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Pharmacological Activity of a Polyherbal Formulation by Haemoglobin Glycosylation Assay

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ABSTRACT

Present study involves the development of a polyherbal formulation by using four different herbs i.e. Chirata (*Swertia chiratia*), Haldi (*Curcuma longa*), Neem (*Azadirachta indica*), Gudmar (*Gymnema sylvestre*), Ashwagandha (*Withania somnifera*), Gokharu (*Pedalium murex*), Methi (*Trigonella foenum-graecum*), Jammun (*Syzygium cumini*), relating to antidiabetic activity. Freshly collected and authenticated herbs were characterized by studying its morphological and pharmacognostic character. Antidiabetic and antioxidant activity of the formulation was determined by *in vitro* haemoglobin glycosylation assay and H_2O_2 radical scavenging method respectively. In the above study it was found that ethanolic extract of polyherbal formulation possess promising antidiabetic and antioxidant activity which can be consider for further biological investigation.

Keywords: polyherbal formulation, *Swertia chiratia, Curcuma longa, Azadirachta indica, Gymnema sylvestre, Withania somnifera, Pedalium murex, Trigonella foenum-graecum, Syzygium cumini*

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INTRODUCTION

The American Diabetes Association classifies diabetes mellitus into four types. Type 1 also known as insulin dependent diabetes mellitus (IDDM) in which genetic deficiency in insulin production as a result of allergic reactions which destroy the pancreatic beta cells. Type 2 also known as non-insulin dependent diabetes mellitus (NIDDM) is combined resistance to insulin-action and insulin-secretory response. Type 3 also known as gestational diabetes causes carbohydrate intolerance with first recognition during pregnancy and type 4 (genetic defect- or medication-induced diabetes). Among all the types NIDDM accounts for approximately 90% of the diabetes cases globally[1-2]. Ayurvedic system of medicine is as old as human civilization. Herbal plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants[3]. Study suggest that polyherbal formulations have promising antioxidant properties in term of H_2O_2 radical scavenging activity and validates their synergistic effect by having an improved activity in most of the formulations[4]. As such developing a polyherbal formulation will definitely produce synergistic effect as needed comparable to standard drugs that are available in market all over the world. In the traditional system of Ayurveda, polyherbal formulations are used as drug of choice rather than individual plant extract. Various herbal formulations such as DIAMED, COAGENT and HYPONID are known for their antidiabetic effect. However, these formulations cannot be said ideal in the treatment of diabetes [5-6]. The polyherbal formulation, which has a combination of medicinal herbs such as Chirata (*Swertia chiratia*), Haldi (*Curcuma longa*), Neem (*Azadirachta indica*), Gudmar (*Gymnema sylvestre*), Ashwagandha (*Withania somnifera*), Gokharu (*Pedalium murex*), Methi (*Trigonella foenum-graecum*), Jammun (*Syzygium cumini*), can serve as antidiabetic agent.

Chirata leaves, Haldi rhizomes, Neem seeds, Gudmar leaves, Ashwagandha stem, Gokharu fruits, Methi seeds, Jammun seeds are used in traditional medicine in the treatment of chronic cases of high blood pressure, obesity, diabetes, various digestive ailments, as well as geriatric and antiarteriosclerosis remedies[7-8]. Literatures revealed that the selected eight herbs have antioxidant activity. Hence an attempt was made to formulate a polyherbal formulation, and to evaluate its *in vitro* antioxidant activity[9]. Aerobic respiration, stimulated polymorphonuclear leukocytes, macrophages and perioxisomes causes formation of reactive oxygen species (ROSs). These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco

smoke, ionizing radiation, certain pollutants, organic solvents and pesticides. Life threatening diseases like cancer, emphysema, cirrhosis, atherosclerosis, and arthritis can be correlated with oxidative damage[10]. Numerous vitamins, minerals and other phytochemicals may act as antioxidant to protect the damage caused by ROS[11-12]. A non-enzymatic reaction between free amino groups of proteins and reducing sugars is known as glycation. The increased glycation is nothing but additional binding of glucose to hemoglobin which may leads to formation of reactive oxygen species (ROS). Such accelerated haemoglobin glycation can also cause pathogenic conditions like angiopathy, nephropathy and neuropathy in diabetic patients. When oxidation occurs in glycation process, it leads to glycoxidation and formation of advanced glycation end products[13].

Impaired glucose metabolism in diabetes is associated with increase free radical generation (oxidative stress) causes serious cell damage leading to a variety of human diseases like Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer, arthritis, immunological incompetence, and neurodegenerative disorders, etc[14]. Antioxidants are intimately involved in the prevention of cellular damage - the common pathway for cancer, aging, and a variety of diseases[15-16]. The purpose of the present study was to investigate the *in-vitro* antioxidant and antidiabetic potential of polyherbal formulation.

