Phytochemical Screening, GC-MS Analysis and Toxicity Evaluation of Polyherbal Drug

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Abstract: The polyherbal drug of six medicinal plants (mixture of *Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini* and *Justicia glabra*) were given by tribal healers of Kolli hills to treat diabetes mellitus. In this study, the polyherbal drug was extracted using chloroform. The phytochemicals of the chloroform extract of polyherbal drug was separated and identified. The phytochemical screening and analysis of the chloroform extract of the polyherbal drug showed the occurrence of the phytochemical compounds such as alkaloids, flavonoids, carbohydrate, glycosides, triterpenoids, emodins and fatty acids, and among those five bioactive compounds recommended as therapeutic activity. Toxicity of chloroform extract of polyherbal drug was studied by the administration of a daily dose of 125, 250 and 500mg/kg of body weight. All doses of polyherbal drug extracts showed no behavior changes of rats and mortality. Further, they showed no significant alteration in the hematological profile and all doses of extract did not cause any toxic oriented effect.

Keywords: Polyherbal Formulation, phytochemical screening, GC-MS study, Toxic effect.

1. INTRODUCTION

Herbs are alternative medicines for treatment of various diseases due to their assumed acceptability, effectiveness, affordability, safety and low cost (Arya *et al*., 2012). There is also an emerging increase in the consumption of herbal formulations by the public because of the strong belief that these products are natural; hence, they are safe for the treatment of ailments (Said et al., 2002). However, herbal preparations assumed to be safe may contain contaminants such as heavy metals (Abou Arab *et al.,* 2000) aflatoxins and pathogenic microbes due to the manner in which they are prepared or as a result of acquisition of metals (e.g. cadmium) from the soil (Thanaboripat *et al*., 2007; Kneifel *et al*., 2002). There is also the belief that because herbal remedies are derived from nature, they are devoid of adverse or toxic side effects often associated with synthetic drugs used in conventional medicine (Pushpalatha *et al*., 2010).

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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 treat diabetes mellitus. They are reported to be useful for the treatment of various ailments such as antidiabetic activity and anti-inflammatory activity (Anil kumat *et al.,* 2010), antibacterial and antimicrobial activity (Saneja *et al.,* 2010) and antidiarraheal effects (Mohan kumar, 1992). However there are no reports available in related to their toxic effect and hypoglycemic effect (Elavarasi and Saravanan, 2012; Elavarasi *et al*., 2013; Revathi *et al.,* 2015). Thus, the present study was carried out to analyse the phytochemical components of the plant extract. GC-MS is one of the best techniques to identify the bioactive constituents of the plant extracts. The aim of the present study is to screen the phytochemicals, identify the compounds through GC-MS analysis and evaluate the toxic effect of a polyherbal drug.

2. MATERIALS AND METHOD

2.1 Collection of plant materials

Whole plant *of A. paniculata, A. alata, G. sylvestre* and *J. glabra,* leaves of *A. zeylanica,* and bark of *S. cumini* were collected from Kolli hills, Namakkal district, Tamilnadu.

2.2 Preparation of extracts

The process of extraction was followed by the method of Swami Handa *et al* (2008). The polyherbal drug was prepared by mixing equal quantity of whole plant *of A. paniculata, A. alata, G.*

sylvestre and *J. glabra,* leaves of *A. zeylanica,* and bark of *S. cumini* powder. Then it was extracted by cold extraction method using chloroform. They were concentrated to a dry mass by vacuum evaporator and stored separately in desiccator until use.

2.3 Preliminary phytochemical screening

Different extracts of polyherbal mixture were subjected to screen the preliminary phytochemicals such as alkaloids, flavonoids, glycosides, phenolic compounds, saponins, terpenenoids, steroids, tannins, fatty acids, protein and carbohydrate according to the standard methods (Kokate, 1994; Harborne, 1973; Rajpal, 2002; Raaman, 2006).

2.4 GC-MS analysis

Presence of individual compounds in the study plant extracts were analyzed using GC-MS/MS of Thermo Fisher make, ITQ900 model. One micro litre of the sample was run in a DB-1 fused silica capillary column with helium (1ml/min) as carrier gas, 250°C injector temperature, 280°C ion-source temperature and isothermal temperature 110°C (2 min), with an increase of 10°C/min to 200°C then 5°C/min to 280°C and 9 min to 280°C. The mass spectrum interpretation was performed using the library of National Institute Standard and Technology (NIST) and the compounds were identified.

