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EVALUATION OF ANTIDIABETIC EFFICACY OF A SIDDHA POLYHERBAL FORMULATION (SUGNIL) IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

SUGNIL, a traditional siddha polyherbal formulation marketed for diabetes, was investigated for its scientific basis of pharmacological action in the management of diabetes and its related complications. We investigated the antihyperglycemic, antioxidant potential of our drug in streptozotocin induced diabetic rats and also quantitatively analysed for the presence of important biochemical components to determine the scientific mode of its pharmacological action. Male albino wistar rats, weighing 150-180 g were used for present study. A single intraperitoneal dose of 55mg/kg body weight of streptozotocin was used to induce experimental diabetes. Aqueous extract of SUGNIL was administered orally (100 mg/kg body weight) for 45 consecutive days. After the treatment, it was observed that the level of blood glucose was significantly high and at the same time the level of plasma insulin and the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, catalase and reduced glutathione, were significantly low in tissues of streptozotocin induced diabetic rats when compared with control rats. On the other hand, the reverse effect was found in rats treated with SUGNIL which was marked by significant decrease in blood glucose and significant increase in plasma insulin and antioxidant status. However, there was no significant effect witnessed in control rats treated with SUGNIL. Hence, the decreased blood glucose and increased insulin, antioxidant levels in SUGNIL treated animals substantiate its antihyperglycemic and antioxidant effects. Our quantitative chemical analysis of SUGNIL reveals the presence of vitamins (D & E), phytochemicals (Flavonoids, Tannins, Phenols), minerals (Zn, Cu, Mn,

Cr, Fe), and heavy metals (Pb, Hg and Co).Presence of these bioactive components may exercise their physiological role in the mechanism of antihyperglycemic and antioxidant effects of SUGNIL.

Keywords: SUGNIL, streptozotocin,streptozotocin, vitamins, minerals, phytochemicals.

Introduction

Polyherbal formulations with various active principles and properties have been used in Siddha System of medicine from ancient days to treat a whole range of human diseases. Generally, they are collection of therapeutic recipes that are formulated and prepared on the basis of the healing properties of individual ingredients with respect to the condition of sickness. Such herbal constituents with diverse pharmacological actions principally work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. Nevertheless, these traditional medicinal preparations gradually lost their popularity and foothold among people due to the fast therapeutic action of allopathic system of medicine. In recent years however, renewed interest has grown on traditional herbal remedies because of the reportage of adverse side effects using synthetic drugs in allopathic medicine. At the same time, WHO also recommends further research on traditional method of treatment. [1]

In traditional system of Siddha medicine, there are many medicinal preparations which are specified in antique literature for treating various ailments. One such traditional poly-herbal formulation meant to treat diabetes has been revived and manufactured as a drug in capsule form by the brand name SUGNIL and is being used by many Siddha medical practitioners to effectively treat their diabetic patients. Eventhough successful clinical results of SUGNIL have been reported, not much is known about its scientific basis of pharmacological actions. Thus, the present study is designed to ascertain the scientific basis of its use in the management of diabetes and its related complications.

Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia which is usually a consequence of insulin resistance and pancreatic B cell failure acting in conjunction with other metabolic disturbances of the insulin resistance syndrome to generate the characteristic chronic complications of diabetes mellitus.[2] Though hyperglycemia is identified as a classical risk factor for development of diabetic complications, there is no consensus regarding the pathogenic link between hyperglycemia and diabetes

complications.[3] Furthermore, hyperglycemia does not act alone to induce diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycemia, which is mediated and complicated through oxidative stress.[4] Numerous studies also suggest that oxidative stress is a common pathway linking diverse mechanisms for the pathogenesis of complications of diabetes.[5]

Based on these scientific facts, we assume that a therapeutic approach focused on reducing hyperglycemia and increasing antioxidant status of an individual may be considered as an effective treatment strategy in the management of diabetes and its related complications.

