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Decoction of *Andrographis paniculata* whole plant and *Gymnema sylvestre* leaves demonstrated noteworthy hypoglycemic activity in Sprague Dawley rat

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ABSTRACT

Mother Nature is an elite source for the exploration of leads with good hypoglycemic potential. Progressing in the direction of research, two well-known plants from the Indian subcontinent; *A. paniculata* (Family: Acanthaceae) and *G. sylvestre* (Family: Apocynaceae) were chosen and evaluated for their hypoglycemic property in alloxan induced diabetic rat model using metformin hydrochloride as the standard drug. In the current investigation, whole plant decoction of *A. paniculata* and decoction of *G. sylvestre* leaves were utilized. The research specified preliminary phytochemical investigations which facilitated better understanding the role(s) of natural bioactive molecules in mediating the activity and their probable mechanism(s). The results represented that the combined decoction of plants significantly exerted the hypoglycemic effect. The glucose lowering activity was mediated by active polyphenol or flavonoid principles present in the decoction which may be believed to enhance the transportation of blood glucose level in the peripheral tissues or via potentiation of insulin secretion from the pancreatic β-cells. The present study reflects the perception to be used clinically according to the toxicity profiles. The research may hold perspective for the development of formulations based on above two natural principles and may open innovative avenues for managing hyperglycemia.

Keywords: Diabetes, Hypoglycemic, *Andrographis paniculata*, *Gymnema sylvestre*, Decoction, Alloxan.

INTRODUCTION

Diabetes Mellitus Type-II (DM-II) is a class of chronic carbohydrate metabolic disorder characterized by high blood glucose level as the cells do not appropriately utilize the produced insulin [1]. The postprandial surge presents the chief cause for aggravation of metabolic commotion in majority of the DM-II issues [2]. At present, 387 million people, a large section of the population are merely affected [3]. As per WHO reports, more than 4 million people of age groups 20-89 have died from DM-II. It has been projected that the death from DM-II will double up by the end of 2030, after just a few years [4]. However, it there are several glucose lowering therapeutic inhibitor classes like protein tyrosine phosphatase 1B, peroxisome proliferator activated receptor-γ, dipeptidyl peptidase-4, aldose reductase, α-glucosidase, etc. are available, but more than 80% of deaths are reported in low and middle-income countries [5]. The exploration of unknown classes of material principles having both efficacy and good safety profile for managing hyperglycemia, Mother Nature signifies an elite source for the exploration of leads with good hypoglycemic potential [6]. From any principle from natural source, an ideal drug would have been such which will afford decrease the uptake of carbohydrates, effective glucose intensity management, offer repression of hyperglycemia raise, decrease serum triglyceride level, anti-obesity activity, etc [7].

Progressing in the direction of research, two well-known plants from the Indian subcontinent; *A. paniculata* (Family: Acanthaceae) and *G. sylvestre* (Family: Apocynaceae) were chosen and evaluated for their hypoglycemic property in alloxan induced diabetic rat model using metformin hydrochloride as the standard drug. The research specified preliminary phytochemical investigations which facilitated better understanding the role(s) of natural bioactive molecules in mediating the activity and their probable mechanism(s).

MATERIALS AND METHODS

Chemicals

The standard drug metformin hydrochloride was received from Zim Laboratories Ltd., Nagpur as a gift sample. The alloxan monohydrate was procured from HiMedia Ltd., India. HiMedia Ltd. remained the chief supplier for the miscellaneous analytical chemicals.

Instruments

One Touch™ Glucose strips were procured from the local Pharmacy from glucose level determination. Double-beam Ultraviolet-Visible spectroscopy (Shimadzu® UV-1800, Japan), electronic balance (Shimadzu® AUW220D, Japan) were utilized during study.

Animals

After prior approval from the CPCSEA (853/AC/04/CPCSEA/2009), the combined decoction was screened for hypoglycemic activity in six Sprague Dawley rat (150-200 g weight; age 5-6 weeks). During the experiment, the animals were kept under controlled temperature rooms (25–26ºC, humidity 50–55%, 12 hr light and 12 hr dark) in clean polypropylene cage. The rats were fed with standard rodent pellets and given free access to water.

Collection and authentication of plant material

The whole plant of *A. paniculata* and leaves of *G. sylvestre* were freshly collected in the month of October from Ramtek region of Nagpur city, Maharashtra state, India. The plant materials were authenticated by Dr. Dongarwar, Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India.

