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Research Article

CHRONIC TOXICITY STUDY OF POLYHERBAL EXTRACT ON ALBINO RATS

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A B S T R A C T

Objective: To determine the toxic effect of polyherbal formulation on albino rats (*Rattus norvegicus*).
Methods: The chronic toxicity study were conducted the limited test dose level of 125mg, 250mg and 500mg/kg body weight of extract administered for 30 days.
Results: No abnormal behavior and no mortality was recorded till the end of the experiment. No significant variation in the related organ weight and biochemical parameters was recorded at the end of the experiment.
Conclusion: The present study revealed that the polyherbal formulation (mixture of *Andrographis paniculata, Andrographis alata, Gymnema sylvestre, Justicia glabra, Adhatoda zeylanica* and *Syzygium cumini*) showed no toxic effect to the rats even at high dose of 500mg/kg body weight, thus the polyherbal plant extract is used to evaluate antidiabetic activity.

INTRODUCTION

Plants have been used by human beings since time immemorial. Plants are significant and perennial sources of food and medicines that are used for the treatment of various human diseases (Revathi *et al.,* 2017).The World Health Organization (WHO) estimates that 80 % of the world's population relies on these "alternative" plant-based medicines as their primary medical intervention especially in the developing and in the developed countries where modern medicines are predominantly used (Ogbonnia *et al*., 2008). Over the years, the use of herbs in the treatment of illnesses has been very successful and its historic usage has been useful in drug discovery development. Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases, this practice being an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Zhu *et al*., 2002). Several medicinal plants have been used to control diabetes in the traditional medicinal systems of many cultures worldwide. Many more medicinal plants have found potential use as hypoglycemic in the Indian system of medicines (Elavarasi *et al*., 2013). However, the lack of standardization has been a major concern regarding use of herbal medicines (Angell and Kassierr 1998; NIEHS, 1998). Although herbal supplements may be considered to be safe, some are known to be toxic at high doses and others may have potentially adverse effect after prolonged use. The general public is largely unaware that adverse health effects can be associated with the use of herbal supplements resulting from overdosing, contaminated formulations to the inherent toxicity of the herbs of choice (Hazel *et al*., 1999). Herbal medicines are an essential part of traditional health care system in many cultures (Vickers and Zollman, 1999). Herbs are products of natural origin most often used as self-treatment of diseased states or less than optimal health conditions. Many are without therapeutic effects and some are toxic (Tyler *et al*., 1988). Herbal medicines are usually thought to be safe due to its natural origin however several reviews summarize their significant side effects and interactions (Skalli *et al*., 2007). 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). 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. Thus, the present study was carried out to evaluate the toxic effect of a polyherbal drug.

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MATERIALS AND METHODS

Collection of plant materials

The plants of *Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini* and *Justicia glabra* were collected from Kolli hills, Namakkal district, Tamilnadu.

Preparation of extracts

The process of extraction was followed by the method of 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.

Test model

Healthy adult male Wistar albino rats, *Rattus norvegicus* (150-200g body weight) were used as test model for the present study. The rats were obtained from Tamilnadu Veterinary and Animal Science University, Chennai and maintained under controlled environment. The rats were grouped into control and experimental groups and housed in different plastic cages. All rats were fed with standard pellet feed 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).

Experimental design

Toxicity studies for polyherbal drug extracts were conducted by modified method of Lorke (1983). The chloroform extract of polyherbal drug were separately tested for their toxic effect. In each extract, 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 divided into 4 groups including one control group.

Blood collection

At the end of the experiment (*i.e.*, 31st day), rats were sacrificed by giving mild anaesthesia and the blood samples were collected by heart punching method. Blood was collected in the blood collecting tube to separate serum. Serum was used for biochemical estimations. Serum was separated by centrifuging the samples at 5000 rpm for 10 minutes.

Assay for liver and renal function enzymes

The increase in the levels of liver marker enzymes (SGOT, SGPT, ALP, ACP and Bilirubin) indicates the organ specific toxicity or potential drug interactions. The renal function was assessed by measuring the levels of creatinine, urea and uric acid in the blood serum using Auto Analyzer.

Organ weight: After sacrifice, vital organs such as pancreas, liver and kidney were dissect out and weighed.