Therefore, during the course of this study we deemed it pertinent to evaluate the anti-hyperglycemic and antioxidant effects of our poly herbal drug, SUGNIL, in Streptozotocin induced diabetic rats. Besides, our major concern in the present study was also to elucidate the scientific basis of pharmacological action of our drug which needs a sound knowledge of the biochemical resident of the drug as the biological functionality of such elements determine the mode of action of therapeutic agents. With this in mind, we quantitatively analyzed the biochemical constituents of SUGNIL and thereby evaluated the scientific basis of its pharmacological action.

Materials and Methods

STZ was purchased from Sigma Chemical Co, st.Louis, MO, USA. All the other chemicals used were of analytical grade and purchased from commercial sources.

The polyherbal drug SUGNIL was prepared by Siddha drug manufacturing unit "Naturo Herbal Remeidies" Salem, Tamilnadu. The formula for its preparation has been revived from ancient literature. (Table - 1).

Table-1: SUGNIL FORMULA (composition and concentration).

S.no	Botanical name	Family	Part used	Concentration (mg/dl)
1.	*Aristolochia bracteat*	Aristolochiaceae	Whole plant	40
2.	*Balsamodrunmukul*	Burseraceae	Gum	40
3.	*Cassia auriculata*	Ceasalpinaceae	Flower	50
4	*Casearia esculanta*	Asclepiadaceae	Leaf	40
5.	*Cosinium fenestratium*	Menispermaceae	Bark	50

6.	*Curcuma longa*	Zingiberaceae	Tubers	40
7.	*Eugenia Jambolana*	Myrtaceae	Seeds	50
8.	*Gymnema Sylvestre*	Asclepiadaceae	Leaves	50
9.	*Three myrobalans*	Euphorbiaceae	Fruits	40

Healthy adult male albino Wistar rats, weighing 150-180g were used for the present investigation. The animals were housed in polypropylene cages, maintained under standard conditions (12h light./12h dark cycle, 25 ± 35°C, 35-60% humidity) and were fed with a standard rat pellet diet supplied by Hindustan Lever Ltd., India. The study was approved by the institutional animal ethical committee of animal care, Raja Muthiah Medical College, Annamalai nagar.

The animals fasted overnight and diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (55mg/kg body weight of rats) in 0.1M citrate buffer (pH4.5).[6] The animals were allowed to drink 5% glucose solution overnight to overcome the drug- induced hypoglycemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, when their blood glucose values were above 250mg/dL on the third day after the STZ injection. The treatment was started on the fourth day after the STZ injection and this was considered the first day of treatment. The treatment was continued for 45 days.

The rats were divided into four groups comprising six animals in each group as follows.

Group – I : Control rats given only buffer

Group –II : Diabetic controls (STZ 55mg/kg body weight of rats)

Group –III : Diabetic rats treated with SUGNIL (100mg/kg body weight of rats/day) in aqueous solution orally for 45 days

Group – IV: Control rats treated with SUGNIL (100mg/kg body weight of rats /day) in aqueous solution orally for 45 days

After completion of treatment, the animals were sacrificed by cervical decapitation under pentobarbitone sodium (60mg/Kg) anesthesia. Blood was collected in the tubes containing potassium oxalate and sodium fluoride. Plasma was used for the estimation of glucose using the O-Toluidine method reported by Sasaki et al.[7]

Plasma Insulin level was assayed by enzyme linked immuno sorbent assay kit (ELISA, Boerhringer Mannheim, Germany).

The liver, kidney and pancreatic tissues were quickly excised and rinsed in ice-cold saline. Tissues were cut into small pieces and homogenised in Tris – HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for measurement of antioxidant status.

The activity of SOD was assayed using the method of Markuland and Markuland.[8] The activity of GPx was assayed using the method of Lawrence and Burk.[9] CAT activity was assayed by using the method of Aebi.[10] GSH was estimated using the method of Sedlak and Lindsay.[11] Protein was estimated using the method of Lowry et al.[12] All spectrophotometric measurements were carried out in a camspec UV-visible spectrophotometer.

All the grouped data were statistically evaluated using the statistical package for social sciences (SPSS) version 7.5 (chicago, IL, USA). Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant differences test. P-Values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± standard deviation (SD) for six animals in each group.

