots (Drug C) were purchased from authentic supplier and were authenticated by Botanical Survey of India, specimen are kept in the Department of Pharmacy. All the drugs were dried, coarsely powdered, and kept in a sealed container. In proposed study, 700 g of each drug was extracted separately with hydro-alcohol (50% ethanol) to using triple maceration method where 2 L of 50% ethanol was used each time for the individual drugs (method was selected on basis of maximum % extractive values). The prepared extracts were lyophilized (Daihan, Korea) the % yield of lyophilized powder was 8.34, 7.84 and 6.90 % w/w for Karela, Gurmar and Aswagandha, respectively and kept until the next investigation in an airtight container.

Experimental Animals

Wistar rats of either sex, weighing 160–220 g, were obtained from the Institute's animal house. The animals became acclimatized to the typical laboratory circumstances, which included a 12 hr light/dark cycle, an ambient temperature of 25±2°C, and relative humidity levels between 55 and 65 %. The rats were fed with readily available commercial feed and provided free access to water. The institution's animal ethical committee gave its approval to the experimentation's protocol, (vide approval no ISF/CPCSEA/IAEC/2010/34).

Preparation of test drug material

The lyophilized powder of the hydro-alcoholic extracts of karela (Drug A), gurmar (Drug B) and ashwagandha (Drug C) were combined in different ratios to find out the most effective anti-hyperglycemic combination as HA: Hydroalcoholic extracts combination A, HB: Hydroalcoholic extracts combination B, HC: Hydroalcoholic extracts combination C. The combination details are as follows:

1. HA Karela (Drug A): gurmar (Drug B):ashwagandha (Drug C), 2:2:1
2. HB Karela (Drug A): gurmar (Drug B):ashwagandha (Drug C), 2:1:2
3. HC Karela (Drug A): gurmar (Drug B):ashwagandha (Drug C), 2:1:1

All these prepared combinations studied for their antihyperglycemic effect on normal Wistar rats in oral glucose tolerance test (OGTT) model at the dose level of 1000 mg/kg body weight each and marketed polyherbal formulation (MHF) in the form of capsules, which contain these three drugs as ingredients along with other drugs. For the OGTT and anti-diabetic tests, all test medications were given to the test subjects by suspending in 1 percent w/v carboxy methyl cellulose (CMC).

Oral Glucose Tolerance Test (OGTT)

The test carried out in order to determine the capacity of the normal rats to tolerate the glucose level. The animals were distributed into five groups (n = 6); Group I as normal control (1% w/v carboxy methyl cellulose (CMC) solution pretreated rats), Group II as Hydroalcoholic extracts combination A (HA, 1000 mg/kg, per oral), Group III as Hydroalcoholic extracts combination B (HB, 1000 mg/kg, per oral), Group IV as Hydroalcoholic extracts combination C (HC, 1000 mg/kg, per oral), and Group V as Marketed herbal formulation (MHF, 1000 mg/kg, per oral). In this model, glucose load (2 g/kg, per oral), given 1 hr after the pretreatment of animals with the prepared herbal combinations i.e. HA, HB and HC at 1000 mg/kg, per oral, and Marketed Herbal Formulation (MHF), 1000 mg/kg. Under a light anaesthetic, *i.e.,* xylazine**,** blood samples were taken from the retro orbital plexus prior to and 0, 60, and 120 minutes after glucose delivery. Within 30 minutes of removing the blood sample, the serum glucose level (SGL) was calculated using the glucose oxidase-peroxidase technique. This study also helped in finding the most effective sugar lowering combination.[26]

