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EVALUATION OF TOXIC EFFECT OF TRADITIONALLY USED ANTIDIABETIC POLYHERBAL FORMULATION ON ALBINO RATS

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ABSTRACT

Polyherbal formulations are available with a wide range of types to treat various diseases and are popular remedies for diabetes. Despite the widespread use, there is a lack of scientific evidence on their efficacy and safety. The present study was undertaken to know the toxic effect of the aqueous extract of antidiabetic polyherbal formulation (includes *Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini and Justicia glabra*) on albino rats. Toxic effect of aqueous extract of antidiabetic polyherbal formulations on male albino rats were investigated by administering at a daily doses of 10mg/kg b.wt., 100mg/kg b.wt. and 1000mg/kg b.wt. for 30 days. Mortality of the rats due to toxic effect if any and abnormal behaviour was noted. Biochemical parameters such as SGOT, SGPT, ALP, ACP, Urea, Uric acid, Creatinine and Bilirubin were measured as indices or organ specific toxicity or potential drug interactions. Toxicity studies revealed the non-toxic nature of aqueous extract of antidiabetic polyherbal formulations. No mortality was observed in aqueous extract of antidiabetic polyherbal formulations treated rats. Similarly, no abnormal behaviours were observed in experimental rats and they also appeared in normal condition. Further, this herbal formulation did not affect any biochemical parameters under the study. Thus the polyherbal formulation may be used for the treatment of diabetes mellitus.

KEYWORDS: **Antidiabetic Polyherbal formulation drug, Toxic effect, Phytochemical compounds.**

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INTRODUCTION

The apparent reversal of trend from western to herbal medicine is partly due to the fact that synthetic drugs have always shown adverse reactions and other undesirable side effects. This has led to the belief that natural products are safe because they are more harmonious with biological systems. In traditional system of medicine, medicinal plants are used in many countries to control many diseases. Diabetes mellitus is associated with hyperglycemia, altered metabolism of carbohydrates, lipids and proteins with an increased risk of complications from vascular disease. In the indigenous system of medicine, a good number of plants were used to cure the diabetes and some of them have been experimentally evaluated and active principles were isolated[1]. Ninety percent population in rural areas of developing countries still relies on traditional medicines for their primary health care[1]. A polyherbal formulation (*Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini* and *Justicia glabra*) was developed by tribal healers of Kolli hills and given to diabetic patient to cure treat diabetes mellitus. They are reported to be useful for the treatment of various ailments such as antidiabetic activity[2], anti-inflammatory[3], antibacterial[4], antimicrobial[5] and antidiarraheal effects[6]. However there are no reports available in related to their toxic effect and hypoglycemic effect. Thus, the present study was carried out to evaluate the toxic effect of a polyherbal formulation (*Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini* and *Justicia glabra*) developed by Kolli hill tribal healers.

MATERIALS AND METHODS

Selection of Plant Materials

The polyherbal formulation (*Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini and Justicia glabra*) is a traditionally used antidiabetic drug which was chosen for the present investigation to evaluate its toxic effect.

Collection of plant materials

The whole plant of *Andrographis paniculata,* whole plant of *Andrographis alata,* leaves of *Adhatoda zeylanica,* leaves of *Gymnema sylvestre,* bark of *Syzygium cumini and* whole plant of *Justicia glabra* was collected from the Mathikettan Solai and Chinna Sadaiyan solai, Kolli hills. The collected plant parts were cut into pieces dried under room temperature and powdered with the help of mechanical grinder.

Preparation of Extracts

The polyherbal formulation was prepared as described by tribal healer of the Kolli hill, Namakkal district, Tamilnadu, India. By this method, the powder of those 6 plants was mixed with equal quantity. The process of extraction was as described previously[7]. The polyherbal drug extracted as a whole preparation in a Soxhlet apparatus using aqueous solvent. Aqueous extraction of polyherbal formulation was concentrated to dry mass by vacuum evaporator and stored in desiccator.

