



Design, Development and Evaluation of a Formulated Antidiabetic Herbal Suspension-SAM

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Research Article

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Abstract

Diabetes causes hyperglycemia. It affects vital organs. Herbal plants in combination have great potential for antidiabetic activity. Therefore a herbal suspension containing alcoholic extract of *Annona squamosa* leaves, *Aegele marmelos* leaves and *Azadirachta indica* leaves was prepared and evaluated for its organoleptic characteristics, physicochemical parameters and antidiabetic activity. The suspension was free flowing and easily dispersible. The organoleptic and physicochemical properties were satisfactory. Antidiabetic activity was studied in streptozotocin induced diabetic rats. The standard drug used was Glibenclamide 600 µg/kg. Herbal suspension at both the doses of 25 and 50µg/ml was having significant activity. The weight gain and decrease in blood glucose level was less than that of standard drug. Thus the prepared oral herbal suspension is safe for use with promising antidiabetic activity.

Keywords: Antidiabetic activity, *Annona squamosa*, *Aegele marmelos* and *Azadirachta indica*, Herbal suspension.

Introduction

Diabetes is a metabolic disorder resulting in hyperglycemia. It is due to insufficient production of insulin or inadequate response of target cells to insulin or both. Chronic hyperglycemia causes damage to vital organs like heart, kidney, and eyes^[1]. As the modern hypoglycemic agents have side effects, there is a need to shift to herbal plants and formulations^[2]. Traditional medicines from readily available medicinal plants have great potential for the discovery of new antidiabetic drugs. These natural products have either insulinomimetic or secretagogues properties^[3]. Plants with hypoglycemic and antioxidant properties are useful as antidiabetic agent^[4]. Polyherbal formulations have been prepared and found to have antidiabetic activity^[5]. A combination of two or more plants give better therapeutic effect as compared to a single plant^[6]. Shravan et al has reported synergistic antidiabetic activity of combinational extracts of Neem leaf and Fenu Greek seed^[7]. Studies on five polyherbal mixtures showed that the glycemic level was restored to normal^[8].

Hence antidiabetic plants were used to prepare a polyherbal formulation as suspension which is easy, rapid and economical to be formulated. The herbs used in suspension were leaves of *Annona squamosa*, *Aegele marmelos* and *Azadirachta indica*. These plants have other medicinal properties too. *Annona squamosa* L. (Annonaceae) has been reported to be antidiabetic^[9], antioxidant^[10], antihyperlipidemic^[11], cytotoxic^[12]. *Aegele marmelos* L. (Rutaceae) has been studied for immunomodulatory^[13], anti-inflammatory^[14] and antifertility^[15] activity. The third drug *Azadirachta indica* L. (Meliaceae) has antimicrobial^[16], larvicidal^[17] and antidiabetic activity^[18-19]. Halim found that *A. indica* leaf extract completely reversed the abnormal change in diabetic rat retina and inflammation of paws. This proves its ability to reverse the complications of retinopathy and cardiovascular changes in diabetes^[20]. The antidiabetic effect of root bark of *A. indica* was less significant than glibenclamide^[21].



Material and Method

Plant material:

The leaves of *Annona squamosa*, *Aegle marmelos* and *Azadirachta indica* were collected from the campus. Their authentication was done from the Department of Botany, Janata PG College, A.P.S. University, Rewa (M.P.). The voucher specimen number is JC/B/PAN/054f,g,c. The leaves were dried in shade and grinded to a coarse powder.

Preparation and evaluation of Herbal suspension (SAM):

The suspension of mixture of all three leaf extracts was prepared by trituration method^[6]. The alcoholic extract of the drugs was prepared individually by maceration. They were taken in the ratio of 1:1:1^[22]. Tween 80, sorbitol, potassium sorbate, lemon oil and distilled water were added. The suspension was evaluated for its organoleptic properties like colour, taste and odour. The physicochemical parameters studied were pH, sedimentation volume, redispersibility, density, flow and viscosity (Brookfield viscometer type III using spindle 2 at 250 rpm). The formulation was kept at room temperature (35±10°C) for one year. Its physicochemical parameters were again examined.

Table 1: Evaluation of Herbal suspension (SAM)

S. No.	Evaluation Parameters	Inference
1.	Colour	Dark green
2.	Odour	Characteristic
3.	Taste	Slight bitter
4.	pH	3.42
5.	Sedimentation Volume	0.17
6.	Viscosity (cps)	47.7
7.	Redispersibility	Very good
8.	Density (gm/ml)	1.0121
9.	Flow	Easy and uniform

Animals:

Wistar albino rats were taken. Their weight was between 150 to 200gm. They were kept in 12:12

light:dark cycle at a temperature 21°C to 25°C. They had free access to water and given standard diet (Golden feed N. Delhi). The protocol was approved by the Institutional Animal Ethics Committee as per CPCSEA guidelines (1413/a/11/CPCSEA, protocol approval no. PBRI/IAEC/13-14/PN-353).