Alloxan-induced diabetes model

This study carried out at two dose levels (500 mg/kg and 1000 mg/kg) on the combination, which had shown the maximum sugar lowering effect in OGTT model. The animals were distributed into five groups (n = 6); Group I as Animals received 1% w/v CMC, per oral (no treatment), Group II as Alloxanized rats received only 1% w/v CMC per oral, Group III as Alloxanized rats received Hydroalcoholic extracts combination B (HB, 500 mg/kg, per oral), Group IV as Alloxanized rats received Hydroalcoholic extracts combination B (HB, 1000 mg/kg, per oral) and Group V as Alloxanized rats received Metformin (500 mg/kg, per oral). The anti-diabetic effect of marketed herbal formulation, MHF on this model compared with the optimized combination. However, metformin (500 mg/kg) used as a standard drug. All the test drugs administered orally daily for 21 days in test animals at 24 hr intervals. Doses were selected on the basis of toxicity studies as per OECD425 guidelines where animals showed no any toxic effect to the highest dose of 5g/kg body weight. And 1/10th of the highest dose was selected (500mg/kg) and 1000 mg/kg was selected just to check effective and/or ceiling dose.

Induction of experimental diabetes

Prior to the administration of a single dosage of freshly produced alloxan 150 mg/kg, intraperitoneally (i.p.), the rats were starved for 16 hours. Alloxan was dissolved in ice-cold sodium citrate buffer to create the solution (pH 4.3). Overnight, animals were given free access to a 5 percent w/v glucose solution to treat their alloxan-induced hypoglycemia. Hyperglycemia had confirmed one week after the alloxan injection *via* serum glucose level measurement of 16 hr fasting rats, the animals with glucose level 230-300 mg/dl were considered as a diabetic for the present study.[27]

Blood and tissue collection

The blood for the sampling purpose is collected from retro-orbital plexus as per the standard protocol. After allowing the blood to coagulate, the sample was subjected to centrifugation (3000 rpm for 15 min) to obtain clear serum. Lipid profile and SGL, total liver glycogen (after sacrificing the animals as per approved protocol) content were estimated.[27]

Biochemical studies

The liver glycogen level, lipid profile, and fasting SGL were assessed in the experimental animals. Serum glucose was measured using the GOD/POD techniques (53). The lipid profiles of the animals were analysed by measuring total cholesterol, triglycerides, High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and very Low Density Lipoprotein Cholesterol (VLDL-C) as per the standard method.[28] However, total liver glycogen content was estimated according to standard procedure.[29]

Body weight

Throughout the study animals were kept in strong observation to diagnose the effect of test samples on body weight, for this all test animals were weighed on weekly interval i.e. 0, 7th, 14th and 21st days of the study.

Statistical analysis

Results of the study are Mean ± S.D, where $n = 6$; One way ANOVA was used to compare the numerical data obtained during the study between test groups. Tukey's multiple comparison test was performed at 95% ($P<0.05$) confidence level to analyze the mean differences.

RESULTS

Molecular Docking Studies

Based upon the available literature ligands like charantin, vicine, withaferin-A, withanone, withanolide-A, withanolide-B, gymnemagenin, withanoside-IV, and isoforms of gymnemic acid 1-10 were shortlisted for generating a ligand library. The two-dimensional structure of these ligands was generated by obtaining isomeric SMILES from PubChem and converting them into two-dimensional structure using ChemDraw 9.0.[30,31] These two-dimensional structures of all the shortlisted ligands were utilized for generation of their three-dimensional structure followed by energy minimization process. The three-dimensional structure model of the selected macromolecular targets was procured from the protein databank. The target protein was set for the docking protocol by confiscating the complex ligand. Validation of the used docking protocol was confirmed by redocking the complex ligand using similar docking parameters.[32,33] Later these validated parameters were further utilized for screening of the prepared ligand library. The docking results obtained after computational screening of the ligand library were

Table 1: Binding score obtained after computational screening of the prepared ligand library against various antidiabetic drug targets.

