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Pharmacological Screening of Some Plant Extract for Hypoglycemic Activity

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ABSTRACT

Globally, the pervasiveness of chronic, non-communicable disease diabetes mellitus is growing by leaps and bounds. It is one of the major causes of premature morbidity and mortality worldwide. Almost 3.2 million people die of diabetes across the world every year. India is one of the major biodiversity centers of the world and enriched by nearly 45,000 plant species. Out of these, about 2,500 species are described in Ayurveda along with over 10,000 formulations. Herbal preparations for various purposes including pharmaceutical and cosmetic form, part of traditional biodiversity uses in India. Even the WHO (World Health Organization) approves the use of plant drugs for different diseases, including diabetes mellitus. Many plants possessing hypoglycemic principles/ properties are known to exist in nature. Some of these are leaves of Gymnema sylvestre, fruits of Withania coagulans and leaves of Ocimum sanctum. Also a large number of polyherbal formulations derived from these plants are presently being prescribed as medicinal/dietary supplements for diabetes mellitus. This paper focuses on pharmacological activity such as serum glucose level, serum total lipids, triglycerides; cholesterol of these plant extract on STZ-Nicotinamide induced diabetic rats for the management of type 2 diabetes and found that G. sylvestre had significantly reduced the serum glucose level in STZ-Nicotinamide diabetic rats while W. coagulans and O. sanctum showed potent hypolipidemic activity in STZ-Nicotinamide rats.

Keywords: Diabetes management; Diabetes mellitus; *Gymnema sylvestre; Ocimum sanctum;* STZ-Nicotinamide; *Withania coagulans*.

INTRODUCTION

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus [1].

Diabetics tend to believe that there is no cure yet for diabetes in any system of medicine anywhere in the world, which is partly because diabetes is not a single disease but is a complex disorder with multiple syndromes making it difficult to cure the cause of the disease. Diabetes is a cluster of symptoms like in an aging process this manifests as graying of hair or wrinkling of skin. As with aging symptoms, diabetes may occur at a very early age in a few, and at some stage of the life in most. Diabetes manifests in different persons in many different ways, and depending on the age, severity of the symptoms and involvement of other organs in the body, medical treatment greatly varies. For example a diabetic, either in young age, during pregnancy (gestational diabetes), if suffering from tuberculosis or a

foot ulcer or, with recent heart attack or paralysis, should be given insulin injections only, and not other anti diabetic drugs of any systems of medicine [1].

Ayurvedic system of medicine

Ayurveda is perhaps the oldest among the organized traditional medicine. It spread with *Vedic* and Hindu culture as far in east as Indonesia and to the west it influenced the Greek, who developed a similar form of medicine. Ayurveda remains one of the most ancient medical systems widely practiced in the Indian subcontinent and has a sound philosophical, experiential and experimental basis [2]. *Charak Samhita* and *Sushrut Samhita* (100–500 B.C.) are main Ayurvedic classics, which describe over 700 plants along with their classification, pharmacological and therapeutic properties. Knowledge of Ayurvedic medicine has unfortunately been confined to India and the west is largely ignorant of it. Even in India, this traditional medical practice has lost a lot of its importance in the urban situations. One of the main reasons for this is that much of the early and core medical literature on Ayurveda is in Sanskrit, the ancient language which ceased to be a day-to-day language. Even today a considerable bulk of Ayurvedic knowledge is in the form of ancient palm leaf manuscripts hidden in remote libraries and private collections, and as treasured personal knowledge of a few individuals. The net result is that Ayurveda has been away from limelight and does not enjoy the importance and popularity it deserves.

Diabetes and Ayurveda

Diabetes mellitus is a condition not new to Ayurveda. In ayurveda, diabetes is mentioned as *Madhumeha* which is a type of *Prameha* characterised by discharge of sweet urine through urethera. Charaka and Sushruta (600 and 400 BC) had classified 20 varieties of *'Meh'* under *'Vata', 'Pitta' and 'Kapha'* syndromes. *Madhumeha* consists of three clinical types identified as *Vataja, Pittaja* and *Kaphaja*. *Kaphaja Madhumeha* may be related to high growth hormone in the system and *Pittaja* is the one which may be related to high level of glucocorticoids. In the progression of diabetes, the initial stage of *Prameha,* the *Kapha dosha* is at high level which later on changes into *Kaph Kashaya*. Similarly *Pittaja Prameha* leads to *Pitta Kashya.* However, there is a common denominator of *Vata Briddhi* in both *Kaphaja* and *Pittaja Prameha,* which in the terminal state lead to a stage of *Madhumeha,* sub 1 type of *Vataja Prameha* [3].