MATERIALS AND METHODS

Haemoglobin glycosylation assay

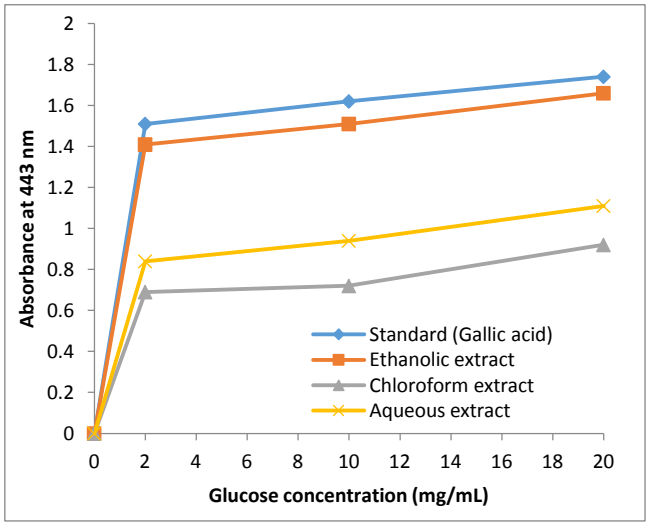
The blood of healthy volunteers was collected in EDTA vials from a pathology laboratory of Ayurved Medical College (Shobhit University, Gangoh). Haemolysate was prepared based on the principle of hypotonic lysis[17]. Collected blood was washed three times with sodium chloride solution. One volume of red blood cells suspension was lysed with 2 volumes of phosphate buffer and 0.5 volume of chloroform. The haemolysate was then centrifuged at to achieve clear hemoglobin solution was obtained by centrifugation of haemolysate at 2300 rpm for 15 min at room temperature. The upper layer containing haemoglobin fraction was separated and kept in refrigerator for use in the assay. Evaluation of haemoglobin glyacation was estimated by the method of Adisa et al[18].

1 ml of haemoglobin solution (obtained by hypotonic lysis), 5µl of gentamycin and 100, 200, 400, 800, and 1000 µg ml^{-1} of polyherbal formulation were added in test tubes. 1 ml of 20 mM glucose in 10mM phosphate buffer of pH 7.4 was added to the mixture to start the reaction and the contents were incubated at room temperature for 72 hrs. Spectrophotometer at 443nm was used for estimation of glycated haemoglobin at the incubation interval of 24, 48 and 72 hrs **(Table 1)**.

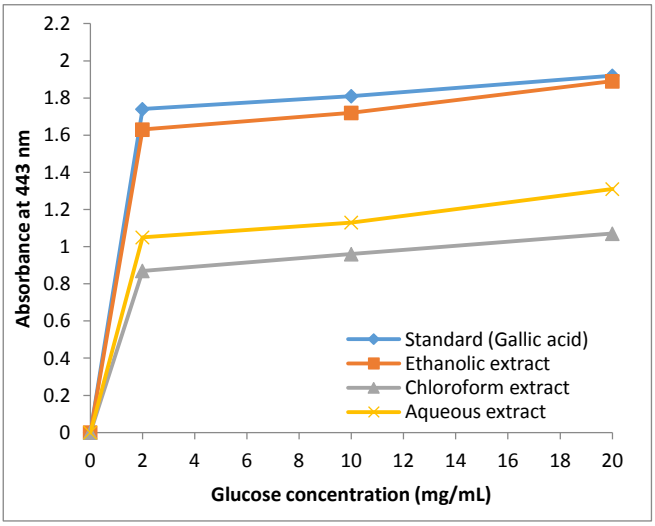
Table 1: Effect of polyherbal formulation on hemoglobin glycosylationat physiological glucose concentrations

S.No	Concentration (µg ml)	% Hemoglobin glycosylation inhibition		
		24 hrs	48 hrs	72 hrs
1	100	16.36	18.43	32.72
2	200	22.23	28.64	52.56
3	400	24.36	36.52	66.58
4	800	27.34	42.54	80.62
5	1000	30.26	46.32	92.18