S. No.	Ligands	α-Glucosidase	DPP4	GPR40	PTP1B	SGLT2
PDB id→		3wy4	5y7k	5tzr	6w30	7vsi
1	Charantin	-0.22	-4.94	-5.80	-3.78	-11.32
2	Vicine	-6.81	-4.86	-4.34	-5.38	-5.96
3	Withaferin A	-0.62	-7.79	-8.00	-6.98	-11.15
4	Withanolide A	-8.86	-8.10	-7.10	-7.64	-11.00
5	Withanolide B	-7.70	-7.94	-8.93	-7.74	-10.66
6	Withanone	-5.67	-7.18	-7.90	-7.05	-10.36
7	Withanoside-IV	----	-4.80	-4.36	-3.70	-9.31
8	Gymnemaganin	-----	-6.27	-5.77	-4.67	-6.41
9	Gymnemic acid 1	-----	-----	-3.90	-2.39	-----
10	Gymnemic acid 2	-----	-----	-4.20	-0.91	-----
11	Gymnemic acid 3	-----	-----	-5.20	-1.57	-----
12	Gymnemic acid 4	-----	-----	-4.83	-2.63	-----
13	Gymnemic acid 5	-----	-1.20	-2.85	-2.04	-----
14	Gymnemic acid 6	-----	-----	-2.03	-0.39	-----
15	Gymnemic acid 7	-----	-4.07	-5.02	-3.69	-----
16	Gymnemic acid 8	-----	-----	-2.73	-1.05	-----
17	Gymnemic acid 9	-----	-----	-2.69	-0.96	-----
18	Gymnemic acid 10	-----	-1.38	-3.79	-3.09	-----
19	Gymnemic acid 11	-----	+1.48	-4.26	-2.50	-----
20	Gymnemic acid 12	-----	-----	-1.95	-0.50	-----
21	Gymnemic acid 13	-----	-1.01	-3.86	-1.93	-----
22	Gymnemic acid 14	-----	-1.23	-4.81	-2.87	-----

*(------ No binding observed)

DPP4: dipeptidyl peptidase IV; GPR40: G-protein-coupled receptor 40; PTP1B: Protein Tyrosine Phosphatase 1B; SGLT2: Sodium-glucose Cotransporter-2

tabulated in Table 1. The three-dimensional binding mode and observed binding interactions for the best lead molecules against their target macromolecule was shown in Figure 1.

OGTT model

In this investigation, after 1 h of glucose administration, animals of vehicle control and Marketed herbal formulation, MHF (1000 mg/kg body weight) administered groups allowed 42.3% and 12.04% rise, respectively in SGL. However, when compared to 0 hr, the animals administered with HB, 1000 mg/kg p.o. had a significant ($P < 0.05$) resistance to the rise of SGL and allowed a 17.13% rise. Additionally, after 2 hr, the animals in all the test groups' SGLs nearly returned to normal. This result leads to author(s) to test HB at 500 mg/kg and 1000 mg/kg per oral in the alloxan-induced diabetes model, HB has been chosen for further research. The results are depicted in the Table 2.

Alloxan Induced diabetes model

In this paradigm, diabetic control rats who received vehicle treatment on days 7, 14, and 21 of the trial have not shown any discernible change in the fasting SGL from day 0 to those days. On the seventh, fourteenth, and twenty-first days of the trial, respectively, the diabetic rats treated with MHF (1000 mg/kg) shown a significant ($p < 0.05$) drop in SGL by 22.44 percent, 44.22 percent, and 55.23 percent when compared with 0 day. Additionally, HB (1000 mg/kg per oral) administered group of rats displayed a substantial ($p < 0.05$) and progressive decrease in SGL by 22, 49, 42, and 49.38 percent on days 7, 14, and 21 of the trial, respectively, in compared to day 0 of the experiment. HB (500 mg/kg per oral), however, demonstrated a lesser decrease in SGL at a modest dose of. Additionally, diabetic rats treated with metformin (500 mg/kg) demonstrated a constant and substantial ($p < 0.05$) decline in serum glucose level. The results depicted in the Table 2.