Statistical Analysis

Values were represented as mean ± standard deviation. To compare the means of different experimental groups with normal groups one way Analysis of Variance (ANOVA) 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).

RESULTS AND DISCUSSION

Liver marker enzymes

SGOT: It was found that the SGOT levels of different doses of extract treated rats were decreased (15.5 ± 2.14U/L; 17.7 ± 1.18U/L and 26.4 ± 5.42U/L of group II, group III and group IV, respectively) when compared to the control rats (39.0 ±2.81U/L). SGOT level showed a significant difference among the different groups (One way ANOVA; $p<0.005$). The SGOT level control group was significantly higher than all doses of extract treated groups (SNK test; $p<0.05$).

SGPT: Similar trend as SGOT level was observed in the SGPT level in extract treated rats showed decrease level compared to the control rats. It exhibited a significant difference among groups (One way ANOVA; $p<0.005$). SNK test revealed that SGPT level was higher in control rats than the extract treated groups (SNK post hoc test; $p<0.05$).

ALP: ALP level was found to be high in all doses of extract treated group rats except group IV, when compared to control rats. But no significant difference was observed among the ALP level of different groups of rats (One way ANOVA; $p>0.005$).

ACP: The ACP was found to be low in different doses of extract treated rats (1.0 ± 0.07IU/L, 1.1 ± 0.04IU/L and 1.0 ± 0.07IU/L of group II, group III and group IV, respectively) when compared to the control rats (15.0 ± 2.51IU/L). One way ANOVA ($p<0.005$) results revealed that there was a significant difference among the different groups of experimental rats. The control rats was significantly higher than that of different doses of extract treated groups of rats (SNK post hoc test; $p<0.05$).

Bilirubin: It was found that the bilirubin level of the extract treated rats was found to be low when compared to the control rats. But, no significant difference was observed among the different groups of experimental rats (One way ANOVA; $p>0.005$).

The results inferred that the liver function enzymes such as SGOT, SGPT, ALP, ACP and bilirubin in all doses of polyherbal drug treated rats showed slight variations compared to control rats but those levels were found within the normal limits.

Table 1 Results of Student-Newman-Keuls (SNK) post hoc test show the hepatic marker enzyme levels among the groups of albino rats with response to various doses of polyherbal drug treatment

Parameters	Groups (Subset for alpha = 0.05)			
SGOT (U/L)	15.5 (II)	17.7 (III)	26.4 (IV)	39.0 (I)
SGPT (U/L)	15.1 (II)	15.5 (III)	18.6 (IV)	34.9 (I)
ALP (U/L)	46.9 (IV)	69.6 (I)	71.0 (III)	102.4 (II)
ACP (IU/L)	1.0 (IV)	1.0 (II)	1.2 (III)	15.0 (I)
Bilirubin (mg/dl)	0.15 (I)	0.2 (IV)	0.3 (II)	0.3 (III)

