ORIGINAL ARTICLE

EVALUATION OF TOXICITY OF MUCUNA PRURIENS, CINNAMOMUM ZEYLANICUM, MYRISTICA FRAGRANS AND THEIR COMBINATION ON RENAL, HEPATIC AND CARDIAC PARAMETERS

Muhammad Arif[1], Bushra Sherwani[2], Muhammad Rashid Ahmed[3], Rafeeq Alam Khan[1]

[1]Department of Pharmacology, Faculty of Pharmacy, University of Karachi

[2]Department of Ophthalmology, AJK Medical College, Muzaffarabad

[3]Department of Anatomy, Baqai Medical University, Karachi, Pakistan

ABSTARCT

Background: Alternative and traditional medicines are regarded as significant but underemployed means against diseases. *Myristica fragrans, Cinnamomum zeylanicum, and Mucuna pruriens*, are among the vital components of herbal medicines used for the treatment of different diseases, including male sexual disorders. However knowledge about their safety is scanty. This study was carried out to evaluate the toxic effects of these drugs, alone and in combination.

Material & Methods: This study was conducted on rabbits, divided into treated and control batches. Drugs were administered orally for 90 days daily, and serum biochemical parameters were analyzed on 91st day. Histopathological examination of kidney, liver, and heart were also done. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by post hoc.

Results: There was no significant difference found in the renal and hepatic parameters, serum proteins and cardiac parameters of treated batches as compared to control ($p > 0.05$). The microscopy of kidney and liver did not show any noteworthy pathology. Pericardium, myocardium, endocardium and cardiac chambers were within normal limits. There was no significant difference in histology of all treated groups i.e. *M. pruriens, C. zeylanicum, M. fragrans,* and combination groups as compared to control.

Conclusion: The drugs were found to be harmless in the doses used in this study in rabbits. However preclinical and clinical research is required to ascertain the safety of these drugs in herbal medicines.

KEY WORDS: Herbal Medicine; Herbals; Phytotherapy; Toxicity.

This article may be cited as: Arif M, Sherwani B, Ahmed MR, Khan RA. Evaluation of sub chronic toxicities of *Mucuna pruriens, Cinnamomum zeylanicum, Myristica fragrans* and their combination on renal, hepatic and cardiac parameters. Gomal J Med Sci 2015; 13: 146-50.

INTRODUCTION

About two hundred years back, practice of medicine was mainly led by herbal medicines.[1] In traditional herbal practice of medicine, diseases are usually treated by herbs combinations, which are supposed to act synergistically to complement useful effects and to counter or reduce the adverse effects of individual herbs. However, this assertion has not been scientifically appraised.[2]

Corresponding Author:
Dr. Rafeeq Alam Khan
Professor Meritorious
Department of Pharmacology, Faculty of Pharmacy, University of Karachi Pakistan
E-mail: rkhan1959@gmail.com

Numerous herbal drugs are reported to have different organ/systemic toxicity including hepatotoxicity, nephrotoxicity, and cardiac toxicity.[3] *Myristica fragrans, Cinnamomum zeylanicum, and Mucuna pruriens,* are among the vital components of herbal medicines used for the treatment of different diseases, including male sexual disorders. However the knowledge about the safety from any detrimental effect, especially in combination of these drugs is scanty. The safety, efficacy and cost effectiveness of these drugs cannot be justified until the relative pharmacology, toxicology and drug-interactions of such herbal drugs in every possible combination with every possible dose is evaluated.

This study was undertaken to evaluate the toxicity of *M. pruriens, C. zeylanicum, and M. fragrans,* alone and in combination.

MATERIAL AND METHODS

The rabbits were chosen for the experimental work to assess the sub chronic toxicities.[4] A total of fifty white male healthy rabbits, body weights in the range of fourteen hundred to eighteen hundred grams, were selected. Environmental conditions were maintained constantly such as humidity (50-60%) and temperature (20-24°C) with twelve hour duration of alternate darkness and light. Green leafy vegetable were given to all rabbits daily with free access to water. All rabbits were kept in the laboratory surroundings for habituation period of one week before the start of dosing period of drugs. Animals were separated into five groups, each having ten rabbits. Random and uniform distributions were done for fifty rabbits. One batch was selected as a control while the three batches were labeled to administer the three tested drugs singly and one batch was assigned to be treated with combination of all three drugs. (Table 1)

Table 1: Groups of rabbits.