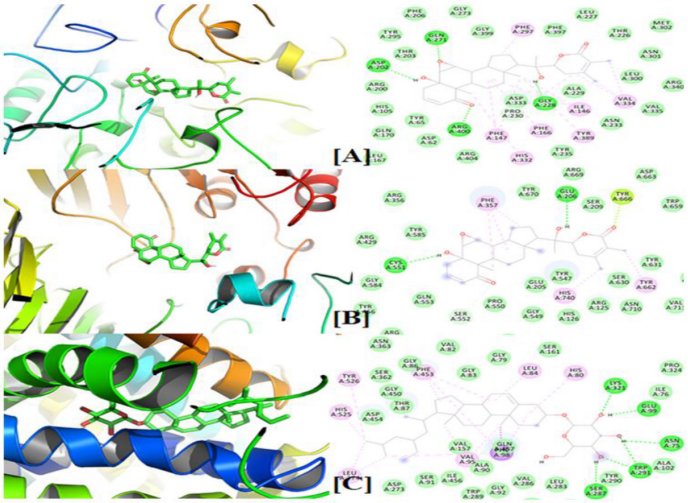

Figure 1: [A] Three-dimensional binding mode and two-dimensional interactions of Withanolide-A against α-Glycosidase enzyme for commencing its antidiabetic effect [B] Three-dimensional binding mode and two-dimensional interactions of Withanolide-A against DPP-4 enzyme for commencing its antidiabetic effect [C] Three-dimensional binding mode and two-dimensional interactions of Charantin against SGCT2 for commencing its antidiabetic effect.

Body weight analysis

The diabetic animals had shown significant ($p<0.05$) loss (13.41%, 28.64 % and 35.51% by the 7th, 14th and 21st days, respectively) of their body in comparison to 0 day. The HB treated animals from both (500 mg/kg per oral and 1000 mg/kg per oral), registered a less gradual, non-significant impact on body weights till the end of study. Animals administered with MHF (1000 mg/kg) recorded a less progressive loss of body weight throughout the experiment. However, until the end of the study, rats given metformin (500 mg/kg) experienced a less progressive loss in body weight. The results tabulated in the Table 4.

Table 2: The effects of pre-treatment of different test drugs on serum glucose level in oral glucose tolerance test (OGTT) in normal Wistar rats.

Group	Blood glucose level mg/dl			
	Basal	0 min	60 min	120 min
Normal	90.67±5.18	92.88±2.94	132.61±3.0 (42.37↑)	99.48±2.57
HA(1000 mg/kg)	83.18±3.16	87.69±10.56	118.18±8.35 (34.47↑)	94.64±6.86
HB (1000 mg/kg)	86.47±6.27	86.91±3.89	101.80±2.33 (17.13↑)[ab]	92.11±1.55
HC (1000 mg/kg)	83.48±4.48	82.56±3.40	102.49±2.36 (24.14↑)[a]	87.07±5.94
MHF (1000 mg/kg)	86.90±5.83	85.31±7.62	95.24±7.09 (12.04↑)[ab]	87.35±5.66

All values are represented as Mean ± SD, n= 6

[a]$p< 0.05$ vs normal group; [b]$p< 0.05$ vs. HA (1000 mg/kg)

*HA: Hydroalcoholic extracts combination A, HB: Hydroalcoholic extracts combination B, HC: Hydroalcoholic extracts combination C, MHF: Marketed Herbal Formulation

Table 3: The effects of 21 days treatment with HB (500 mg/kg and 1000 mg/kg) and MHF (1000 mg/kg) and Metformin (500 mg/kg) on serum glucose level in alloxan-induced diabetic rats.