Acute toxicity:

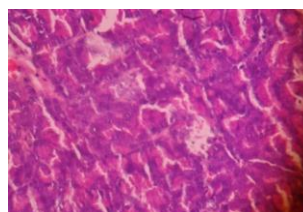
The rats were administered oral dose of herbal suspension till 2000mg/kg. Mortality or behavioural changes were observed.

Assessment of Antidiabetic activity:^[23-24]

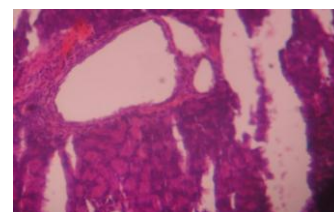
For the study six groups of rats were taken. Each group contained six rats. All the animals were given their dose orally except the dosing of streptozotocin (STZ - 60mg/kg) which was given intraperitoneally. The groups for the activity were administered their doses as follows:

- Group I - normal control - normal saline 5ml/kg only
- Group II – diabetic control - STZ + normal saline
- Group III – Standard - STZ + glibenclamide 600µg/kg per day
- Group IV – SAM 25 - STZ + Herbal suspension 25mg/kg per day
- Group V – SAM 50 - STZ + Herbal suspension 50mg/kg per day

The rats of Group II to V were made diabetic with STZ. From 4th day all the rats were given their respective doses. This 4th day was considered as the 1st day of treatment. The dosing continued till 28 days. The parameters taken to study diabetes were body weight and blood glucose level. These were observed on 0, 7, 14, 21 and 28 day of post treatment. (Table 2,3 and Fig.2,3). On 28th day the rats were sacrificed. The pancreas were removed and processed by the paraffin technique to get fine sections which were stained by haematoxylin and eosin (H & E). They were observed under microscope. (Fig.1).



a. Vehicle control



b. STZ diabetic control

**Table 2. Effect of Herbal suspension SAM on Body weight**

Group	Treatment	Body Weight (gm)					Gain in weight %
		0 Day	7 Day	14 Day	21 Day	28 Day	
I	Vehicle (Saline) 5ml/kg	193.5±17.581	196.2±18.324	197.3±19.065	198.7±19.449	199.0±18.953	2.8
II	STZ +Saline (5ml/kg)	188.7±16.110	182.7±14.528	174.3±16.931	171.0±17.170	168.3±16.403	-10.8
III	STZ + Glibenclamide (600 µg/kg)	181.0±25.195 ^{NS}	188.5±24.785 ^{NS}	193.7±26.621 ^{NS}	196.5±28.212 ^{NS}	199.5±27.009 ^{NS}	10.2
IV	STZ + SAM (25 mg/kg)	184.8±11.868 ^{NS}	187.9±12.058 ^{NS}	192.2±11.231 ^{NS}	194.6±9.846 ^{NS}	197.2±10.637 ^{NS}	6.7
V	STZ + SAM (50 mg/kg)	177.9±15.402 ^{NS}	180.1±14.118 ^{NS}	182.9±14.579 ^{NS}	185.3±12.948 ^{NS}	189.8±11.371 ^{NS}	6.7

Values are expressed as MEAN±SD at n=6, *P<0.001 compared to the Streptozotocin (STZ), NS – non significant

Table 3. Effect of Herbal suspension SAM on Blood Glucose Level (mg/dl)

S. No.	Treatment	Blood Glucose Level (mg/dl)				
		0 Day	7 Day	14 Day	21 Day	28 Day
1	Vehicle (Saline) 5ml/kg	88.2±8.424	89.6±7.261	90.1±7.855	89.2±8.288	89.5±7.793
2	STZ + Vehicle 5ml/kg	264.5±12.721	269.7±13.171	274.3±12.863	278.2±11.873	277.7±13.604
3	STZ + Glibenclamide (600 µg/kg)	257.8±11.374 ^{NS}	170.3±9.156*	134.0±7.483*	102.5±10.015*	86.0±6.197*
4	STZ + SAM (25 mg/kg)	257.8±09.432 ^{NS}	234.7±8.824*	208.2±10.998*	192.7±11.021*	166.2±12.254*
5	STZ +SAM (50 mg/kg)	261.0±10.334 ^{NS}	206.3±12.111*	186.2±14.919*	163.8±14.006*	129.2±11.215*

Values are expressed as MEAN±SD at n=6, *P<0.001 compared to the Streptozotocin (STZ), NS – non significant

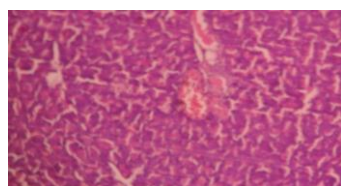
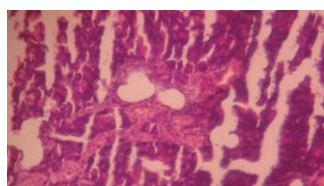
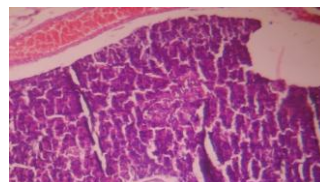
**c. Standard Glibenclamide****d. SAM-25mg/kg****d. SAM- 50mg/kg**

Fig. 1: Histology of rat pancreas for antidiabetic activity of Herbal suspension SAM. Statistical analysis: All data are presented in Mean±SD and analyzed by One way ANOVA followed by Bonferroni test. The value of p<0.05 was considered as significant.