In quantitative biochemical analysis, vitamin compositions were determined spectrophotometrically, using the standard method of AOAC (2000).[13] Quantitative phytochemical compositions were determined using the methods described by Trease and Evans (1996) for flavonoids[14], Kirk and sawyer (1998) for tannins[15] and AOAC (1990) for phenols.[13] The perkin Elmer atomic absorption spectrophotometer was used for the determination of Fe, Zn, Cu, Mn, Cr, Pb, AS, Hg, Se and Co using the method of AOAC (2000).[13]

Results

The data in table 2 represents the level of blood glucose and plasma insulin after 45 days of SUGNIL treatment in control and experimental group of rats. From the data it was observed that the level of blood glucose was significantly high and at the same time the level of plasma insulin was significantly low in streptozotocin induced diabetic rats when compared with control rats. On the other hand, the reverse effect was found in rats treated with SUGNIL which was marked by significant decrease in blood glucose and significant increase in plasma insulin. However, there was no significant effect witnessed in control rats treated with SUGNIL.

Table-2: Effect of sugnil treatment on blood glucose and plasma insulin in control and experimental rats.

Parameters	**CON**	**STZ**	**STZ+SUG** (100 mg/kg)	**CON+SUG** (100 mg/kg)
Glucose (mg/dL)	76.81 ± 5.85	258.02 ± 19.65[a]	98.16 ± 7.47[b]	75.54 ± 4.23
Insulin (U/ml)	12.19 ± 0.93	4.06 ± 0.31[a]	8.96 ± 0.68[b]	11.28 ± 0.86

Values are means ±SD of 6 rats from each group. CON-control rats; STZ-Streptozotocin induced diabetic rats; STZ +SUG - streptozotocin induced diabetic rats treated with SUG (100 mg/kg b.w/day); CON + SUG – control rats treated with SUG (100 mg/Kg b.w/day). [a]Significant as compared with control rats ($P<0.05$) (DMRT); [b]Significant as compared with streptozotocin induced diabetic rats ($P<0.05$) (DMRT).

The data in table 3 shows the level of antioxidants in kidney, liver, and pancreas of control and experimental group of rats. From the data it could be seen that the Activities of SOD, CAT, GPX, and GSH in tissues were significantly low in STZ induced diabetic rats when compared to control rats. Whereas the activity of these antioxidants were significantly high in SUGNIL treated rats. However, the control rats showed no effect upon SUGNIL treatment.

Table-3: Effect of sugnil on antioxidant status of tissues in control and experimental group of rats.

Parameters	**CON**	**STZ**	**STZ+SUG (100 mg/kg)**	**CON+SUG (100 mg/kg)**
SOD[A]				
Kidney	15.70 ± 1.2	8.26 ± 0.63[a]	13.11 ± 1.15[b]	15.24 ± 1.15
Liver	11.77±0.90	6.18 ± 0.57[a]	10.93 ± 0.87[b]	11.96 ± 0.85
Pancreas	7.81 ± 0.59	4.18 ± 0.39[a]	6.94 ± 0.48[b]	7.96 ± 0.67
CAT[B]				
Kidney	39.37 ± 3.00	23.85 ± 1.82[a]	31.72 ± 2.18[b]	40.48 ± 3.08
Liver	82.07 ± 6.25	43.69 ± 3.33[a]	74.11 ± 6.27[b]	82.84 ± 6.11
Pancreas	14.33 ± 1.09	6.48 ± 0.49[a]	11.60 ± 0.88[b]	14.90 ± 1.22

GPx[C]				
Kidney	8.55 ± 0.65	4.35 ± 0.33[a]	5.91 ± 0.45[b]	8.50 ± 0.65
Liver	14.64 ± 1.11	7.88 ± 0.60[a]	11.20 ± 0.70[b]	14.18 ± 1.08
Pancreas	8.13 ± 0.62	4.45 ± 0.34[a]	7.21 ± 0.55[b]	8.07 ± 0.61
GSH[D]				
Kidney	11.66 ± 0.89	6.80 ± 0.52[a]	8.50 ± 0.57[b]	11.99 ± 0.91
Liver	13.88 ± 1.06	8.70 ± 0.66[a]	11.52 ± 0.80[b]	13.83 ± 1.05
Pancreas	18.18 ± 1.38	13.80 ± 1.05[a]	16.48 ± 1.26[b]	18.55 ± 1.41