Group	Blood glucose level mg/dl			
	0 day	7th day	14th day	21st day
Normal	72.93 ± 3.63	75.97 ± 5.20	79.75 ± 3.95	77.32 ±4.44
Diabetic control	268.62 ±9.05[a]	275.02 ±7.45[a]	292.11±7.26[a]	295.30±6.6[bc]
HB (1000 mg/kg)	268.86±9.07[a]	208.39 ± 9.07[ab] (↓22.49%)	154.04±11.36[abc] (↓42.00%)	136.08±7.27[bc] (↓49.38%)
HB (500 mg/kg)	250.42±9.01[a]	217.19±10.17[ab] (↓13.26%)	188.92±9.25ab (↓24.55%)	156.92±7.99 (↓37.33%)
MHF (1000 mg/kg)	259.16 ± 14.6[a]	200.99 ± 8.81[ab] (↓22.44%)	144.55 ±16.41[abc] (↓44.22%)	116.02 ± 5.78[bc] (↓55.23%)
Metformin (500 mg/kg)	265.68 ±10.37[a]	210.51 ±12.86[ab] (↓20.76%)	151.05 ±12.47[abc] (↓43.14%)	111.56 ± 7.74[bc] (↓ 58.02%)

All values represent Mean±SD of the mean (n=6).

[a]$p< 0.05$ vs. normal group; [b]$p< 0.05$ vs. diabetic control group; [c]$p<0.05$ vs. HB (500 mg/kg)

*HB: Hydroalcoholic extracts combination B, MHF: Marketed Herbal Formulation

Table 4: Effect of 21 days treatment with test drugs on the body weight of alloxan induced diabetic rats.

Group	Body weight (g)			
	0 day	7th day	14th day	21th day
Normal	205.80± 11.90	214.80±3.34	214.40± 5.54	215.8± 3.96
Diabetic control	186.40±10.89	161.40±9.12[a] (↓13.41%)	133.00± 8.06[a] (↓28.64)	120.2±6.97[a] (35.51↓)
HB (1000 mg/kg)	185.00±18.54	173.40± 16.92[a] (↓6.27%)	164.4±17.12[ab] (↓11.13%)	142.2±7.39[abc] (↓ 23.13%)
HB (500 mg/kg)	192.00±10.93	166.60±8.56[a] (↓13.22%)	148±5.52[ab] (↓22.68%)	133.2±9.78[ab] (↓30.62%)
MHF (1000 mg/kg)	179.60± 14.11	160.80±12.98[a] (↓10.50%)	152.2 ±11.69[b] (↓15.25%)	143.6 ± 7.09[abc] (↓20.04%)
Metformin (500 mg/kg)	190.20±9.33	173.80±11.0[a] (↓8.62%)	161.4 ±3.78[bc] (↓15.14%)	152.8±4.54[abc] (↓19.66%)

All the values represent Mean±SD (n=6)

[a]p<0.05 vs. normal group; [b]p<0.05 vs. diabetic control group and [c]p< 0.05 vs. HB (500 mg/kg)

*HB: Hydroalcoholic extracts combination B, MHF: Marketed Herbal Formulation

Table 5: Effect of 21 days treatment of HB (500 mg/kg and 1000 mg/kg), MHF (1000 mg/kg) and Metformin (500 mg/kg) on bio-chemical parameters in alloxan induced diabetic rats.

Group	Lipid profile					
	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Tissue glycogen (mg/g)
Normal	94.50±10.50	71.8±3.42	44.00±20.02	36.30±7.70	14.90±0.96	47.34±1.18
Diabetic control	229.03±11.52[a]	152.49±3.87[a]	24.30±40[a]	174.34±11.4[a]	30.36±0.96[a]	18.48±2.00[a]
HB (1000 mg/kg)	119.53±7.47[ab]	93.28±5.69[ab]	33.6±3.40[ab]	67.23±9.58[ab]	18.65±1.13[ab]	39.66±1.50[b]
HB (500 mg/kg)	123.06±6.68[ab]	101.86±11.90[ab]	28.5±1.90[a]	74.18±6.66[ab]	20.36±2.39[ab]	35.33±2.65[ab]
MHF (1000 mg/kg)	115.47±7.19[ab]	87.45±9.11[b]	35.01±5.20[ab]	62.98±6.34[ab]	17.48±1.82[b]	42.24±1.45[b]
Metformin (500 mg/kg)	100.98±9.14[b]	80.88±11.60[b]	38.07±2.02[bc]	46.71±10.10[b]	16.194±2.29[bc]	45±1.29[b]

