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INVESTIGATION OF HYPOGLYCAEMIC ACTIVITY OF SOME MYANMAR MEDICINAL PLANTS

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Abstract-The hypoglycaemic activities of three ethanolic extracts prepared from leave of Orthosiphon aristatus (Blume) Miq., Andrographis paniculata (Nees) and bark of Azadirachta indica (A.Juss) were investigated in this study. According to preliminary phytochemical examination, the three plants showed the absence of cyanogenic glycosides and the presence of antibiotic potential of constituents. In vitro and vivo toxicity test, these selected plants were potentially safe for medicinal use. Among these three extracts, O. aristatus showed the most significant reduction in fasting blood glucose (FBG) levels on tested mice according to the results from hypoglycaemic activity. But A.indica extracts could not be lowers the fasting blood glucose levels significantly. The extracts of O. aristatus and A. paniculata were further fractionated by trituration method into three solvent portions. According to overall results, A. paniculata plant has validated for the treatment of diabetes mellitus.
Keywords: Orthosiphon aristatus (Blume) Miq., Andrographis paniculata (Nees), Azadirachta indica (A.Juss) and hypoglycaemic activity.

1. INTRODUCTION

From the dawn of human history, the early ancients have been known to use herbal medicines to cure all diseases and sicknesses. For instance, ancient Egyptian, Indian, early North and South Americans were known to use plant extracts and their products as medicines. Today, this tradition of using Folk-lore medicines is still being practiced by all developing countries. However, when specific synthetic medicinal products were produced, the modern men had come to rely on it mainly for their specific therapeutics pharmacognostic and remedial properties. But today, because of the higher costs of synthetic medicines, some countries have retorted to use Fork-lore medicines [1].

Myanmar is rich in varieties of medicinal plants as well as plants due to the presence of different climates zones. There are 7,000 different known plants growing on Myanmar and most of them have been recognized as medicinal plants. The utilization of traditional medicine is growing day by day, not only in Myanmar but also in part of the world including both developing and developed countries.

Nowadays, herbal medicines are popular. They are extensively used in the developing world, where in many places they offer a more widely available and more affordable alternative to pharmaceutical drugs. In Africa, for example, up to 80% of the population depends on them according to WHO estimates. Moreover, herbal medicines are also profitable. Worldwide, they represent a market value of about US $43 billion a year, according to WHO [2].

Many of the active ingredients in chemically manufactured drugs were originally derived from plant compounds. In the field of drug discovery, tradition and indigenous medicines have long been basis to medicinal plant research. The use of traditional medicine in developing countries is increasing. Because of increasing population, governments want to encourage indigenous forms of medicine rather than rely on imported drugs, and there are strong moves to revive traditional cultures [3].

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration-hyperglycemia (fasting plasma glucose > 7.0 mmol/L, or plasma glucose > 10 mmol/L, 2 hours after a meal) due to insulin deficiency and /or insulin resistance [4].

There are two types of Diabetes mellitus; Type I and II. Type I diabetes is treated with exogenous insulin and Type II with oral hypoglycaemic agents (sulfonylureas, biguanides etc.). WHO estimated that about 30 million people suffered from diabetes in 1985 and the number increased to more than 171 million in 2000. It is estimated that the number will increase to over 366 million by 2030 and that large increases will occur in developing countries, especially in people aged between 45 and 64 years [5].

According to the world ethanobotanical information reports, almost 800 plants may possess antidiabetic potential. In this study, three Myanmar medicinal plants; Orthosiphon aristatus (Blume) Miq., Andrographis paniculata (Nees), Azadirachta indica (A.Juss) are used for the treatment of diabetes mellitus. Andrographis paniculata is a bitter shrub that is widely used as traditional medicine having many antibacterial, anti-inflammatory, immunological, antivenin, antihepatotoxic, antidiabetic and hypotensive properties [6]. The leaves of Orthosiphon aristatus are locally reputed as an antidiabetic drug. Roots and leaves are also used as stomactic tonic and antihelmintic [7]. Extracts from the leaves, stem bark and seeds of Azadirachta indica also have hypoglycaemic activity. Oral administration of nimbidin demonstrated significant hypoglycaemic effect in fasting rabbits [8]. The aim of my research is to evaluate the role of Myanmar medicinal plants as traditional medicines.

2. METHODS

The role of Myanmar medicinal plants is instrumental in the development of novel drug to be used in antimalarial and antimicrobial projects. Natural products from plants may have different properties such as antidiabetic, anticancer, hypertension etc.

2.1 Sample Preparation

The collected samples were cleaned and air-dried at room temperature and subjected to pulverization to get coarse powder. Each of the air-dried powdered plant samples were percolated in 95% ethanol. Maceration was carried out in ethanol solvent for one month at room temperature. The solvent of extract material was filtered with Whatmam No.1 filter paper. The filtrates were concentrated by rotary evaporator. The concentrated plant extracts were dried and evaporated on water bath.