Experimental design and toxicity study

Healthy adult male Wistar albino rats, *Rattus norvegicus* (150-200 mg/kg b.wt.) were used throughout the study. The albino rats were obtained from Tamilnadu Veterinary and Animal Science University, Chennai. The principles of animal care (Ethical Committee's Approval No.BDU/IAEC/2014/NE/31/Dt. 18.03.2014) were followed throughout the experimental period. They were randomly divided into 4 groups (3 test groups and one control group) in each group 3 rats were included. Before administration of extract, the experimental rats were fasted for 12 hrs. The polyherbal drug extraction was prepared in 3 concentrations *viz.,* 10mg/kg b.wt., 100mg/kgb.wt. and 1000mg/kg b.wt. They were separately administered orally to the rats for 30 days. Then the rats in both the test and control groups were allowed to access food and water. Behavioural changes, daily food intake, water intake and changes in body weight were observed over a period of 30 days. The mortality caused by the drug within this period of time was observed and noted. At end of the experimental day all rats were

sacrificed and blood samples were collected for biochemical estimations. Further serum urea level, serum uric acid level, serum creatinine level, total bilirubin level, SGOT and SGPT levels, ALP and ACP levels in the extract treated rats and compared with that of normal rats. All Biochemical estimations were performed in Star Plus Semi Auto Analyser. Haematological changes in the control and extract treated rats were analyzed by Auto Hematology Analyzer (Mindray BC-2800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

Hypoglycemic effect

Blood was collected from the tail vein at every 7 days once for estimation of blood glucose level to know the hypoglycemic effect of drugs. During the experimental period the blood glucose level was estimated by glucometer (AP+Plus blood glucose monitoring system).

Statistical Analysis

Values were represented as mean ± standard deviation. To compare the means of different experimental groups with normal groups the One Way Analysis of Variance (ANOVA) was performed. The post hoc test (SNK) was performed to investigate the influence of the plant extracts on various biochemical parameters in the extract treated rats. All statistical analyses were performed by using Windows based SPSS 16.0 (Statistical Packages for Social Sciences, and now it is called Statistical Product and Service Solutions).

RESULTS AND DISCUSSION

No mortality was observed on oral administration even at the dose of 1000mg/kg body weight of the aqueous extract of polyherbal formulation and all the animals were found to be normal throughout the experimental period (30 days). There was no notable changes in the body weight of experimental rats were observed. Similarly the food intake and water intake did not show remarkable changes during the experimental period (Table 1). No abnormal behaviour or no other abnormal physiological activities in the polyherbal extract administered rats were observed. Blood as an index of physiological and pathological status in humans and animals is well documented[8, 9, 10] and the most frequently investigated hematological parameters include hemoglobin, packed cell volume, white blood cell count, and platelets count[8]. In accord with this principle, total RBC, total and differential leukocyte counts, and haemoglobin were measured in the present study. One way ANOVA test showed no significant differences (P>0.05; One Way ANOVA) in all haematological parameters (Total WBC, WBC DC, RBC count and haemoglobin) except basophil among the various group of rats (Table 2). The percentage of basophil was remarkably increased in 1000mg/kg extract treated rats. Results of biochemical investigations conducted on 30 days showed no significant (p>0.05) changes in the values of different parameters studied except basophil when compared with control. Further the values obtained were within normal biological and laboratory limits. Liver marker enzymes level showed no significant (P>0.05; One way ANOVA) changes in SGOT, SGPT, ACP and bilirubin among the rat groups (Table 2). However a significant decreased (SNK post hoc test) in ALP levels of group III rats when compared to the control rats was observed. Even though the ALP levels in group III rats were found to be a normal range. During hepatic damage, cellular enzymes like SGPT, SGOT, ALP, ACP and bilirubin will leak into the serum concentration, the increase of the liver weight and volume. SGOT and SGPT are largely used in the assessment of liver damage by drugs or any other hepatotoxin[11]. However, SGOT is more specific to the liver and is thus a better parameter for detecting liver injury[12]. Herbal supplements may contain a variety of chemicals that are toxic when ingested at high doses and often causes sub clinical injury to the liver which manifest only as abnormal liver enzyme test[13]. Although the liver has the capacity to completely regenerate itself when damaged during short periods, chronic liver injury can cause a gradual progression of fibrotic or cirrhotic changes or even cancerous transformation that may result in dysfunction of the liver itself with whole-body consequences[14]. Since the levels of liver marker enzymes did not show any remarkable abnormalities in the polyherbal formulation