All the values are represent to Mean±SD (n=6)

[a]p<0.05 vs normal group; [b]p<0.05 vs diabetic control group; [c]p<0.05 vs H B (500 mg/kg)

*HB: Hydroalcoholic extracts combination B, MHF: Marketed Herbal Formulation

Biochemical Parameters

During study, the serum of diabetic control group's animals displayed significant (p<0.05) increase in triglycerides (152.49 mg/dl), total cholesterol (229.03 mg/dl), LDL-C (174.34 mg/dl) and VLDL-C (30.36 mg/dl). Further significant decrease in HDL-C (24.3 mg/dl) and liver glycogen contents. The serum of animals received HB (1000 mg/kg p.o.) for 21 days, recorded significant (p<0.05) decrease for triglycerides (93.28 mg/dl), total cholesterol (191.53 mg/dl), LDL-C (67.23 mg/dl), and VLDL-C (18.65 mg/dl). Whereas, improvement in serum HDL-C (33.6 mg/dl) and liver glycogen (18.65 mg/g) were recorded in the same group of animals.

However, the animal lipid profile levels did not significantly decreased when HB (500 mg/kg) was used at low doses, and the serum HDL-C and liver glycogen levels were also not significantly affected. A substantial (p<0.05) decrease in blood total cholesterol, triglycerides, LDL-C, and VLDL-C levels was seen in the MHF (1000 mg/kg) treated group. Additionally, it demonstrated significantly increased HDL-C and hepatic glycogen levels (p<0.05). The effect of HB, 1000 mg/kg per oral was found at par with metformin (500 mg/kg per oral) on total serum cholesterol, triglycerides, LDL-C, VLDL-C, and in serum HDL-C and liver glycogen levels. The results are described in Table 5.

DISCUSSION

The present work carried out with an aim of optimizing the polyherbal anti-diabetic formulation with the maximum number of three ingredients. The drugs combined in this study are selected on the basis of their proposed mechanism of action, availability and predicted safety profile. Molecular docking simulation-based screening of the prepared ligand library by considering the active chemical constituents of the concerned plants was performed against the anti-diabetic drug targets to identify the most potent anti-diabetic compound and their probable mechanism of action. Physicochemical properties of all the compounds of the ligand library were analyzed to predict their pharmacokinetic and toxicological profile. In the OGTT model, animals administered with HB (1000 mg/kg per oral) reported significantly lower SGL than the normal group; the findings were similar to those of MHF (1000 mg/kg per oral). α-glucosidase enzymes inhibition low down the rate of absorption of glucose in the intestine, which may be responsible for HB (1000 mg/kg per oral) effect on normal rats in the OGTT paradigm, or may attributed its effect while promoting insulin secretagogue effect from the pancreas. Insulin stimulates the liver to metabolise glucose, muscle and fat cells that leads to elimination of glucose from the circulation. Alloxan is a diabetogenic agent, selectively destroys the cells of β islets which results in insulin deficiency, high SGL and ketosis.[34] In alloxanized rats, administration of HB (1000 mg/kg per oral) significantly (P < 0.05) decreased SGL on 7th, 14th and 21st days in comparison to 0 day (Table 3). The outcomes of the proposed formulation, HB were observed similar to those of metformin (500 mg/kg per oral) and MHF (1000 mg/kg per oral). HB (1000 mg/kg per oral) may have stimulating impact on remaining β-cells or improve insulin sensitivity at the cellular level, which would explain its antihyperglycemic efficacy. However, the developed formulation is having Karela fruit extract as an ingredient, has Insulin secretagogue effect and helps in removal or control the glucose level from the blood circulation.[35] Along with this in the same ratio,