S. No.	Groups	Drugs Administered
1	Control A	0.5% CMC (Carboxy methylcellulose)
2	Treated B	M. pruriens
3	Treated C	C. zeylanicum
4	Treated D	M. fragrans
5	Treated E	M. pruriens + C. zeylanicum + M. fragrans

Drugs were cleaned, dried, and then crushed to form fine powder. Herbal drugs were given daily for 90 days through oral route in the form of suspension with 0.5% carboxy methylcellulose. *M. pruriens* was administered at a dose of 70 mg/kg, *C. zeylanicum and M. fragrans* were administered at doses of 30 mg/kg and 20 mg/kg body weight respectively. While in combination group the same doses were used together.

At the end of dosing period on 91st day, samples of blood were taken and serum was obtained to execute different biochemical tests utilizing standard reagent kits. Serum biochemical tests were performed on Siemens Dimension Clinical Chemistry System and Merk Microlab 300, semi-automated clinical chemistry analyzer.

Biochemical parameters included were renal and hepatic parameters, serum total protein, and cardiac parameters. Serum urea and creatinine were estimated to assess the renal function. Serum total bilirubin, direct bilirubin, alanine aminotransferase, Alkaline phosphatase, γ-Glutamyl transferase were estimated to assess the hepatic function. Serum LDH, CK, and AST were estimated to assess any cardiac injury.

Animals were sacrificed after taking the blood for laboratory tests after the completion of study period on 91st day. Kidney, liver, and heart of rabbits were removed and processed to form slides for histopathological examination.

All observations were expressed as Mean ± SEM. Statistical analysis was performed on SPSS by using ANOVA followed by post hoc analysis. Values of $p<0.05$ were measured as significant and <0.01 as highly significant.

RESULTS

All drug groups showed a decrease in urea as compared to control. Creatinine level was low in combination group, and in other drug groups it was high as compared to control. (Table 2) All these changes in serum biochemical renal parameters were in normal physiological limits and found to be statistically not significant as compared to control.

There was a slight decrease in serum total bilirubin in *M. fragrans and C. zeylanicum* groups and slight increase in combination and *M. pruriens* groups as compared to control. All drug groups showed decrease in serum direct bilirubin and alanine aminotransferase as compared to control. There was a slight decrease in serum alkaline phosphatase in *C. zeylanicum* and *M. pruriens* groups and slight increase in combination and *M. fragrans* groups as compared to control. There was slight decrease in serum γ-GT in *C. zeylanicum* and combination groups and slight increase in *M. pruriens* and *M. fragrans* groups as compared to control. (Table 2) All these changes in serum biochemical hepatic parameters were in normal physiological limits and found to be statistically not significant as compared to control.

There was slight increase in serum total protein in all the drug groups as compared to control. (Table 2) All these changes in serum total protein were in normal physiological limits and found to be statistically not significant as compared to control.

There was slight decrease in serum LDH in all drug groups as compared to control. There was increase in serum creatine kinase and AST in combination and *M. pruriens* groups and decrease in serum creatine kinase and AST in *M. fragrans* and *C. zeylanicum* groups as compare to control. (Table 2) All these changes in serum biochemical cardiac parameters were in normal physiological limits and found to be statistically not significant as compared to control.

The microscopy of kidney did not show any noteworthy pathology. Glomeruli, interstitium, tu-

Table 2: Comparison of serum biochemical parameters in treated and control groups.