treated rats, it is concluded that the polyherbal formulation has no toxic effect in liver. Renal function test showed no significant (P>0.05; One way ANOVA) changes in urea among the rat groups. On the other hand, a significant dose dependent increase (P<0.05; One Way ANOVA, SNK Post hoc test) was observed in uric acid and creatinine (Tables 2). Study on hypoglycemic effect of the polyherbal formulation showed no significant (P>0.05; One way ANOVA) difference in the blood glucose level among the groups. It reveals that the polyherbal formulation under study have no hypoglycemic effect in normal rats. The result shows that a very high oral dose (1000 mg/kg b.wt.) of polyherbal drug was tolerated by the rats without producing any toxicity symptoms. The ingredients present in the formulation are purely phytochemical in origin and contain sterols, triterpenoids, saponins, aminoacids, triterpenes and flavonoids (Revathi and Saravanan[15]). The presence of compounds like phenols, tannins, flavonoids, alkaloids, glycosides and triterpenes in the extracts of *A. paniculata* are responsible for the antimicrobial activity and antidiabetic activity[16]. Since a number of phytoconstituents are present in the formulation, these experiments were designed to screen for any toxic effects. No toxic effects were observed in the present, it could be inferred that the basic principle in the use of crude plant products or polyherbal preparations in traditional medicine, is that the toxic effect of one component is nullified by the protective effect of the other components, Without int erfering with their therapeutic properties[17].

Table 1

Effect of aqueous extract of polyherbal formulation in body weight, food intake and water intake in different groups of albino rats

Parameter	Group	Week I	Week II	Week III	Week IV
Body Weight (g)	I	134.1 ± 5.19 (130 - 147)	149.5 ± 8.79 (135 - 160)	157.6 ± 5.62 (145 - 165)	164.4 ± 5.43 (155 - 175)
	II	133.4 ± 6.43 (130 - 150)	143.1 ± 5.80 (135 - 150)	145.0 ± 4.18 (140 - 150)	146.7 ± 4.16 (140 - 150)
	III	133.8 ± 5.68 (130 - 150)	157.9 ±12.41 (140 - 180)	176.9 ± 10.42 (165 - 190)	182.2 ± 11.63 (165 - 195)
	IV	155.5 ± 6.10 (150 - 170)	175.0 ± 7.07 (160 - 190)	184.5 ± 10.83 (170 - 200)	187.8 ± 13.75 (170 - 210)
Food intake (g)	I	19.2 ± 3.45 (15 - 25)	35.0 ± 5.77 (25 - 40)	26.4 ± 3.78 (20 - 30)	26.6 ± 4.33 (20 - 35)
	II	20.7 ± 3.14 (15 - 25)	30.7 ± 10.5 (20 - 50)	27.4 ± 6.02 (22 - 40)	26.6 ± 6.12 (15 - 35)
	III	35.7 ± 10.6 (25 - 57)	42.5 ± 5.88 (35 - 50)	30.7 ± 6.07 (20 - 35)	32.1 ± 7.83 (20 - 40)
	IV	32.0 ± 9.03 (20 - 45)	41.4 ± 6.90 (35 - 50)	35.0 ± 11.90 (25 - 60)	36.1 ± 7.40 (30 - 50)
Water intake (ml)	I	45.7 ± 8.15 (35 - 60)	69.2 ± 19.45 (45 - 100)	61.5 ± 9.39 (55 - 82)	48.0 ± 6.36 (40 - 60)
	II	54.4 ± 14.63 (35 - 70)	48.2 ± 10.59 (30 - 60)	64.0 ± 25.07 (34 - 105)	51.1 ± 5.41 (41 - 60)
	III	58.2 ± 17.46 (29 - 80)	66.1 ± 8.80 (52 - 80)	68.5 ± 6.90 (60 - 80)	54.3 ± 10.19 (41 - 72)
	IV	52.8 ± 16.6 (30 - 75)	69.2 ± 7.18 (62 - 80)	70.0 ± 12.54 (50 - 85)	67.8 ± 11.31 (51 - 80)

Values in the parentheses are ranges of respective mean.