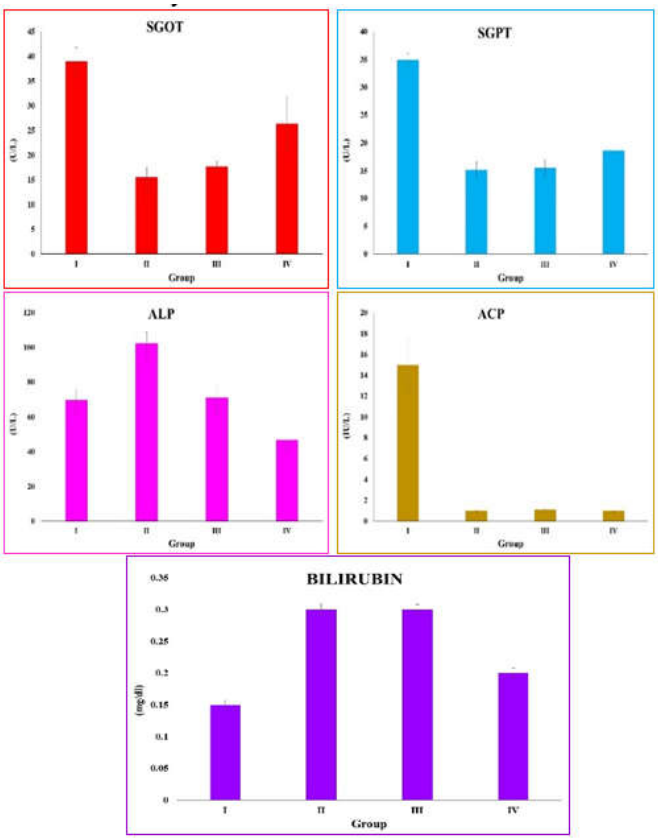


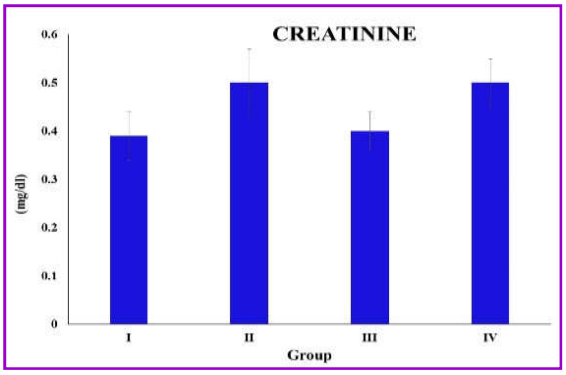
Figure 1 Effect of polyherbal drugs on hepatic marker enzymes in albino rats

Groups-I: Control rats,
II: 125 mg/kg b.wt. of polyherbal drug treated rats,
III: 250 mg/kg b.wt. of polyherbal drug treated rats,
IV:500mg/kg b.wt. of polyherbal drug treated rats

RENAL FUNCTION ENZYMES

The creatinine and uric acid level of the extract treated rats were increased compared to the control rats. However, no significant difference was observed among the creatinine level of different groups (One way ANOVA; $p>0.005$) but significance was observed among the level of uric acid of different groups ($p<0.005$). The uric acid level of the control rats was significantly differed from the extract treated rats (SNK test; $p<0.05$). In contrast, the urea level of various doses of extract treated rats was more or less similar to control rats and there were no significant difference among different groups (One way ANOVA; $p>0.005$).

The overall results of the renal function test showed no significant variation due to treatment of polyherbal drug extracts even at high dose (500mg/kg b.wt.) except uric acid.



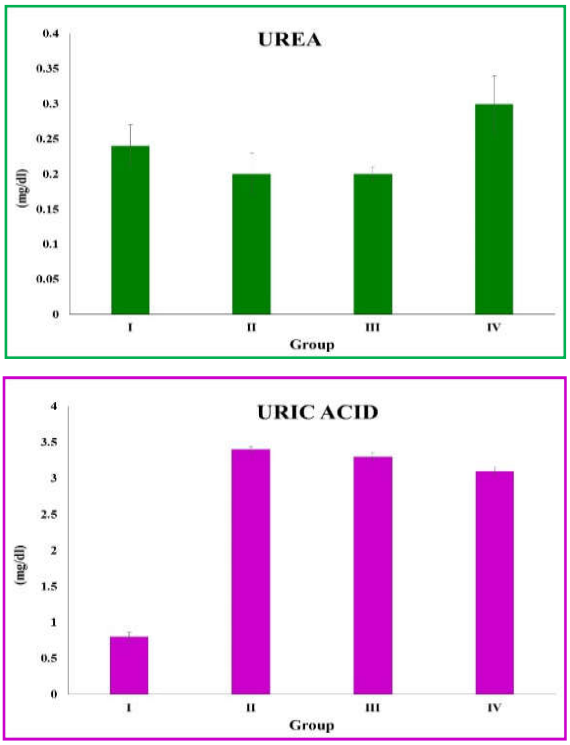


Figure 2 Effect of polyherbal drugs on renal profiles in albino rats

Table 2 Results of Student-Newman-Keuls (SNK) post hoc test show the urea, uric acid and creatinine levels among the groups of albino rats with response to various doses of polyherbal drug treatment

Parameters	Groups (Subset for alpha = 0.05)			
Creatinine (mg/dl)	0.39 (I)	0.4 (III)	0.5 (II)	0.5 (IV)
Urea (mg/dl)	0.2 (III)	0.2 (II)	0.24 (I)	0.3 (IV)
Uric acid (mg/dl)	0.8 (I)	3.1 (IV)	3.3 (III)	3.4 (II)