Parameter	Control Mean±SEM (p value) n=10	M. pruriens Mean±SEM (p value) n=10	C. zeylanicum Mean±SEM (p value) n=10	M. fragrans Mean±SEM (p value) n=10	Combination Mean±SEM (p value) n=10
Urea (mg/dl)	45.5 ± 4.49	36.7 ± 4.23 (0.64)	38.2 ± 3.63 (0.78)	41 ± 6.32 (0.95)	36.8 ± 3.31 (0.65)
Creatinine (mg/dl)	1.15 ± 0.07	1.45 ± 0.13 (0.49)	1.27 ± 0.14 (0.96)	1.37 ± 0.21 (0.76)	1.01 ± 0.06 (0.94)
Total Bilirubin (mg/dl)	0.5 ± 0.04	0.52 ± 0.05 (0.99)	0.45 ± 0.02 (0.82)	0.4 ± 0.02 (0.28)	0.51 ± 0.04 (1.00)
Direct Bilirubin (mg/dl)	0.2 ± 0.02	0.14 ± 0.02 (0.14)	0.16 ± 0.02 (0.57)	0.15 ± 0.02 (0.18)	0.18 ± 0.01 (0.90)
Alanine Transaminase (U/L)	68.8± 11.19	56 ± 4.62 (0.75)	45.7 ± 5.01 (0.87)	56.7 ± 8.04 (1.00)	55.7 ± 7.08 (1.00)
Alkaline Phosphatase (U/L)	87.2 ± 2.24	84 ± 4.86 (0.99)	82.8 ± 6.81 (0.98)	101 ± 7.5 (0.43)	90.7 ± 5.63 (0.99)
γ-GT (U/L)	11.74 ± 1.03	12.26 ± 1.57 (0.99)	10.63 ± 0.78 (0.95)	12.5 ± 0.84 (0.98)	11.42 ± 0.99 (1.00)
Total Protein (g/dl)	5.80 ± 0.14	6.22 ± 0.11 (0.31)	6.15 ± 0.17 (0.49)	6.10 ± 0.17 (0.63)	6.29 ± 0.17 (0.17)
LDH (U/L)	227.1± 2.44	212.3 ± 28.46 (0.98)	206.2 ± 20.62 (0.95)	222.1 ± 15.03 (1.00)	224.3 ± 12.65 (1.00)
CK (U/L)	244.9±24.06	268.2 ± 24.76 (0.95)	225.4 ± 20.46 (0.97)	234.8 ± 24 (0.99)	253 ± 26.67 (0.99)
AST (U/L)	64.4 ± 7.88	70.3 ± 10.9 (0.99)	56.1 ± 11.97 (0.98)	63.1 ± 9.1 (1.00)	65.2 ± 13.11 (1.00)

p value as compared to control Combination: M. pruriens + C. zeylanicum + M. fragrans

bules and blood vessels were within normal limits. There was no significant difference in histology of all treated groups that is *M. pruriens, C. zeylanicum, M. fragrans,* and combination groups as compared to control. (Fig. 1-A)

The microscopy of liver did not show any striking changes in the architecture, portal tract and lobules. There was no significant difference in histology of all treated groups that is *M. pruriens, C. zeylanicum, M. fragrans,* and combination groups as compared to control. (Fig. 1-B)

No significant pathology was seen. Pericardium, myocardium, endocardium and cardiac chambers were within normal limits. There was no significant difference in histology of all treated groups that is *M. pruriens, C. zeylanicum, M. fragrans,* and combination groups as compared to control. (Fig. 1-C)

DISCUSSION

With respect to renal parameters, results of present study are in agreement with Suresh et al, in which *M. pruriens* given to adult male rats for forty five days, showed no marked histological changes in kidney and liver.[5] Accordingly, Ram et al reported that ethanol extract of *M. fragrans* given for sixty days in rabbits, produced no significant change in urea, creatinine, kidneys, ALP, ALT, AST, liver, and heart.[6]

Similarly, serum biochemical hepatic parameters in present study are in accordance with Ngatchic et al, in which male growing rats, aged between twenty five to thirty days, fed raw mucuna bean flour for 28 days, showed no significant change in ALT, AST, and no noteworthy alteration in histopathology of liver.[7]

There was small decline in serum ALT in present study which is in agreement with the study by Chukwadi et al, in which normal adult male rats after a period of six weeks of administration of hot water crude extract of *M. pruriens* seeds caused a noteworthy ($p<0.05$) decline in serum ALT judged against the control animals.[8] The disparity in significance may be related to alterations in doses, animal species, and study period.