2.2 Preliminary Phytochemical Examination of Plant Samples

Preliminary phytochemical examination for the selected medicinal plants was carried out. Presence of some classes of compounds were determined namely alkaloids, glycoside, carbohydrate, reducing sugar, tannin, α-amino acid, cyanogenic glycoside, phenolic compound and flavonoids.

2.3 Examination of Toxicity Tests

Toxicity test was carried out according to the method of Teng Wah Sam.

2.4 Detection of Hypoglycaemic Activity

The selected mice were prepared to cause hypoglycaemic effect by using adrenaline injection. For giving adrenaline injection, the selected mice were fasted overnight. The animals were given intraperitoneally with adrenaline 0.2 ml/kg/ body weight in distilled water. They were starved for 4 hours after injection and then they were given 0.5 ml of glucose solution orally at hourly interval to prevent hypoglycaemic shock. They were offered unlimited amounts of standard laboratory diet food and water. After one week, the mice were used to test the hypoglycaemic activity. Blood sampling was carried out by cutting their tails and the resulting blood drops were tested by Glucometer and test strips. The four groups fasted mice were used to give orally the plant extracts of 1g/kg of body weight. The standard drug; glibenclamide 0.5mg/kg of body weight was administrated orally for one group. No fasting and no adrenaline injection group of mice were used as normal group. During the experimental procedure, three observations were performed at three times of 45 minutes interval after of the adrenaline by using Glucometer. The results were collected from each group for the data analysis.

3. RESULTS

All selected plants were found to observe the absence of lead, arsenic and zinc. Preliminary phytochemical constitutents of the selected plant samples were shown in Table 3.1. In every test plant sample, cyanogenic glycoside was not detected.

Table-3.1 Preliminary Phytochemical Examination of Selected Plants

S. No.	Constituents	Orthosiphon aristatus (Blume) Miq.,	Andrographis paniculata (Nees),	Azadirachta indica (A.Juss)
1.	Alkaloid	+	+	+
2.	Glycoside	+	+	+
3.	Reducing sugar	+	-	+
4.	Phenolic compound	+	+	+
5.	Flavonoid	+	-	+
6.	Saponin glycoside	-	+	+
7.	Cyanogenic glycosides	-	-	-
8.	Amino acid	+	+	+
9.	Carbohydrate	-	+	-
10.	Acid or Base or Neutral	B	B	N
11.	Tannin	+	+	+

(+): Present, (-): Absent, B: Base, N: Neutral

Three selected extracts were chosen for use in the determination of toxicity test because these extracts were tested for hypoglycaemic activity. In acute toxicity test, the extracts were tested with 2000, 1500, 1000 and 500 mg/kg/day. Lethal effect being not found in this test that these extracts had extremely low toxicity.

From the measured glucose levels, the mean blood glucose values were graphically presented in Table 3.2. The significant hypoglycaemic activity was observed in *O.aristatus* and comparable to diabetic control.

The crude plant extracts were triturated by using the solvent of n- Hexane, ethyl acetate and methanol. . Most of the crude extracts are more soluble in methanol. The resulting fractions obtained by triturating method. The solvents were used to detect the hypoglycaemic activity according to the same procedure describes before. From the measured glucose levels, the mean blood glucose values were graphically presented in Table 3.3 and Table 3.4.

Table-3.2 The Hypoglycaemic Activity of Ethanolic Selected Plant Extracts

Groups	Dose	Blood Glucose Level Mean (mg/dL) ±SD				
		0hr	45min	90min	135min	180min
O.aristatus	1g/kg	115.5± 8.35	278.25±29.10[b]	212±53.15[a]	153.25±16.78[a]	134±13.90[ab]
A.paniculata	1g/kg	114±4.55	297.25±24.84[b]	237.75±98.65[bc]	175.25±50.33[bc]	153.5±16.5[bc]
A.indica	1g/kg	118.25±6.13	363.75±39.79[bc]	272.5±40.31[bc]	202.5±45.73[bc]	165±19.15[bc]
Diabetic Control	0.2ml/kg	109.75±3.30	301.25±43.06[bc]	311±85.43[bc]	226.5±49.68[bc]	176.5±31.55[bc]
Positive Control	0.5mg/kg	115.25±14.84	185.75±41.76[ac]	133±21.70[a]	105±3.74a	98.75±9.00[a]
Normal Control	0.2ml/kg	110.25±6.70	115.25±0.5[ab]	124±6.48[a]	121±0.82a	116.5±1.29[a]

SD = Standard Deviation,

a = The mean difference between diabetic control is significant at the 0.05 level.

b = The mean difference between positive control is significant at the 0.05 level.

c = The mean difference between normal control is significant at the 0.05 level.