According to Charaka, *Prameha* resulted from over eating of' *Havish'* (ghee) offered in the *'Yagya'. Tridosha (Vata, pitta* and *kapha)* concept of Ayurveda, regarded as the functional basis of body mechanism controls the state of health of an individual (Prakriti) by maintaining the functional stability of the body. Individual *dosha* maintains equilibrium amongst them in a healthy state. Vitiation of this equilibrium will induce a state of ill health which may proceed up to the stage of disease [3]. It is interesting to note that although symptoms of the disease were known to ancient Hindu physicians, clear cut knowledge about the treatment of the disease was not handy although suitable dietary therapy i.e. use of oils (unsaturated fat) instead of animal fat (saturated fat), was recommended.

The therapeutic importance of diet was not unknown to Ayurveda which modern system of medicine emphasizes now. It is evidenced by the fact that as early as Charaka-Sushruta era (600 B.C.), it was recommended that addition and restriction of certain foods were an adjunct to treatment of *Madhumeha.* It was recommended that the low carbohydrate diet (low sugar -low starch) and almost total withdrawal of animal fats and ghee should be taken by patients suffering from *Madhumeha*. However, it was also felt that lean and young patients should be prescribed nourishing diet whereas, obese adults should live on low calorie diet. Use of vegetable oil was generally recommended. Apart from the diet restrictions, Ayurveda had also identified and recommended a number of plant based therapies for the control and treatment of diabetes either as single or in a synergistic combination [4, 5].

Plants used to treat diabetes

Ayurvedic physicians recognized and categorized clinical entities corresponding to diabetes. Pharmacopoeia of ancient India listed specific treatments for diabetes, including dietary modifications, medicinal plant remedies. Chinese medical books written as early as 3000 B.C. spoke of diabetes and therapies for this diseases. These historical accounts reveal that diabetes was well known among the ancients and the medicinal plants have been used for over millennia to treat this disease. Before the advent of insulin, diabetes was treated with success by use of plant medicines. In 1980, WHO urged researchers to examine whether traditional medicines produced any beneficial clinical results? Few of the medicinal plant treatments for diabetes have received scientific scrutiny, for which WHO has also recommended attention [6]. Over the last decades, several comprehensive reviews have been written on the evidence that higher plants are of use in the treatment of diabetes, providing discussions of the botany, phytochemistry, and pharmacology of botanical agents. Literally hundreds of extracts of higher plants used in folk

medicine for diabetes have been screened for their biologic activity in both *in vitro* and *in vivo* assays [7, 8, 9, 10, 11] which further proves the effectiveness of plant in treating diabetes. There are number of herbal formulations in the market for the treatment of diabetes [12].

EXPERIMENTAL SECTION

Candidate plants were collected from their natural habitats in and around Lucknow and also procured from the local herbal drug market. The plants were authenticated by comparison with the herbarium and voucher specimen (NBRI/CIF/2372011) was lodged in the departmental herbarium of National Botanical Research Institute, Lucknow.

Chemicals

Streptozotocin (STZ) (Sigma Chemicals St louis, USA), nicotinamide was procured from K. B. & Sons Charbagh, Lucknow, India. Glucose diagnostic kit (Qualigens, Mumbai), Total lipids diagnostic reagent kit (Merck, Mumbai), cholesterol diagnostic reagent kit (Span Diagnostics, Surat, India), Triglyceride kit (Qualigens Diagnostics, Mumbai), all the other chemicals used were of the analytical and highest purity grade from standard companies. Water represents the distilled water; standard orogastric cannula was used for oral drug administration.

Animals

Wistar albino rats (180-250g) of either sex were procured from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at temperature 26 ± 2 °C and relative humidity 44 – 56 %, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Dayal Industries, Lucknow) and the food was withdrawn 18-24 h before the experiment though water was allowed ad libitum.

Experimental induction of diabetes in rats

NIDDM was induced by a single intraperitoneal injection of 65 mg/kg streptozotocin (Sigma Aldrich, Germany) followed by nicotinamide (K. B. & Sons Charbagh, Lucknow, India) 110 mg/kg, i.p, 15 min afterwards. Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline [13]. Diabetes was confirmed in STZ-Nicotinamide rats by measuring the fasting blood glucose concentration after 96 h after the injection of STZ-Nicotinamide. The rats with blood glucose level above 220 mg/dl were considered to be diabetic and were used in the experiment. 65 mg/kg body weight was found to cause stable diabetic animals with the blood glucose level at 220-240 for around 4 weeks.