Aswagandha is present in the proposed combination, enhances insulin secretion by increasing the pancreatic beta cells production or stimulate the release of endogenous insulin and/or increase in glucose utilization. Additionally, Gurmar, a well-known antioxidant and rejuvenator, is a component of the chosen mixture and has the ability to treat diabetes by regenerating pancreatic cells.[36,37]

In 21 days study, HB (1000 mg/kg per oral) administered group significantly decreased serum total cholesterol, triglycerides, VLDL-C and LDL-C levels and increased HDL-C levels, while compared with diabetic untreated animal group. The antidiabetic effect may be attributed to the upregulation of Peroxisome proliferator activator receptors and/or improve insulin secretion may both have a lipid-lowering impact.[13]

This shows that HB (1000 mg/kg per oral) might block the HMG-CoA reductase or reduce the NADPH necessary for the synthesis of fatty acids and cholesterol. This medication's ability to decrease cholesterol in alloxan-induced diabetic rats may also aid in reducing secondary consequences of diabetes mellitus, such as atherogenesis and other cardiovascular diseases.[35]

Additionally, the level of liver glycogen was enhanced considerably ($p< 0.05$) after 21 days of HB (1000 mg/kg per oral) treatment. Improved insulin levels and up-regulated GLUT-4 and PPAR- activity, which make it easier for peripheral organs to absorb glucose, are the two potential mechanisms by which liver glycogen levels can be improved. Metabolic disorder in the diabetic control group may be the cause of body weight loss, HB (1000 mg/kg per oral) did not significantly reduce body weight in diabetic rats, possibly because of increased insulin secretion and food intake. [37,38]

CONCLUSION

In alloxanized rats, HB (1000 mg/kg per oral) significantly ($p < 0.05$) reduced hyperglycemia, improved lipid profiles, and stopped weight loss. All such results suggest that the developed formulation is having same anti-diabetic potentials as MHF (Market formulation), that is having many number of ingredients that is difficult to be got standardized. Further study is required to evaluate its effect on various diabetes related complications. This type of study can help in minimizing the incorporation of the number of ingredients, which further help in the establishment of various polyherbal formulation(s) in the international market. Analysis of docking results clearly concludes that the withanolide-A is found to be most potent molecule present in the bioactive herbal extract of all three plants used in this study. Withanolide-A is supposed to exert its antidiabetic effect by targeting multiple biomolecules like α-Glucosidase and DPP4 those are considered as major contributors to the pathophysiology of diabetes. Charantin is also found to be one of the potent lead molecules in the used herbal extract and it is found to exert its antidiabetic effect by targeting SGCT2 protein.

ACKNOWLEDGEMENT

The author(s) would like to thank IES Institute of Pharmacy, Bhopal, Madhya Pradesh and M.M. College of Pharmacy, MM (Deemed to be University), Mullana, Ambala, Haryana, India for providing institutional facilities and support to carry out the research work.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

ABBREVIATIONS

HA: Hydroalcoholic extracts combination A; **HB:** Hydroalcoholic extracts combination B; **HC:** Hydroalcoholic extracts combination C; **MHF:** Marketed Herbal Formulation; **OGTT:** Oral Glucose Tolerance Test**; CMC:** carboxy methyl cellulose; **DPP4:** dipeptidyl peptidase IV; **GPR40:** G-protein-coupled receptor 40; **PTP1B:** Protein Tyrosine Phosphatase 1B; **SGLT2:** Sodium-glucose Cotransporter-2.

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Article History: Received: 17-05-2022; Revised: 28-07-2022; Accepted: 27-08-2022.
Cite this article: Gauttam VK, Munjal K, Mujwar S, Sawale J, Rohilla M, Gupta S. Comparative Study of Developed Formulation and Market Formulation for Antidiabetic Potential in Alloxan Induced Diabetic Wistar Rats. J Young Pharm. 2022;14(4):387-93.