Values are means ±SD of 6 rats from each group. CON-control rats; STZ-Streptozotocin induced diabetic rats; STZ +SUG - streptozotocin induced diabetic rats treated with SUG (100 mg/kg b.w/day); CON + SUG – control rats treated with SUG (100 mg/Kg b.w/day). [a]Significant as compared with control rats ($P<0.05$) (DMRT); [b]significant as compared with streptozotocin induced diabetic rats ($P<0.05$) DMRT).

A-Amount of enzymes which gave 50% inhibition NBT reduction/mg protein.

B-µmol of H_2O_2 consumed/min/mg protein

C-µmol of GSH utilized/min/mg protein.

D-µmol/mg protein.

Table 4 represents the quantitative analysis for vitamins and phytochemicals in SUGNIL, which revealed the presence of Vit E, Vit C and phytochemicls such as flavonoids, tannins and phenol at different concentrations.

Table-4: Quantitative analysis of vitamins and phytochemicals in sugnil.

Parameters	Concentration (%W/W)
Vit C	10.06±0.10
Vit E	10.87±0.32
Flavonoids	11.09
Tannins	16.54
Phenols	17.74

Results are expressed as mean of three determinations ±SEM.

Table 5 shows the quantitative analysis for minerals and heavy metals in SUGNIL which revealed the presence of Cr, Zn, Fe, Mn, Cu, Pb, Hg and Co at different proportions and As,Se were not detectable in analysis.

Table-5: Quantitative analysis of minerals and heavy metals in sugnil.

Parameters	Concentration(Ppm)
Cr	0.015
Zn	0.018
Fe	0.855
Cu	1.977
Mn	0.129
Pb	0.063
As	ND*
Hg	1.344
Se	ND*
Co	0.042

ND*-Not detectable.

Results are expressed as mean of three determinations ±SEM.

Discussion

Recent scientific developments in understanding the pathophysiology of diabetes clearly reveals its multi-factorial pathogenicity and demands for multi-model therapeutic approach, which involves the use of multiple therapeutic agents to address different features of the disease at various stages of its development.[4] Such therapeutic strategy have been practiced from ancient of days in Siddha system of medicine through polyherbal therapy using combination of various medicinal plants and so called polyherbal drugs. The individual medicinal plant extracts in these drugs expresses diverse pharmacological actions that act together on various pathogenic targets and thereby possibly increase the therapeutic efficacy of the drug and minimize the side effects.

This possibility is evidenced by our present study on traditional Siddha polyherbal formulation called SUGNIL which exerts its potent antihyperglycemic and antioxidant effects in STZ induced diabetic rats. Since SUGNIL is a polyherbal drug supplemented with various medicinal plants, we assume that the pharmacological action of individual medicinal plants may act in tandem upon disease conditions through various mechanisms like manipulating carbohydrate metabolism, preventing and restoring integrity and function of β cells, Insulin releasing activity, improving glucose uptake and utilization, and antioxidant properties. Our assumption is supported by previous scientific reports on individual medicinal plant extracts incorporated in SUGNIL formula.

Gymnema Sylvestre, an ingredient of SUGNIL was found to regenerate β cells in pancreatic islets[4]. Since, we used STZ- chemical to induce diabetes in experimental animals which selectively destroyed β cells of pancreas, the pharmacological action of *G.Sylvestre* may have played a role at this juncture and this was evidenced by blood sugar values in SUGNIL treated animals. Other medicinal plants like *Eugenia Jambolana,*[16,17] *Casearia esculanta,*[18] and *Cassia auriculata*[19] that are present in SUGNIL have also been shown to stimulate insulin release from pancreatic β cells. The therapeutic action of these plants may account for the increased level of insulin in SUGNIL treated animals. Hence, the decreased blood glucose and increased insulin levels in SUGNIL treated animals substantiate its antihyperglycemic effect.