Hypoglycemic activity of selected plant extracts in STZ-Nicotinamide induced diabetic rats

The hypoglycemic effects of the aqueous and alcoholic extracts of G. sylvestre, W. coagulans, O. sanctum were studied in STZ-Nicotinamide diabetic rats.

Group I - Control rats received vehicle solution (2% gum acacia)
Group II - Diabetic control rats received vehicle solution (2% gum acacia)
Group III - Diabetic rats treated with extract 100 mg/kg body weight in 2% gum acacia
Group IV - Diabetic rats treated with extract 250 mg/kg body weight in 2% gum acacia
Groups V - Diabetic rats treated with glibenclamide 3 mg/kg body weight in aqueous solution

The vehicles and the drugs were administered orally using intra gastric tube daily for three weeks. After three weeks of treatment the rats were fasted overnight, the blood samples were analyzed for blood glucose content.

Biochemical estimations

Glucose estimation

The collected serum samples of different study group were subjected to the serum glucose level estimation by enzymatic GOD-POD method by using glucose diagnostic kit (Qualigens Diagnostics).

Total lipids estimation

The Total lipid was estimated by Phosphovainilline method using Total lipids diagnostic reagent kit (Merck, Mumbai).

Total cholesterol estimation
The serum cholesterol level was estimated by wybenga and pileggi method using cholesterol diagnostic reagent kit (Span Diagnostics, Surat, India)

Triglyceride estimation
Triglyceride was estimated by glycerol phosphate oxidase method using Triglyceride kit (Qualigens Diagnostics, Mumbai).

Blood sample collection
The blood was collected from the eye canthus of rats using capillary tubes (microhaemotocrit capillaries) and transferred into empty fresh centrifuge tubes. Care was taken during collection and transferring of blood samples to prevent haemolysis. The collected blood samples were immediately centrifuged at 2500 rpm for 15 mins. The serum separated was collected in fresh serum tubes and stored in refrigerator (2-4 ^{o}C) after tightly capped.

Statistical analysis
The statistical analysis of all the pharmacological analysis was carried out using GraphPad Prim version 3.03 for windows. The values are represented as mean ± SEM for six rats data were analyzed by Student *t* test and ANOVA with post-hoc difference was analysed using Newman-keuls method.

Pharmacological Evaluation of selected plants
Effect of extracts on blood glucose level in STZ diabetic rats
Seventy two hours after administration of STZ-Nicotinamide, rats with serum glucose level more than 250 mg/dl were included in this study. Two dose levels 100 mg/kg/day, 250 mg/kg/day of test drug in appropriate quantity of water were given orally by gavage for three weeks. A normal control group (non-diabetic) and diabetic control group (diabetic induced) were included in this study. The positive control group for synthetic drug also included and received glibenclamide of 3 mg/kg/day in the same manner described above. All the doses were given 72 hrs after Streptozotocin injection. The blood samples were drawn at 0, 7 and 21 days of the study.

Table 3 shows the effect or oral administration of 100 mg/kg/day, 250 mg/day of Ethanolic extract of *O. sanctum* (OSEt) on serum glucose level in 21days, at the end of the treatment, reduction in serum glucose level in treated rats with dose 250mg/kg was 44.47% and 100mg/kg was 39.13%. The difference in percentage reduction of serum glucose level of different groups are significantly ($p<0.01$). After 14 days and 21days, 50.4% and 55.21% significant fall in serum glucose level were found in 250mg/kg aqueous extract of *G. sylvestre* (GSAq) treated STZ diabetic rats ($p<0.001$**)** and 54.9% and 50.45% significant reduction with glibenclimade (Table1). Table 2 shows aqueous extract of *W. coagulans* (WCAq) also showed significant serum glucose activity at 250mg/kg was 32.35% and 34.88% after 14 and 21days treatment ($p<0.01$). GSAq had the maximum glucose lowering activity at 250 mg/kg (102.26 ± 23.12 mg/dl as compared to the 228.33 ± 23.03 mg/dl in untreated diabetic rats). OSEt also had potent activity, which was followed by WCAq with the blood glucose.