Groups-I: Control rats,
II: 125 mg/kg b.wt. of polyherbal drug treated rats,
III: 250 mg/kg b.wt. of polyherbal drug treated rats,
IV:500mg/kg b.wt. of polyherbal drug treated rats

Relative organ weight

Pancreas: The relative weight of pancreas in group II (0.4 ± 0.12g/100g body weight) was found to be similar, and in group III (0.5 ±0.06g/100g body weight) was found to be slight increase, and in group IV it was found to be low (0.26 ± 0..03g/100g body weight) when compared to control rats (0.4 ± 0.06g/100g body weight). However, no significant difference was observed among the different groups of experimental rats (One Way ANOVA; $p>0.005$).

Liver: It was found that the relative weight of liver was found to be increased when compared to the control rats. However, there was no significant difference was observed among the different groups of rats (One way ANOVA; $p>0.005$).

Kidney: The mean kidney weight (both left and right kidney) of extract treated rats was increased as compared to that of control rats. There was a significant difference among the right kidney of the experimental groups (One way ANOVA; $p<0.005$) but no significant difference was observed among the left kidney of the experimental groups (One way ANOVA;

p>0.005). The relative weight of right kidney of the rats treated with 500mg/kg b.wt. (group III) significantly differed from the other experimental groups (SNK test; p<0.05). Results of different doses of various extracts of polyherbal drug treatment to the normal albino rats revealed that no significant changes in the relative weight of pancreas, liver and kidney.

In overall results of toxicity study no mortality was observed and showed no remarkable changes in general behavior, hepatic marker enzymes and renal function tests. Even though some abnormalities were found in biochemical profile of extract treated rats compared to control rats, they were not exceeded the normal range.

Herbal medicine is the oldest form of healthcare and had been used by all cultures throughout history. The WHO has assessed that 80% of the world's population stays to use traditional therapies, a main part of which are obtained from plants, as their primary health care tools (Khan *et al.,* 2013). The estimation of AST and ALP is suitable in the early diagnosis of viral or toxic hepatitis and thus patients exposed to hepatotoxic drugs (Zimmerman, 1984). Hence animals were tested for ALP, AST, γ-GT and Bilirubin levels to check for hepatic toxicity. There were no significant changes ALP, AST, γ-GT and Bilirubin (Total and Direct) in animals of either group received herbal formulation (Khan *et al.,* 2016).

Table 3 Results of Student-Newman-Keuls (SNK) post hoc test show the relative organ weight among the groups of rats with response to various doses of polyherbal drug treatment

Parameters	Groups (Subset for alpha = 0.05)			
Pancreas (g/100 g b. wt.)	0.26 (IV)	0.4 (I)	0.4 (II)	0.5 (III
Liver (g/100 g b. wt.)	3.5 (I)	3.9 (IV)	3.9 (III)	4.3 (II)
Right Kidney (g/100 g b. wt.)	0.32 (IV)	0.39 (I)	0.42 (III)	0.44 (II)
Left Kidney (g/100 g b. wt.)	0.37 (I)	0.37 (IV)	0.42 (III)	0.44 (II)

Alteration in organ-to-body weight ratio may be as a result of organ damage (Busari *et al*., 2015). The result is an indication that *N. campestris* may not elicit any deleterious effect on the weight of kidney, liver and heart, and the result is in consonance with the findings of Olorunnisola *et al* (2012). They reported that 28-day oral administration of methanol extract of *Tulbaghia violacea* rhizomes at doses of 125, 250 and 500 mg/kg body weight was not toxic to the heart, liver, kidney and pancreas of the experimental subjects. Assessment of liver and kidney function is a very vital index in evaluating the toxicity of drugs and plant extracts. Kidney function indices evaluated in this study were serum urea, creatinine and electrolyte concentrations. This correlates with the findings of Muhammad *et al* (2011) who carried out an investigation on the acute and sub-chronic toxicity of kernel extract of *Sclerocarya birrea* in rats. They reported that a significant increase in serum urea and creatinine was observed when the experimental rats received higher doses of the kernel extract of *Sclerocarya birrea* ranging from 3000 to 4000 mg/kg body weight. NH3 released during deamination is removed from the blood by conversion into urea. Increase in urea may be the result of high glomerular filtration. Creatinine is not supposed to be reabsorbed but all creatinine that is filtered in the glomerular filtrate passes on through the tubular system and is excreted in the urine. In this situation, creatinine is reabsorbed rather than excreted in urine. Although there are many traditional herbal medicines available, only a few have been verified by clinical trials, their efficacy and safety are still questioned by consumers (Cheng *et al.,* 2009).

