REPORT

Impact of herbal drug combination on lipid profile, renal and cardiac parameters

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Abstract: For the past two decades, there has been an increasing interest in the investigation of medicinal plants as potential sources of new therapeutic agents; hence it is crucial to recognize the apparent toxicity that might occur while using herbal medicines. This study was undertaken in rabbits to assess the safety profile of an herbal drug combination. It was tested in 3 different doses for a period of 45 days, each group comprising of seven rabbits of either sex. Biochemical test and histopathological assessment were performed at the completion of dosing using standards reagent kits. The result shows that high dose of herbal drug (10ml/kg) revealed significant increase in serum lactate dehydrogenase, total protein and creatinine ($p<0.05$); more over there was highly significant decrease in triglycerides ($p<0.005$) at the completion of dosing.

Keywords: Herbal drug, biochemical markers and histopathological examination.

INTRODUCTION

Herbal drugs had been extensively used for medical purposes since prehistoric times and even today most of the medicinal preparations are derived from plants. The world health organization estimated that 80% of the people worldwide rely on herbal medicines for their primary health care (Verma and Singh, 2008; Patrick-lwuanyanwu *et al*., 2012).

Eisenberg and colleagues in 1993 reported that 34% of adults in the United States use at least 1 unconventional form of health care during the previous year (Astin, 1998). Representing an annual global market of US$ 60 billion every year, herbal medicines account for approximately 20% of the overall drug market (WHO, 2004). Despite the increased popularity of herbal treatments, the safety and effectiveness of alternative medicines have not been scientifically corroborated. Besides that the use of herbal remedies and supplements together with prescribed medication increases the risk of potentially lethal drug-herb interaction (Elvin-Lewis, 2001).

Conventionally, herbs are usually considered to be non-toxic by the general public owing to their natural origin however there is little information concerning the toxicity of herbal drugs to declare them safe for clinical use. Due to increased morbidity and mortality, poisonings related with the use of herbs have augmented universal attention in the last few years (Deng, 2002); therefore considering complexity of herbals, it is now necessary to evaluate their safety before the clinical use (Zhou and Yao, 2013). Hence present study has been specifically designed to evaluate the safety profile of herbal drug combination frequently used in our society without any pharmacological evaluation.

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MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology, Faculty of Pharmacy, University of Karachi, after approval from Board of Advance Study and Research (BASR) University of Karachi, Karachi.

Experimental design and dosing

The study was carried out on 28 healthy New Zealand white rabbits of either sex weighing from 1100-1600 gm. Animals were housed separately in cages, under controlled climatic room (23±2°C) and humidity (50-60%), during the entire study in an alternating 12-h of light/dark cycle.

Animals were treated on daily dosing basis for a period of 45 days. Herbal drug combination *Eclipta alba hassk* (35 mg leaves and stem), *Picrorrhiza curroa* (35/mg root and rhizome), *Solanum nigrum* (35/mg fruits), *Cichorium intybus* (35/mg seeds), *Glycyrrhyza glabra* (35/mg roots),

Tamarix dioica (7/mg fruits), *Raphanus sativus* (18/mg seeds), *Berberis aristata* (fruits 14/mg), *Silybum marianum* (70/mg leaves, seeds and fruits), *Spheranthus indicus* (7/mg fruits), *Boerhaavia diffusa* (14/mg leaves and stem per 10/ml) was administered by mouth through oral feeding tube. The prescribed daily dose in the leaflet of the formulation was considered as normal dose. All animals were uniformly divided into four groups in the following pattern:

1. Control: Received saline
2. ND:Normal dose of herbal drug combination(0.43 ml/kg)
3. MD:Moderate dose of herbal drug combination(5 ml/kg)
4. HD:High dose of herbal drug combination (10 ml/kg)

Sample collection

Blood sample of about 5 ml were collected in gel tubes through cardiac puncture technique (Feroz *et al*., 2011b) at the completion of dosing i.e. 46th day. Serum was separated by centrifugation (Heraeus, Christ Labofuge A) at 4000 rpm for 8 minutes.

Measurement of renal, cardiac parameters and lipid profile

Serum total protein (Thomas, 1998), urea, creatinine (Bartels and Boehmer, 1971), Lactate dehydrogenase (LDH), creatinine kinase (CPK-MB) (Szasz and Persijin, 1974), cholesterol, triglycerides (Trinder, 1969), high density lipoprotein-cholesterol (HDL-C) (Friedewald *et al*., 1972) and low density lipoprotein-cholesterol (LDL-C) were analyzed on vita lab eclipse automatic analyzer (Merck) at 37^{o}C using standard reagent kits.