Table-3.3 The Hypoglycaemic Activity of O.aristatus Fractions

Groups	Dose	Blood Glucose Level Mean (mg/dL) ±SD				
		0hr	45min	90min	135min	180min
n-Hexane	1g/kg	106.5± 8.70	191.25±49.56[ac]	177±26.60[abc]	138.75±8.22[abc]	135.75±20.16[abc]
EtOAc	1g/kg	112.25±6.34	174±66.67[a]	154.5±54.75[a]	126.5±17.16[ac]	120.25±16.13[ac]
MeOH	1g/kg	110.25±6.70	238.75±66.76[bc]	155.5±24.24[a]	120.25±6.55[a]	106±15.68[a]
Diabetic Control	0.2ml/kg	118.75±7.63	290.75±7.89[bc]	252.25±17.08[bc]	230.5±21.60[bc]	182.5±6.45[bc]
Positive Control	0.5mg/kg	113.5±11.03	178.75±6.29[a]	132.5±6.45[a]	113.75±11.09[a]	101.5±7.42[a]
Normal Control	0.2ml/kg	118.25±6.13	112.75±2.06[a]	116.25±6.24[a]	120±6.78[a]	104±16.75[a]

SD = Standard Deviation,

a = The mean difference between diabetic control is significant at the 0.05 level.

b = The mean difference between positive control is significant at the 0.05 level.

c = The mean difference between normal control is significant at the 0.05 level.

Table-3.4 The Hypoglycaemic Activity of A.paniculata Fractions

Groups	Dose	Blood Glucose Level Mean (mg/dL) ±SD				
		0hr	45min	90min	135min	180min
n-Hexane	1g/kg	106.5± 8.70	214.5±53.17^{ac}	160±41.26^{ac}	154.75±36.60^{abc}	137.75±20.92^{abc}
EtOAc	1g/kg	112.25±6.34	223.75±39.00^{ac}	173.75±26.61^{ac}	157.25±12.34^{ab}	151.5±14.73^{ab}
MeOH	1g/kg	110.25±6.70	260.5±70.68^{bc}	181.25±34.33^{bc}	144.75±27.02^{a}	142±24.39^{ab}
Diabetic Control	0.2ml/kg	118.75±7.63	290.75±7.89^{bc}	252.25±17.08^{bc}	230.5±21.60^{bc}	182.5±6.45^{bc}
Positive Control	0.5mg/kg	113.5±11.03	178.75±6.29^{a}	132.5±6.45^{a}	113.75±11.09^{a}	101.5±7.42^{a}
Normal Control	0.2ml/kg	118.25±6.13	112.75±2.06^{a}	116.25±6.24^{a}	120±6.78^{a}	104±16.75^{a}

SD = Standard Deviation,
a = The mean difference between diabetic control is significant at the 0.05 level.
b = The mean difference between positive control is significant at the 0.05 level.
c = The mean difference between normal control is significant at the 0.05 level.

CONCLUSION

Diabetes mellitus is a serious health problem with continuously increasing rates of incidence and mortality. Diabetes mellitus is characterized by elevated plasma glucose concentrations resulting from insufficient insulin and resistance, or both, leading to metabolic abnormalities in carbohydrates, lipids and proteins.

This investigation had revealed that out of three traditionally used herbal plants which are selected to exhibit hypoglycaemic activity. The selected plants were extracted by using 95% ethanol. The ethanolic extracts were studied for hypoglycaemic activity. The therapeutic effects of herbal drugs are due to chemical substances they contain. Many kinds of substances such as terpenoids, alkaloids, flavonoids, phenolic and glycosides have shown antidiabetic potential. Before detecting hypoglycaemic activity, there was taken the study of presence or absence of toxic metals in this plant samples. According to the mineral analysis data, all selected plants did not contain lead and arsenic. In fact, all samples are safety for medicinal use. Absence of cyanogenic glycoside in each sample was evidenced by preliminary phytochemical examination.

Many drugs have been withdrawn from clinical use owing occurrence of serious toxic effects in man. Toxic effect of drugs may be due to actions that are not desired effect. According to toxicity test, no toxic effect was observed therefore it is potentially used as herbal medicine. Hypoglycaemic activity of selected plant extracts and fractions were tested. In this research, the crude extract of O.aristatus was observed as the hypoglycaemic agent among the tested Myanmar medicinal plants. The medium and polar portions of O. aristatus were also found out the more hypoglycaemic activity than non-polar portion of this plant. Similarly, in A.paniculata, n-Hexane and methanolic portions were observed as the more hypoglycaemic activity. To sum up, the crude extract of O. aristatus can produce as hypoglycaemic agent. The medium and polar portions were also used as the hypoglycaemic agents.

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