Effect of extracts on lipid profile in STZ diabetic rats
GSAq (250 mg/kg) again had potent effect on the lipid profile of the diabetic rats by significant decreasing the total lipid (88.19 ± 1.26 mg/dl as compared to diabetic control 142.56 ± 2.10 mg/dl) ($p< 0.001$), cholesterol (74.35 ± 2.64 mg/ dl as compared to control 159.42 ± 2.86 mg/ dl) ($p< 0.001$) and triglycerides level (67.43 ± 1.25 mg/dl as compared to control 116.42 ± 1.24) ($p< 0.001$). WCAq (250 mg/kg) again had potent effect on the lipid profile of the diabetic rats by significant decreasing the total lipid (108.12 ± 3.56 mg/dl as compared to diabetic control 142.56 ± 2.10 mg/ dl) ($p< 0.001$), cholesterol (78.65 ± 1.98 mg/dl as compared to control 159.42 ± 2.86 mg/ dl) ($p< 0.001$) and triglycerides level (71.46 ± 1.25 mg/dl as compared to control 116.42 ± 1.24) ($p< 0.001$). WCAq was found have potent lipid lowering activity followed by OSEt (Table 4-6).

DISCUSSION

Effects of extract of all the three selected plants on serum glucose and lipid profile in STZ-Nicotinamide induced diabetic rats were studied. *G. sylvestre* caused a significant reduction in serum glucose and had potent lipid lowering activity. Lipid lowering and serum glucose lowering was very profound when compared to the other plants. *W. coagulans* caused a significant lipid lowering effect along with moderate serum glucose lowering effect in STZ-

Nicotinamide induced diabetic rats. *O. sanctum* also caused a significant serum glucose lowering effect along with very moderate lipid lowering effect. The serum glucose lowering effect of the plants was found to be in the following order, GSAq>OSEt>WCAq, while the serum lipid lowering effect was found in the order of GSAq>WCAq>OSEt.

Table 1. Effect of *G. sylvestre* extract on serum glucose level in STZ-Nicotinamide diabetic rats

Groups	Blood glucose (mg/ dl)			
	0 day	7th day	14th day	21st day
Group 1 (Normal)	85.45 ± 11.41	86.21 ± 10.33	84.35± 12.62	83.66 ± 12.76
Group 2 (Diabetic control)	240.78 ± 24.68	235.98 ± 18.23^{z}	232.56±22.24^{z}	228.33 ± 23.03^{z}
Group 3 (GSAq 100mg/kg)	244.38 ± 18.42	152.72 ± 22.16^{b}	126.48± 16.33^{c}	115..88 ± 22.18^{c}
Group 4 (GSAq 250mg/kg)	241.46 ± 23.24	138.24 ± 20.24^{b}	115.26± 23.32^{c}	102.26 ± 23.12^{c}
Group 5 (Glibenclamide)	239.59 ± 32.71	128.29 ± 16.25^{b}	104.82± 12.63^{c}	94.33 ± 13.66^{c}

The values represent the means ± S. E. M for six rats per group.. $^{a}p<0.05$, $^{b}p<0.01$ *and* $^{c}p<0.001$ *compared to diabetic control group,* $^{z}p<0.001$ *as compared to normal*

Table 2. Effect of *W. coagulans* extract on serum glucose level in STZ-Nicotinamide diabetic rats

Groups	**Blood glucose (mg/ dl)**			
	0 day	**7th day**	**14th day**	**21st day**
Group 1 (Normal)	78.45 ± 12.31	76.02 ± 11.70	77.44± 10.32	78.86 ± 9.36
Group 2 (Diabetic control)	248.22 ± 32.46^{z}	227.22 ± 36.14^{z}	229.45±32.12^{z}	212.31 ± 26.11^{z}
Group 3 (WCAq 100mg/kg)	239.23 ± 11.43	186.71 ± 13.16	174.46± 12.33^{a}	152..88 ± 12.18^{a}
Group 4 (WCAq 250mg/kg)	252.23 ± 13.54	182.25 ± 12.42	155.21± 14.21^{a}	138.26 ± 11.12^{b}
Group 5 (Glibenclamide)	257.52 ± 2.22	154.24 ± 12.34	106.12± 12.56^{c}	92.18 ± 12.28^{c}

The values represent the means ± S. E. M for six rats per group. $^{a}p<0.05$, $^{b}p<0.01$ *and* $^{c}p<0.001$ *compared to diabetic control group.* $^{z}p<0.001$ *as compared to normal*

Table 3. Effect of *O. sanctum* extract on serum glucose level in STZ-Nicotinamide diabetic rats