Results and Findings

The herbal suspension had good organoleptic and physicochemical properties. Even after one year it was stable. In acute toxicity studies LD₅₀ was found to be 300mg/kg.

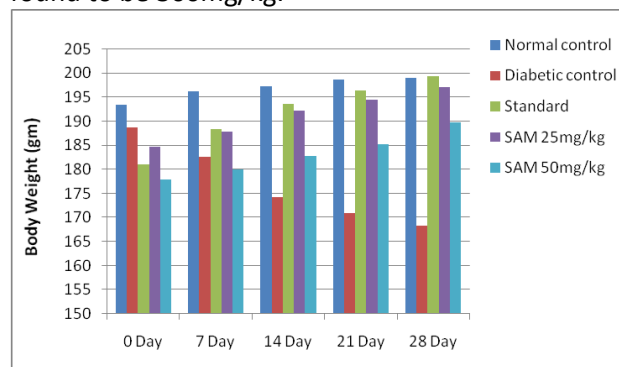


Fig.2: Effect of Herbal suspension SAM on rats Body weight.

In STZ diabetic control group the body weight of rats of standard glibenclamide and SAM groups did not increase significantly when compared to the diabetic control group but the percentage increase of SAM at both the doses was similar. However the blood glucose level decreased significantly $P < 0.001$ in the standard and SAM groups from 7th day onwards till the 28th day.

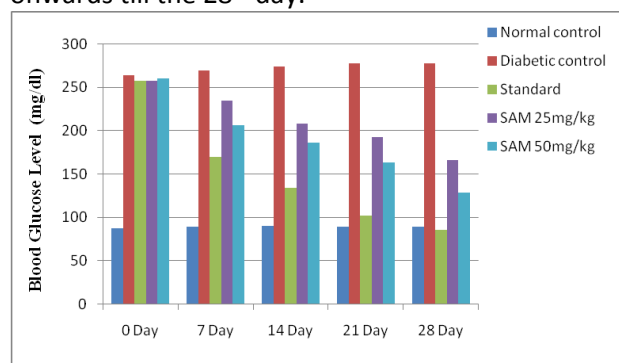


Fig.3: Effect of Herbal suspension SAM on rats Blood Glucose Level (mg/dl).

The decrease in glucose level was gradual but it did not go below the normal value. Both the doses of SAM showed very good antidiabetic activity. Histology of the fine sections of pancreas of rats revealed that all the sections of pancreas were normal in the vehicle control group. The blood vessels, connective tissues, islet of langerhans, acinar cells, inter and intralobular duct were clearly seen. There was no inflammation. The structure and arrangement of islet of langerhans was normal and they were tightly arranged. They were distributed in the lobule unevenly. In STZ diabetic

control group the cells of pancreas were inflamed with a decrease in number of islet, increased gaps between islets and their size. The interlobular and intralobular duct had clear widening. In standard glibenclamide and SAM treated groups there were lesser number of islets as compared to vehicle control group. The gap between the islets was more. But it was significantly much better than the STZ diabetic control group. The dose of SAM 50mg/kg had protected and regenerated the cells which looked similar to normal and standard group. Thus the histological examination confirms very good protective and regenerative property of herbal suspension.

Discussion and Conclusion

Globally a large number of people are suffering from diabetes. The conventional allopathic medicines have undesirable effects. Phytomedicines have been traditionally used from long time without side effects. The constituents present in medicinal plants have a potential to produce therapeutic effect in diabetes by acting on targets through different mechanisms^[25]. Development of phytomedicines is relatively inexpensive, less time consuming and economical rather than allopathic drug development. The synthetic drugs are considered as more toxic having side effects as compared to plant drugs and formulations^[7,26]. This provoked an urge to prepare antidiabetic polyherbal suspension. Sorbitol was added to mask the bitter taste. Studies have shown that there was no significant change in blood glucose level of diabetic people when sorbitol was used in ice cream^[27]. This quantity of sorbitol is much larger as compared to its use in suspension. The standard drug glibenclamide, promotes insulin secretion by closure of potassium-ATP channels, membrane depolarization and stimulation of calcium ion influx^[28]. The antihyperglycemic effect of plants are due to their ability to increase in insulin level or inhibit the intestinal absorption of glucose or the facilitation of metabolites in insulin dependent processes^[29].

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.