2.5 Experimental animals

Healthy adult male Wistar albino rats, *Rattus norvegicus* with body weight ranged between 150 and 200 g were used as test model for the present study. The rats were obtained from Tamilnadu Veterinary and Animal Science University, Chennai and brought to the laboratory and maintained under controlled environment. The rats were grouped into control and experimental groups, and housed in different plastic cages (Tarsons make 43 x 27 x 15cm size cage). All rats were fed with standard pellet feed (Sai Durga Feeds and Foods, Bangalore) and water *ad libitum*. The principles of animal care were followed throughout the experimental period and the experimental protocols of the present study were approved by the Institutional Animal Ethical Committee (Ethical Committee's Approval No.BDU/IAEC/2014/NE / 31/Dt.18.3.14).

2.6 Experimental design

Toxicity studies for chloroform extract of polyherbal drug was conducted by modified method of Lorke (1983). Three concentrations *viz.,* 125mg/kg body weight; 250mg/kg body weight; 500mg/kg body weight were used. In this regard, normal healthy male albino rats fasted for 12 hours were randomly divided into 4 groups including one control group. However, the same group was maintained as a control (group I). Each group consists of 3 rats. Experimental groups were labelled and maintained as follows.

Group – I: Control
Group – II: A dose of 125 mg /kg body weight
Group – III: A dose of 250 mg /kg body weight
Group – IV: A dose of 500 mg /kg body weight

2.7 Chronic toxicity test

The group II rats were treated orally with 125mg/kg body weight, group III rats were treated with 250 mg /kg body weight and group IV rats were treated with 500 mg /kg/ body weight of rats by oral gavage needle for 30 days. The rats in both the test and control groups were allowed to access food pellets and water easily.

2.8 Evaluation of toxic effect of polyherbal drug

Rats of all groups were observed for clinical signs and symptoms of toxicity and mortality from the time of extract administration to 30th day. Further, behavioral changes, changes in body weight, daily food intake and water intake were also observed over a period of 30 days. At the end of the experiment all animals were sacrificed.

Haematological profile: Haematological changes were taken into account for the indication of toxic effect. The haematological parameters measured were WBC (Total count and Differential count), RBC count, Haemoglobin content, Packed cell volume (PCV), Red cell indices (*viz.,* Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin concentration (MCHC) and Platelet count. Haematological profile was analysed by Auto Hematology Analyzer (Mindray BC-2800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

2.9 Statistical Analysis

To compare the means of different experimental groups with normal groups, One Way Analysis of Variance (ANOVA) was performed and Student Newman-Keul's 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 using Windows based SPSS 16.0 (Statistical Packages for Social Sciences, and now it is called Statistical Product and Service Solutions).

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis

Chloroform extract of the polyherbal drug showed the occurrence of the phytochemical substances such as flavonoids, carbohydrate, triterpenoids, emodins and fatty acids.

Table 1: Preliminary phytochemical screening of Chloroform extracts of Polyherbal formulation

S. No.	Name of the compound	Ethanol extract
1	Flavonoids	(+)
2	Alkaloids	(-)
3	Tannin	(-)
4	Protein	(-)
5	Carbohydrate	(+)
6	Saponin	(-)
7	Glycosides	(-)
8	Phenols	(-)
10	Sterols	(-)
11	Triterpenoids	(+)
12	Coumarins	(-)
13	Emodins	(+)
14	Fatty acid	(+)

(+) = Present (-) = Absent

GC-MS analyses

The results pertaining to GC-MS analysis led to the identification of number compounds from the GC fractionations of the chloroform extract of polyherbal drug. The identified compounds, molecular formula, molecular weight and retention time presented in table 2, and peaks and their RT are shown in GC-MS chromatogram (Figure 1).

The GC-MS results confirmed the presence of five compounds and they were 3, 7,3,'4,-Tetrahydroxyflavone, (6- oxo- 2,3- diphenyl -1-piperrazinyl) acetic acid, Phenol , 4,4'-methylenebis [2,3,5,6- tetramethyl, 1,6-Heptadiene- 3,5- dione, 1,7- bis (4- hydroxyl-3-methoxyphenyl)- , Paniculidine. Retention times of the above compounds were 17.82, 19.52, 19.72, 21 and 22.42 respectively. The fragmentations of the components are illustrated in GC-MS chromatogram (Figure 1).

Table 2: Identified compounds from chloroform extract of polyherbal formulation by GCMS.