STATISTICAL ANALYSIS

All values were compared with control by taking mean and standard error to the mean using one sample t-test, values of $p<0.05$ were considered as significant and $p<0.005$ as highly significant. All statistical methods were performed using SPSS software version 14.0.

RESULTS

Renal parameters

Table 1 reveals the comparison of total protein, urea and creatinine at normal, moderate and high doses of herbal drug for a period of 45 days.

Animals received moderate dose did not reveal any significant alteration in total protein, urea and creatinine levels at the end of dosing. Conversely animals received normal and moderate dose showed highly significant and significant increase in the level of total protein i.e. 6.88±0.18 g/dl and 6.51±0.59 g/dl as compared to control animals i.e. 4.71±0.37 g/dl. There was also a highly significant increase in creatinine level in animals kept on high dose of herbal drug i.e. 0.80±0.09 mg/dl as compared to control i.e. 0.31±0.07 mg/dl; however there was no significant changes in the concentration of urea at the completion of dosing.

Table 1: Comparison of renal parameters following administration of herbal drug combination in different doses for 45 days

Parameters	Animal groups			
	Control	Herbal drug combination		
		ND	MD	HD
Total. Protein (g/dl)	4.71± 0.37	6.88± 0.18**	4.36± 0.71	6.51± 0.59*
Urea (mg/dl)	50.0± 8.52	32.57± 4.22	52.71± 12.2	49.00± 6.56
Creatinine (mg/dl)	0.31± 0.07	0.40± 0.06	0.414± 0.180	0.80± 0.090**

n=7 Average values ± SEM
*p<0.05 significant as compared to control
**p<0.005 highly significant as compared to control

Cardiac parameters

Table 2 reveals the comparison of CPK and LDH at normal, moderate and high doses of herbal drug for a period of 45 days.

Table 2: Comparison of cardiac parameters following administration of herbal drug combination in different doses for 45 days

Parameters µ/l	Animal groups			
	Control	Herbal drug combination		
		ND	MD	HD
CPK-MB	437.86 ±110.14	658.29± 161.16	668.57 ±87.97	513.14 ±73.08
LDH	134.29 ±6.25	167.43 ±54.72	89.71 ±35.34	459.71± 85.15*
AST	52.71 ±10.55	74.57± 21.00	35.86 ±7.21	59.86± 12.76

n=7 Average values ± SEM
*p <0.05 significant as compared to control

Animals received normal and moderate dose of herbal drug did not reveal any significant alteration in CPK-MB and LDH levels at the end of dosing**.** Conversely animals received high dose showed significant increase in the level of LDH 459.71±85.15 µ/l in comparison to control animals i.e. 134.29±6.25 µ/l, However there were no significant changes in the level of CPK and AST at the completion of dosing.

Lipid profile

Table 3 reveals the comparison of cholesterol, triglycerides, HDL-C and LDL-C at normal, moderate and high dose of herbal drug for a period of 45 days.

Animals kept on moderate dose did not reveal any significant alteration in cholesterol, triglycerides, HDL-C and LDL-C levels at the completion of dosing. Conversely animals received normal dose showed highly significant and significant increase in the level of triglyceride and cholesterol i.e. 292.57±32.69 mg/dl and

114.57±23.78 mg/dl as compared to control animals i.e. 76.28±4.93 mg/dl and 51.57±6.46 mg/dl respectively. On the contrary animals kept on high dose showed highly significant decrease in the level of triglycerides i.e. 47.43 ±5.24 mg/dl as compared to control animals i.e. 76.28±4.93 mg/dl.

Table 3: Comparison of lipid profile following administration of herbal drug combination in different doses for 45 days

Parameters (mg/dl)	Animal groups			
	Control	Herbal drug - combination		
		ND	MD	HD
Cholesterol	51.57 ±6.46	114.57± 23.78*	89.57± 16.54	57.00± 11.21
Triglycerides	76.28 ±4.93	292.57± 32.69**	126.57± 22.66	47.43± 5.24**
HDL	24.28 ±4.06	58.43± 25.19	49.14± 15.94	23.71 ±4.42
LDL	15.25 ±0.98	20.20 ±8.06	21.60 ±8.31	25.86 ±9.99

n=7 Average values ± SEM
*p<0.05 significant as compared to control.
**p<0.005 highly significant as compared to control.

Histopathological assessment

Gross examination of kidney did not show any macroscopic changes in any group. Similarly microscopic examination of renal tissue of control animals and animals kept on normal and moderate dose of herbal drug did not reveal any microscopic changes (fig. 1).