Further, *Eugenia Jambolana*,[17] *Curcuma longa*[20], *Cosinium fenestratium,*[21] *Aristolochia bracteata,*[22] and *Three myrobalans,*[23] the constituents of SUGNIL have been reported for their potent antioxidant properties in different animal models and might have expressed the same pharmacological action in SUGNIL treated animals which was evidenced by increased level of antioxidants such as SOD, CAT, GSH and GPx and thus may have helped to avoid free radicals generated during disease condition. Numerous studies also suggest that improvement in the antioxidant status of an individual could reduce the risk of diabetic complications.

Moreover, *Tripala,*[22] *Coninium fenestratam,*[24] *Eugenia Jambolna,*[16] *BalsamodrunMukul,*[25] *G.Sylvestre*[26] were shown to posses antihyperlipidemic action which could help prevent the development of cardiovascular diseases, a major consequence of diabetes. Thus improved antioxidant status in SUGNIL treated animals and the lipid lowering activity of ingredients of SUGNIL supports its use in the management of diabetic complications.

The goal of the present study is not only to confirm the antidiabetic efficacy of SUGNIL but also to elucidate the mechanism of pharmacological action in diabetes treatment. As a general rule, pharmacological actions of medicinal plants and drugs have been attributed to their biochemical residents. Therefore we quantitatively analyzed the presence of important bioactive compounds in SUGNIL and thereby we determined the possible mechanism of pharmacological action.

Our quantitative chemical analysis of SUGNIL reveals the presence of Vitamins (D & E), Phytochemicals (flavonoids, tannins, phenols), Minerals (Zn, Cu, Mn, Cr, Fe), and Heavy metals (Pb, Hg, Co). Presence of Vitamins such as D & E is well known dietary antioxidants which may play a partial role in the antioxidant effect of SUGNIL. [27,28,29] Phytochemical residents such as flavonoids, tannins and phenols have already been reported for their potent free radical scavenging activities and may act along with vitamins to strengthen the antioxidant potency of SUGNIL.[30,31,32] Besides Scavenging, flavonoids may preserve β cell function by reducing oxidative stress.[33] Phenols are reported to inhibit α-amylase and sucrase, the principle substances for suppressing Post prandial hyperglycemia. Furthermore, phenols and tannins inhibit glucose transport across the intestine by suppressing sodium glucose co-transporter–1 (S-GLUT-1).[4] Hence the presence of these phytochemicals not only exerts antioxidant effect by scavenging ROS but also involve in antihyperglycemic action of SUGNIL by inhibiting the enzymes.

People with uncontrolled hyperglycemia, especially those on chronic diuretic therapy are prone to develop deficiencies in some minerals.[34] For instance, deficiency of chromium has been implicated as one of the causes of diabetes mellitus;[35,36] and people with uncontrolled diabetes have increased zinc losses in the urine;[34] and copper deficiency have been reported to elevate serum cholesterol and triacylglycerol.[37] Hence, the presence of these minerals in SUGNIL and its daily intake could address deficiency related complications and restore their normal level. Moreover, the biological functionality of these elements may influence the therapeutic mode of action of SUGNIL. For example, Cr & Zn act in normalizing glycemia and are postulated to function as antioxidants and may exert the same in therapeutic action of SUGNIL.[38] Also presence of other minerals in SUGNIL may help in modulating the immune system and the pancreatic insulin secretion and action.

Presence of heavy metals such as Pb, Hg, Co may be considered toxic. However, their actual level in SUGNIL is way below the daily permissible limits recommended by DRI which is an indication of its non-toxicity.

Therefore, from the present study, it may be concluded that the presence of various medicinal plants and their diverse pharmacological actions work together in a holistic way and increase the antidiabetic efficacy of SUGNIL. Besides, the bioactive components of these medicinal plants exercise their physiological role in the mechanism of antihyperglycemic and antioxidant effects of SUGNIL.

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