S.No	Compound name	Molecular formula	Molecular Weight	Retention time
1	3,7,3',4'-Tetrahydroxyflavone	$C_{15}H_{10}O_6$	286	17.82
2	(6-Oxo-2,3-diphenyl-1-piperazinyl) acetic acid	$C_{16}H_{16}O_2$	419	19.52
3	Phenol, 4,4'-methylenebis[2,3,5,6-tetramethyl	$C_{17}H_{20}O_2$	256	19.72
4	1,6-Heptadiene-3,5-dione, 1,7-bis(4-hydroxy-3-methoxyphenyl)-	$C_{21}H_{20}O_6$	368	21
5	Paniculidine	$C_{26}H_{43}NO_3$	417	22.42

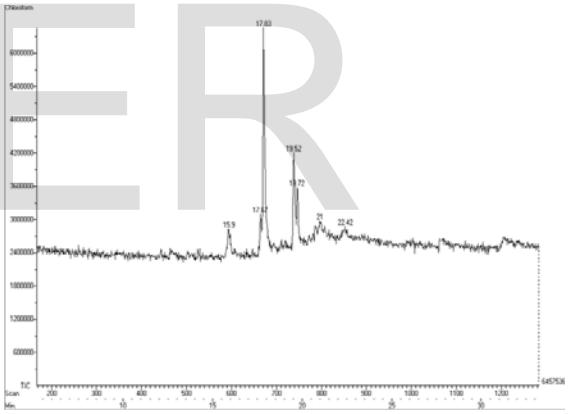

Fig 1: GC-MS Chromatogram of Chloroform extract of polyherbal formulation

3.2 Evaluation of toxic effect of polyherbal drug

Changes in body weight, food intake, water intake and haematological profile due to poly herbal extract were given in tables 3 and 4.

Body weight: The extract treated rats showed normal increase in body weight throughout the experimental period. Especially the chloroform extract treated rats at a dose of 250 mg/kg body weight showed increased body weight compared to low and high dose of the extract treated rats. However, it was low when compared to control rats. The mean body weight exhibited no significant differences (Two way ANOVA; $p>0.005$)

among the different groups of rats of the experiment. The mean body weight of group IV and group II rats were significantly differed from each other and also from other groups (SNK test; $p<0.05$), but group I and group III rats showed no significant difference ($p>0.05$). The week wise percentage changes in the extract treated rats showed up and down trend. But there was increase in the body weight of all groups of the experimental rats. There was significant difference in the body weight among the different weeks of the experiment (Two way ANOVA; $p<0.05$).

Food intake: The mean food intake of polyherbal drug treated rats showed fluctuations throughout the experimental period. The mean food intake showed no significant differences ($p>0.005$) among the groups but it showed significant difference among the weeks (Two way ANOVA; $p<0.005$). The percentage change of food intake among the different groups and weeks showed no significant difference (Two way ANOVA; $p>0.005$). The present result revealed that the test polyherbal drug showed up and down trend of food intake throughout the experimental period. However, at the end of the experimental period the food intake by the extracts treated rats was near to the control level.

Water intake: The mean water intake of control and extract treated rats were increased during the second week of the experiment and gradually decreased. Two way ANOVA results showed that there was a significant difference among the different groups and no significant difference among the different weeks of the experiment ($p<0.005$). The week wise percent change of water intake showed high fluctuations throughout the experimental period in all the groups. However, there were no significant differences in water intake of rats among the groups of rats and different weeks of the experiment (Two way ANOVA; $p>0.005$).

Haematological profile: The total WBC count showed an increase in group II rats, the other two groups (rats treated with 250 and 500 mg/kg b.wt.) were found to be low as compared to control rats. But, they showed no significant differences (One way ANOVA; $p>0.005$) among the different groups. In the case of WBC differential count, increased levels were observed in basophil, eosinophil and lymphocyte of various doses of chloroform extract of polyherbal drug treated rats compared with control rats. In reverse, the percentage of neutrophil and monocyte was found to be low. However, there were no significant differences (One way ANOVA; $p>0.005$) among the different group of experimental rats. The RBC count and haemoglobin levels were slightly lower in all doses of extract treated rats than the control rats. The RBC count and haemoglobin showed no significant differences among the different groups of the experimental rats (One way ANOVA; $p>0.005$). MCHC level was lower in groups of extract treated rats compared to control and` showed a significant difference among the groups (One way ANOVA; $p<0.005$). SNK test showed that the MCHC level of control rats was significantly differed from the extract treated rats ($p<0.05$). But the levels of MCH in the rats treated with 500 mg/kg body weight was slightly higher while in group II and group III rats were more or less equal to the control rats and it showed no significant difference among groups (One way ANOVA; $p>0.005$). MCV and PCV of various doses of chloroform extract treated rats were slightly higher than the control rats. However, the levels of MCV and PCV showed no significant differences among the different groups of rats (One way ANOVA; $p>0.005$). Similarly the platelet count of group III and group IV rats was higher than the control rats but they were not statistically significant (One way ANOVA; $p>0.005$).