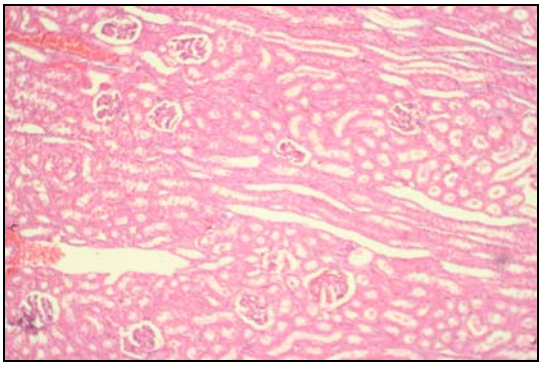

Fig. 1: Kidney showing no remarkable change.

Gross examination of heart did not show any macroscopic changes in any group. Similarly microscopic examination of cardiac tissue in all animal groups including control did not revealed any microscopic changes in cardiac tissue (fig. 2).

DISCUSSION

A variety of disease in the world are treated by herbal medicines such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue and irritable bowel syndrome. However, herbs are advised to be used under the supervision of expert health care professionals to gain maximum effect and fewer side effects. A large and increasing number of patients use medicinal herbs; hence present study has been designed to assess the safety profile of herbal drug combination commonly used in our society.

In present study rabbits were elected as experimental animals because of several reasons i.e. biochemical changes produced in rabbits are relatively similar as observed in humans; rabbits are easily obtainable, easy to handle and cheap (Feroz *et al*., 2009, 2010 & 2011a; Qamar *et al*., 2011).

The human kidneys account for less than 1% of body weight, yet receive approximately 20% of the cardiac output (Loh and Cohen, 2009). Renal function tests are vital in evaluating the extent of renal damage. Creatinine is the most frequently used marker to estimate glomerular filtration rate (GFR) (Tschuppert *et al*., 2007), therefore creatinine levels in blood may be used to estimate the creatinine clearance, which divulge GFR.

Present study generally did not revealed any significant changes in the level of urea and creatinine in animals kept on normal and moderate doses, however there was highly significant increase in the level of total protein in animals kept on normal dose. Rise in protein may be due to increased synthesis or decrease loss. While microscopic examination of renal tissue did not reveals any remarkable microscopic changes in the renal tissues, hence it is not an indication of renal damage (fig. 1). On the contrary animals kept on high dose showed significant elevation in the level of total protein and creatinine. Elevated creatinine level implies decreased creatinine clearance which is a consistent indicator of reduced glomerular filtration rate owing to renal damage. While microscopic examination of renal tissue shows congestion only illustrating no remarkable changes, therefore biochemical changes do not correlate to histopathological changes in renal tissue, hence it is not an indication of renal damage.

Cardiac enzymes are proteins that escape out of injured myocardial cells ensuing increased levels in blood. Elevated levels of AST were initially employed to evaluate cardiac damage, nevertheless now CK is thought to be more precise for myocardial damage. Present study generally did not revealed any significant changes in the level of CPK and LDH in animals on normal and moderate doses of herbal drug combination, however there was significant increase in the level of LDH in animals on high dose but these changes do not correlate with histological changes (fig. 2),

Present study did not reveal any significant changes in the level of HDL and LDL in animals on normal, moderate and high doses, however there was significant increase in the level of cholesterol and triglycerides which may be

due to the disturb lipoprotein metabolism. Conversely animals on high dose of herbal combination showed highly significant decrease in the level of triglyceride, which might be due to presence of several constituents in the herbal combination like Eclipta *alba hassk*, Picrorrhiza *curroa* and Solanum *nigrum* which have been reported to reduce triglycerides (Ahir *et al*, 2008; Elizabeth and Neeraja, 2012; Vijender, Ashok and Gunjan, 2012).

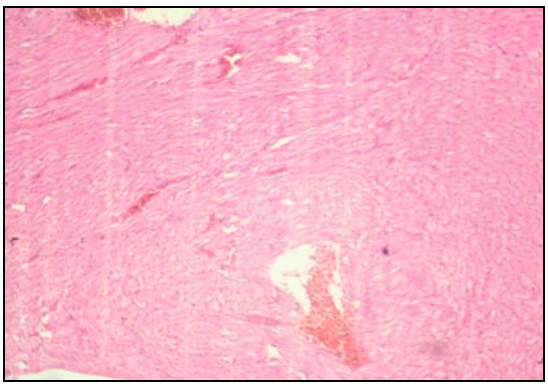

Fig. 2: Heart showing no remarkable change.

CONCLUSION

Present study was conducted to evaluate the toxicity of herbal drug combination in high dose on renal and cardiac parameters and lipid profile. The preparation was administered at three different dose levels 0.43ml/kg, 5ml kg, 10 ml/kg of body weight. The overall results of the study reveal that animals kept on high dose of herbal combination revealed higher toxicity than as compared to animals received normal and moderate doses of the herbal combination.

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