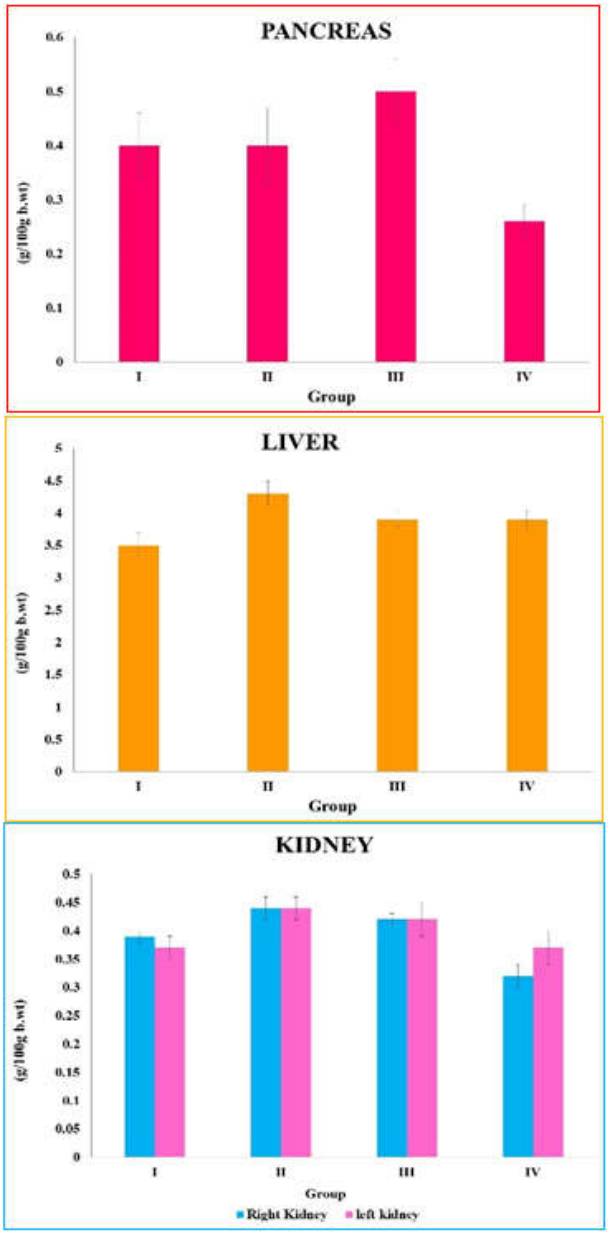


Figure 3 Effect of polyherbal drugs on relative weight of liver and pancreas in the albino rats

Groups-I: Control rats,
II: 125 mg/kg b.wt. of polyherbal drug treated rats,
III: 250 mg/kg b.wt. of polyherbal drug treated rats,
IV:500mg/kg b.wt. of polyherbal drug treated rats

The relationship between the function of cells and organs is reflected in the organisation of tissues, visualised under the microscope. Hence histology supports the study of cell biology at all levels. Histology is also very important in diagnosis of disease and hospitals have associated laboratories and systems for examining and reporting on tissue resections and biopsies. Histopathology evaluation of *Ficus religiosa* on the liver and kidney was done after it was fed to the female albino rats and indicated that the extract did not adversely affect the morphology of the rats' organs. As indicated earlier, kidney,

heart, lungs and liver tissues from control group for all toxicity studies showed normal renal, cardiac, lung tissue and hepatic morphology as well as its internal cells appearance. In fact, all animals in group treated with 2000 mg/kg extract for acute toxicity presented no morphology and physiological changes in kidneys and liver tissues as well (Elavarasi *et al*., 2018).

CONCLUSION

The present findings revealed that the polyherbal formulation did not show any adverse effects in the extract treated albino rats. Thus, it is concluded as polyherbal formulation is safe and used for further antidiaibetic evalution.

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