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds, toxins, chemicals and plant extracts on the blood constituents of animals (Maglhaes *et al.*, 2008; Oyedemi *et al.*, 2011). Rise in WBC (TC and DC) reflects toxic effect or hypersensitive response of immune system. The present results inferred that WBC (TC and DC), RBC and Hb levels in different doses of extract of polyherbal drug treated rats were more or less similar to control rats. Further the other parameters such as MCH, MCHC, MCV, PCV and platelet levels in extract treated rats were slightly deviated from control rats but the levels were not exceed to normal level. Thus, it is understood that the test herbal drug did not affect the haematological parameters when treated with even high dose (500mg/kg body weight).

CONCLUSION

Daily administration of polyherbal drug at different doses of 125, 250 and 500 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 (*Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini and Justicia glabra*) in the treatment of diabetes.

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Table 3: Result of student Newman-Keuls Post hoc show the difference and similarities in the body weight, food intake and water Intake among the group of rats with response to various to doses of herbal drug treatment.

Name of the extracts	Percent changes in body weight (G)
Chloroform extract of polyherbal drug	Among the Groups 0.03 (IV) 0.06 (II) 0.12 (III) 0.15 (I) Among the Weeks 0.03 (III) 0.06 (IV) 0.19 (II) Intake of food (g) Among the Groups 8.94 (I) 9.91 (II) 10.05 (III) 10.28 (IV) Among the Weeks 9.23 (I) 9.23 (III) 9.44 (IV) 11.39 (II) Intake of water (ml) Among the Groups 14.30 (IV) 16.29 (II) 17.40 (III) 18.53 (I) Among the Weeks 15.96 (IV) 16.34 (I) 16.90 (III) 17.50 (II)

(Horizontal line connects similar means).

Table 4: Results of student- Newman-Kelus (SNK) post hoc test show the differences and similarities in heamatological parameters in the groups of albino rats with response to various dose of chloroform extract of polyherbal drug treatment. Mean values are arranged in ascending order.

Parameters	Student- Newman- Kelus post hoc test Groups (Subset for alpha = 0.05)			
	Groups (Subset for alpha = 0.05)			
Total WBC (Thousand/mm3)	7.6 (IV)	8.4 (III)	11.3 (I)	13.8 (II)
Basophil % (Arcsine)	0.1 (0.00) (I)	0.1 (0.00) (II)	0.4(0.002) (III)	11.7 (0.05) (IV)
Eosinophil % (Arcsine)	1.3 (0.006) (I)	2.9 (0.01) (IV)	3.7 (0.01) (III)	6.9 (0.03) (II)
Neutrophil % (Arcsine)	51.4 (0.2) (II)	56.5 (0.2) (IV)	73.1 (0.3) (III)	75.3 (0.3) (I)
Lymphocyte % (Arcsine)	16.0 (0.08) (I)	17.7 (0.08) (III)	27.4 (0.13) (IV)	40.9 (0.20) (II)
Monocyte % (Arcsine)	0.5 (0.00) (II)	1.4 (0.007) (IV)	4.9 (0.02) (III)	6.2 (0.03) (I)
Total RBC (million/mm3)	6.7 (IV)	7.6 (II)	7.8 (III)	8.1 (I)
Haemoglobin (g/dl)	12.3 (IV)	13.2 (III)	13.5 (II)	14.3 (I)
MCH (pg)	17.1 (III)	17.7 (II)	17.8 (I)	18.2 (IV)
MCHC (gms/dl)	31.4 (II)	31.9 (III)	32.5 (IV)	33.6 (I)
MCV (fl)	52.7 (I)	53.4 (III)	56.0 (Iv)	56.4 (II)
PCV (%)	43.3 (I)	45.9 (IV)	46.1 (III)	46.8 (II)
Platelet (Lakhs/mm3)	7.1 (II)	7.5 (I)	8.4 (IV)	8.6 (III)

Horizontal line connects similar means.