Groups	**Blood glucose (mg/ dl)**			
	0 day	**7th day**	**14th day**	**21st day**
Group 1 (Normal)	85.44 ± 11.42	86.20 ± 10.32	84.35± 12.62	83.66 ± 12.76
Group 2 (Diabetic control)	240.78 ± 24.68^{z}	235.98 ± 18.23^{z}	231.56±22.24^{z}	228.33 ± 23.03^{z}
Group 3 (OCET 100mg/kg)	242.36 ± 22.97	202.37 ± 33.96	157.46± 31.16^{a}	138.98 ± 29.58^{b}
Group 4 (OCET 250mg/kg)	240.69 ± 36.24	187.99 ± 24.17	140.35± 26.33^{a}	126.77 ± 24.22^{b}
Group 5 (Glibenclamide)	239.59 ± 32.71	128.29 ± 16.15^{a}	104.82± 12.63^{b}	94.33 ± 13.66^{b}

The values represent the means ± S. E. M for six rats per group. $^{a}p<0.05$, $^{b}p<0.01$ *and* $^{c}p<0.001$ *compared to diabetic control group.* $^{z}p<0.001$ *as compared to normal*

Table 4. Effect of *G. sylvestre* extract on the level of Serum Total lipids, Triglycerides, Cholesterol in STZ-Nicotinamide diabetic rats

Group	Total lipids (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
Group 1(Normal)	85.14 ± 1.53	68.41 ± 1.54	73.45 ± 2.63
Group 2 (Diabetic control)	142.56 ± 2.10 ^{z}	116.42 ± 1.24 ^{z}	159.42 ± 2.86 ^{z}
Group 3 (GSA 100mg/kg)	108.12 ± 2.65^{c}	92.26 ± 2.47^{c}	98.12 ± 2.33^{c}
Group 4 (GSA 250mg/kg)	88.19 ± 1.26^{c}	67.43 ± 1.25^{c}	74.34 ± 2.64^{c}
Group 5 (Glibenclamide)	92.15 ± 1.54^{c}	77.25 ± 1.47^{c}	81.32 ± 1.38^{c}

The values represent the means ± S. E. M for six rats per group. $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ compared to diabetic control group. $^{z}p<0.001$ compared to normal group.

Table 5. Effect of *W. coagulans* extract on the level of Serum Total lipids, Triglycerides, Cholesterol in STZ-Nicotinamide diabetic rat

Group	Total lipids (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
Group 1(Normal)	85.13 ± 1.73	68.41 ± 1.54	73.45 ± 2.63
Group 2 (Diabetic control)	142.56 ± 2.10 ^{z}	115.42 ± 1.23 ^{z}	159.42 ± 2.86 ^{z}
Group 3 (WCA 100mg/kg)	128.16 ± 3.46^{b}	94.42 ± 2.64 ^{b}	104.46 ± 1.36^{c}
Group 4 (WCA 250mg/kg)	108.12 ± 3.56^{c}	71.46 ± 2.56^{c}	78.64 ± 1.98^{c}
Group 5 (Glibenclamide)	92.15 ± 1.54^{c}	77.35 ± 1.46^{c}	81.31 ± 1.38^{c}

The values represent the means ± S. E. M for six rats per group. $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ compared to diabetic control group. $^{z}p<0.001$ compared to normal group.

Table 6. Effect of *O. sanctum* extract on the level of Serum Total lipids, Triglycerides, and Cholesterol in STZ-Nicotinamide diabetic rat

Group	Total lipids (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
Group 1(Normal)	89.25 ± 2.73	64.52 ± 1.46	75.62 ± 1.28
Group 2 (Diabetic control)	136.42 ± 2.16 ^{z}	112.54 ± 1.26 ^{z}	162.42 ± 1.14 ^{z}
Group 3 (OCET 100mg/kg)	134.36 ± 1.17	108.54 ± 2.37	159.36 ± 2.56
Group 4 (OCET 250mg/kg)	126.54 ± 2.48^{b}	104.66 ± 2.12^{a}	152.23 ± 2.25^{b}
Group 5 (Glibenclamide)	98.43± 2.16^{c}	68.12 ± 1.65^{c}	86.46 ± 1.76^{c}

The values represent the means ± S. E. M for six rats per group. $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ compared to diabetic control group. ^{z} $p<0.001$ compared to normal group.

CONCLUSION

In this present study, based on the pharmacological screening of three selected plant extracts for hypoglycemic activity, it was found that *G. sylvestre* had significantly reduced the serum glucose level in STZ-Nicotinamide diabetic rats while *W. coagulans* and *O. sanctum* showed potent hypolipidemic activity in STZ-Nicotinamide rats.

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