Table 2

Effect of aqueous extract of polyherbal drug on liver, renal, haematological profiles and blood glucose levels in albino rats

Parameter	Groups			
Liver Profiles				
***SGOT (U/L)**	34.6 ± 9.56 (3)	39.0 ± 4.88 (1)	54.4 ± 21.66 (2)	55.0 ± 29.57 (4)
***SGPT(U/L)**	26.7 ± 1.04 (3)	28.4 ± 13.42 (1)	34.4 ± 8.65 (4)	49.9 ± 17.73 (2)
****ALP (U/L)**	31.6 ± 1.82 (3)	42.3 ± 8.92 (4)	53.0 ± 4.93 (2)	69.6 ± 11.9 (1)
***ACP(U/L)**	10.3 ± 1.53 (4)	14.3 ± 9.82 (2)	14.6 ± 4.73 (3)	15.0 ± 4.36 (1)
***Bilirubin (mg/dl)**	0.7 ± 0.18 (4)	0.7 ± 0.11 (3)	0.8 ± 0.12 (1)	0.9 ± 0.13 (2)
Renal Profiles				
***Urea (mg/dl)**	0.2 ± 0.05 (1)	0.3 ± 0.05 (2)	0.3 ± 0.03 (3)	0.3 ± 0.08 (4)
****Uric acid (mg/dl)**	0.3 ± 0.59 (1)	0.6 ± 1.09 (2)	2.1 ± 0.46 (3)	2.6 ± 0.78 (4)
****Creatinine (mg/dl)**	0.2 ± 0.09 (2)	0.4 ± 0.08 (1)	0.5 ± 0.11 (3)	0.7 ± 0.18 (4)
Haematology				
***Total WBC (Cells/Cumm)**	11.7 ± 1.90 (1)	12.3 ± 11.11 (2)	12.4 ± 3.50 (3)	13.3 ± 5.70 (4)
***Neutrophil (%)**	4.3 ± 0.00 (4)	4.4 ± 7.68 (3)	4.8 ± 3.96 (1)	7.5 ± 6.59 (2)
***Eosinophil (%)**	0.8 ± 1.44 (3)	1.3 ± 1.44 (1)	1.8 ± 0.00 (4)	3.5 ± 3.27 (2)
***Basophil (%)**	0.3 ± 0.38 (2)	1.8 ± 3.06 (3)	6.0 ± 10.10 (1)	20.6 ± 0.00 (4)
***Lymphocytes (%)**	25.9 ± 44.92 (3)	41.1 ± 39.09 (2)	71.5 ± 0.00 (4)	72.7 ± 17.06 (1)
***Monocytes (%)**	0.4 ± 0.64 (3)	1.8 ± 0.05 (4)	14.3 ± 20.57 (2)	15.0 ± 14.94 (1)
***Total RBC (Millions/Cumm)**	6.1 ± 1.56 (2)	6.9 ± 2.20 (3)	8.1 ± 0.00 (4)	8.1 ± 1.03 (1)
***Haemoglobin (gms %)**	13.0 ± 1.28 (2)	14.2 ± 1.08 (3)	14.3 ± 1.36 (1)	14.5 ± 0.00 (4)
***Blood glucose (mg/dl)**	78.9 ± 34.91 (3)	101.4 ± 0.98 (1)	104.6 ± 1.65 (2)	110.1 ± 5.82 (4)

No significant difference at 0.05 level; ** significant difference at 0.05 level (One way ANOVA and SNK post hoc test). Similar Horizontal line connects similar means

CONCLUSION

Daily administration of polyherbal formulation at different doses of 10, 100 and 1000 mg/kg for 30 days was well tolerated and there was no cumulative toxicity of the drug. Since there are no toxic effects produced by the drug under study, further clinical studies would be conducted to prove the efficacy of polyherbal drug formulation (*Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini and Justicia glabra*) in the treatment of diabetes.

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