Extraction protocol

The whole plant of *A. paniculata* and leaves of *G. sylvestre* were dried for two weeks and grounded to obtain a fine powder. 200 g of powdered content was macerated with 500 mL of methanol for 15 days and the solvent was evaporated to dryness employing a rotator vacuum evaporator. The % yield for *G. sylvestre* and *A. paniculata* were found to be 11.8 and 12.3, respectively.

Phytochemical estimation

Preliminary screening

The preliminary phytochemical screening of the decoction was performed to determine the primary and secondary metabolites as per the given standard test procedures [8].

Determination of total flavonoid content

In majority of the natural compounds, biologically active flavonoids and phenol components are present diverse and have radical scavenging characteristics. The total flavonoid content of the decoction samples was determined as per aluminium chloride method. Quercetin was used as the standard and a calibration plot was generated using known concentrations at 415 nm. In this method, 50 mg of dried extract of the sample was taken in a volumetric flask and 3 mL of methanol was added, followed by sequential addition of 0.1 mL $AlCl_3$ (10%), 0.1 mL Na-K tartarate, and 2.8 mL distilled water. Subsequently, the test solution was shaken vigorously and incubated for 30 minutes. The absorbance was recorded at 415 nm and the concentration of flavonoid was calculated from the calibration plot. The total flavonoid content was expressed as mg quercetin equivalent per gram of sample [9].

Pharmacological screening

Oral acute toxicity studies

The oral acute toxicity, as per OECD guidelines helped selecting the minimum concentration of dose required for exhibiting therapeutic action without mortality. The LD_{50} of test samples estimated according to the OECD guideline 423 in Sprague Dawley rats with dose ranging from 5 to 5000 mg/kg [10]. The dose selection was determined based on the minimum drug concentration required for therapeutic action with no observed toxic effects.

Anti-hyperglycemic activity screening

In a batch of starved normoglycemic rats of 150 mg/kg body weight, alloxan monohydrate (dissolved in physiological saline solution) was injected intraperitoneally to induce diabetes mellitus. 20% glucose solution was administered to the rats intraperitoneally after 6 hr. To prevent hypoglycemia, 5% glucose solution was administered for the next 24 hr. After 48 hr time lapse of alloxan injection, the animals with a blood glucose level > 250 mg/dl were selected for the study and divided into different groups with 6 rats in each group. Based on the ED_{50} value, the decoction of whole plant of *A. paniculata* and leaves of *G. sylvestre* at a dose of 100 mg/kg were orally administered to the animals. The blood glucose level was estimated at the end of 1 hr, 3 hr, and 5 hr after the dose administration. According to the AUC method, the potential(s) of the combined decoction in lowering the blood glucose level was calculated [11].

Statistical analysis

The study was performed in a triplicate manner. The results were expressed in n = 6 experimental animals in each groups and expressed as mean ± SEM with P-values < 0.05. The obtained data were evaluated statistically using Software Prism application v5.0. The hypothesis testing method was performed utilizing two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests to compare the replicate means.

RESULTS AND DISCUSSION

Phytochemical investigations

Preliminary phytochemical screening

Both decoctions of *G. sylvestre* leaves and whole plant of *A. paniculata* displayed the presence of flavonoids, saponins, carbohydrates, and glycoside. No steroid, alkaloid, amino acid, and tannins were detected. Table 1 presents the preliminary phytochemical screening of plant decoctions.

Table 1: Preliminary phytochemical screening of decoction of *A. paniculata* dried plant and *G. sylvestre* dried leaves.

Component	Tests	Decoction of *G. sylvestre*	Decoction of *A. paniculata*
Sterol	Salkowaski test	-	-
	Liebermann's test	-	-
	Liebermann-Burchard test	-	-
Sugar	Molisch's test	+	+
	Fehling's test	+	+
	Barford test	+	+
Flavonoid	Shinoda test	+	+
Saponin	Froth test	+	+
Tannin	Lead acetate test	-	-
	Bromine water test	-	-
	$FeCl_3$ test	-	-
	Potassium dichromate test	-	-
Glycoside	Borntrager's test	+	+
	Keller-Killiani test	+	-
	Legal test	-	-
	Modified Borntrager's test	+	+
Amino acids	Biuret test	-	-
	Million's test	-	-
	Xanthoproteic test	-	-
	Ninhydrin test	-	-
Alkaloid	Wagner's test	-	-
	Dragendroff test	-	-
	Mayer's test	-	-
	Hager's test	-	-

Total flavonoid content

The flavonoid contents of *A. paniculata* whole plant and *G. sylvestre* leaves was found to be 18.75 and 40.23 mg quercetin equivalent/g of dry sample, respectively, as estimated using the standard plot of quercetin ($r^2 = 0.9911$).