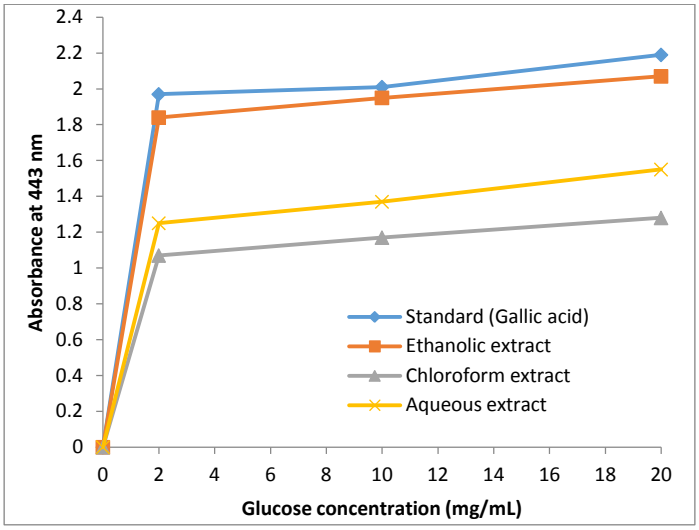
Similarly for haemoglobin glycosylation at physiological glucose concentration, a mixture of 1 ml of glucose solution (2, 10 and 20 mg ml^{-1}), 1 ml of haemoglobin solution, and 5µl of gentamycin in 20 ml of 10 mM phosphate buffer (pH 7.4) and 100, 200, 400, 800, and 1000 µg ml^{-1} of Gallic acid and polyherbal formulation respectively were taken in test tube. Contents were incubated at room temperature for a period of 72 hrs as an indicator of haemoglobin glycosylation. The assay was carried out in triplicates and percent haemoglobin glycosylation was calculated **(Graph 1-3).**



Graph 1: The comparative effects of plant extracts on haemoglobin glycosylation at physiological glucose concentration after 24 hrs of incubation.



Graph 2: The comparative effects of plant extracts on hemoglobin glycosylation at physiological glucose concentration after 48 hrs of incubation.



Graph 3: The comparative effects of plant extracts on haemoglobin glycosylation at physiological glucose concentration after 72 hrs of incubation.

Hydrogen peroxide assays

The hydrogen peroxide scavenging was determined according to the method of Ruch et al. The absorbance value of the reaction mixture was recorded at 230 nm. Hydrogen peroxide scavenging activity was calculated using the equation: (1 - Absorbance of sample/ absorbance of control) × 100. Each experiment was carried out in triplicateand results averaged expressed as mean ± SD **(Table 2)**.

Table 2: H_2O_2 Radical Scavenging Assay

Different Extract	Concentration (µg/mL)						
	50	100	200	400	800	1000	IC_{50}
Ascorbic Acid	60.13	71.56	79.51	82.30	83.44	83.86	9.16
Ethanolic	48.27	54.17	58.15	59.23	60.28	62.96	11.12
Chloroform	66.25	69.15	71.89	72.90	73.67	75.34	14.78
Aqueous	77.16	83.20	86.72	86.84	87.83	89.17	10.23

RESULTS AND DISCUSSION

Haemoglobin glycosylation assay

The glycosylation hemoglobin assay is essential for the diagnosis and management of diabetes because it provides the best estimate of a patient's average blood glucose (AG) over the preceding 2–3 months and is the best predictor of disease complications. Generally the haemoglobin glycosylation is determined mainly to identify the average glucose concentration over a prolonged period of time. Higher values of glycated haemoglobin indicate poor control of blood glucose level under diabetic condition. The *in-vitro*haemoglobin glycosylation inhibition assay showed considerable inhibition of glycosylation over a period of 72 hrs as compared to gallic acid. The *in vitro* assays of the present study indicated that all the three extracts; ethanolic, chloroform and aqueous possess good anti diabetic activity.

Hydrogen peroxide assays

Oxygen free radical can begin peroxidation of lipids, which in turn stimulates glycation of proteins, inactivation of antioxidant enzymes and play a role in long term complication of diabetes. Regardless of the presence of well-known antidiabetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease possibly because they are believed to be less toxic and free from side effects compared to synthetic one. Our polyherbal formulation contains eight different ingredients which are known to possess antidiabetic and antioxidant activity. In general, H_2O_2 radical scavenging assay is widely used to evaluate the antioxidant properties of extracts from different plant materials. Due to their natural origin of the extracts, the use of natural antioxidant from plants does not induce side effect. The higher antioxidant properties in polyherbal formulation might be due to the properties of the plant that conferred with high amount of flavones, polyphenols, glycosides, bioactive active proteins, a volatile oil, and massive quantities of potassium[19]. Ethanolic extract showed better antioxidant property followed by aqueous and chloroform extract.

CONCLUSIONS

Findings of *in-vitro* glycosylation of haemoglobin and H_2O_2 radical scavenging activity assay clearly indicate that polyherbal formulation possesses considerable antidiabetic and antioxidant activity. It was observed that different extracts of polyherbal formulation inhibited the formation of glycated end products. Identification and characterization of bioactive compounds liable for such activities along with *in vivo* investigations should be done further. Free radicals play an important role in the development of degenerative diseases like diabetes hence supplementation with antioxidant may overcome diabetic complications. This polyherbal formulation can serve Therapeutic agents in maintaining blood glucose levels in diabetic patients.

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