Pharmacological screening

Determination of LD_{50}

The acute toxicity studies were estimated to determine the *in vivo* safety and therapeutic index. The study presented no toxic signs, symptoms, or mortality that on while escalating the plant decoction dose from 5 to 5000 mg/kg. For screening the *in vivo* anti-diabetic, 100 mg/kg body weight was selected.

Hypoglycemic potential

The oral administration of whole plant of *A. paniculata* and leaves of *G. sylvestre* alone and in combination, facilitated antihyperglycemic effect in alloxan induced diabetic rats [$F(4,60) = 6.87$, $P < 0.001$]. The post hoc Bonferroni multiple comparison revealed that metformin HCl (120 mg/kg) significantly decreased the blood glucose level after 3 hr and 5 hr ($P < 0.001$). *A. paniculata* (100 mg/kg), *G. sylvestre* (100 mg/kg), and its combination (200 mg/kg) also significantly decreased the blood glucose level after 3 hr and 5 hr ($P < 0.001$) (Figure 1). In the present study, an especially considerable reduction in blood glucose level was observed after 5 hr of oral administration of whole plant of *A. paniculata* and leaves of *G. sylvestre*. The significant glucose lowering activity was mediated by active polyphenol or flavonoid principles present in the decoction which may be believed to enhance the transportation of blood glucose level in the peripheral tissues or via potentiation of insulin secretion from the pancreatic β-cells. Table 2 describes the hypoglycemic potential of *A. paniculata* and *G. sylvestre* decoctions.

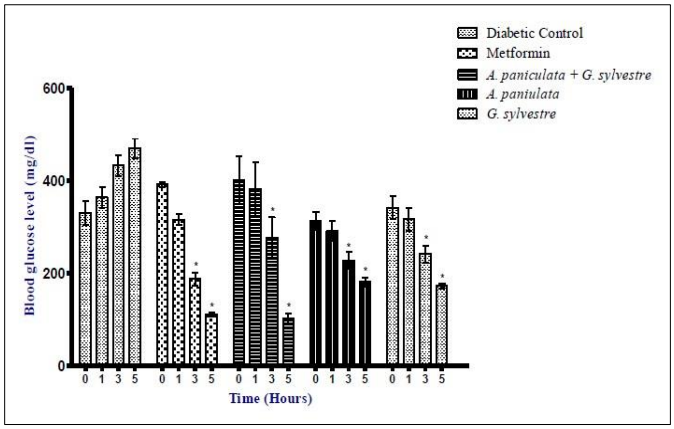

Figure 1: Effects of *A. paniculata* dried plant and *G. sylvestre* dried leaves decoction in alloxan induced diabetic rats.

Table 2: Hypoglycemic potential of combined decoction of *A. paniculata* dried plant and *G. sylvestre* dried leaves.

Groups	Dose (mg/kg)	Blood glucose level (mg/dl)			
		0 hr	1 hr	3 hr	5 hr
Saline	1 mL	330.6 ± 25.19	364.6 ± 21.92	398.3 ± 25.97	433.4 ± 22.8
Metformin HCl	120	391.8 ± 5.86	316.5 ± 12.20	250.2 ± 13.66	188.2 ± 14.29
Decoction of *G. sylvestre*	100	376.8 ± 17.23	350.2 ± 15.42	293.6 ± 18.36	251.9 ± 15.99
Decoction of *A. paniculata*	100	313.7 ± 19.41	291.2 ± 18.71	254.9 ± 14.47	219.6 ± 10.28
Combined decoction of *A. paniculata* and *G. sylvestre*	200	329.6 ± 15.57	279.2 ± 11.94	237.8 ± 13.88	201.1 ± 10.64

n = 6; ED_{50} values were found to be 100, 200, 400 mg/kg b.w.; $P < 0.05$

CONCLUSION

The current investigation represented that the combined decoction of *A. paniculata* whole plant and *G. sylvestre* leaves exhibited the noteworthy antihyperglycemic effect in alloxan-induced diabetic rats. The present study reflects the perception to be used clinically according to the toxicity profiles. The research may hold perspective for the development of formulations based on above two natural

principles and may open innovative avenues for managing hyperglycemia.

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