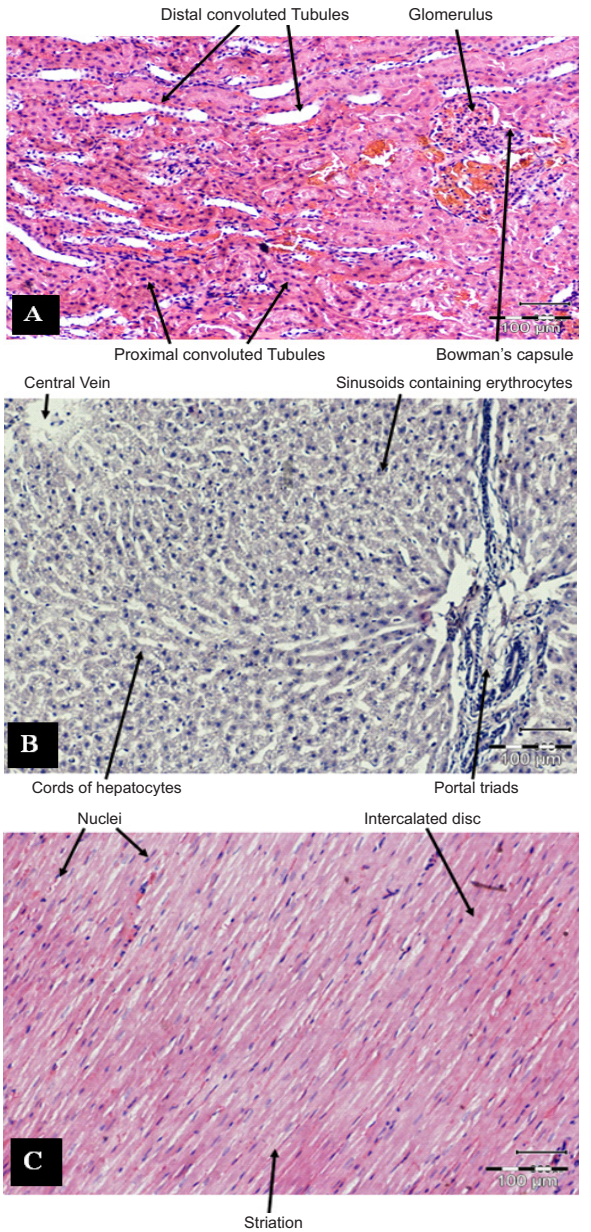

Figure 1: H&E stained, 3 μm thick paraffin section of (A) Kidney, (B) Liver, and (C) Heart revealing no significant pathology (Photograph 10x).

The justification for the decline in serum level of ALT enzyme has been related to the recognized antioxidant action of *M. pruriens.*[8] Treatment with *M. pruriens* showed a decline in lipid peroxidation and a raise in the superoxide dismutase, glutathione, and catalase, implying considerable antioxidant and antitumor actions in mice.[9] Therefore, the suppression of ALT liver enzyme might be elucidated by the amplified suppressive effect of some constituents of *M. pruriens* that put off the over-sensitization of the enzymes.[8] Similarly, *M. pruriens* seed extract injected to rats once a week for 3 weeks, at 21 mg/kg body weight did not cause any observable damage to liver.[10]

In agreement with our results, Subash Babu et al reported a hepato-protective effect by restoring the near normal level of the altered AST, ALT, LDH, and ALP by Cinnamaldehyde, an active component of *C. zeylanicum* when administered orally for forty five days to male rats.[11] Similarly, ethanol extract of Cinnamon orally one time every day for seven days showed hepato-protective activity against lipid peroxidation and liver damage in rats due to CCl_4. It restored the elevated ALT and AST to near normal. It reduced the liver Malondialdehyde (MDA) level and enhanced the antioxidants enzymes, CAT and SOD which may be related to scavenging of free radicals by polyphenol compounds. The hepato-protective effects were further supported by liver microscopy.[12]

Similarly, another research work showed that myristicin, a constituent of *M. fragrans* had most powerful protective activity for liver in rats against the hepatic injury by LPS D-GalN. In mice, hepatic DNA breakup in liver TNF-α serum concentration was raised by LPS/D-GalN and myristicin established to distinctly repress this effect. Hence it is indicated that protective effect of myristicin on liver possibly be, at least partly, related to the blockade of liberation of TNF-alpha from macrophages.[13] Likewise, Sohn et al. claimed about macelignan, constituent of *M. fragrans,* the protective effect on liver, and it was linked to the MAPK signaling pathway activation, especially JNK and c-Jun.[14]

A decrease in serum protein may be due to decrease synthesis in case of liver damage or increase loss in urine in case of renal damage. Hence a normal serum protein level is an indicator of normal function of liver and kidney. Results of present study about *M. pruriens'* effect on serum total protein were in accordance with the study by Chukwadi et al.[8] Likewise in another study, *M. fragrans* extract was administered to rats for pretreatment of thirty days. The heart tissue of *M. fragrans* treated animals, demonstrated no alterations in heart structure. Pretreatment with *M. fragrans* also protected heart to a large degree against the isoproterenol-induced damaging effects.[15]

CONCLUSION

No noteworthy disparity was found in the parameters related to the adverse effects of all treated drug groups. All the drugs were shown to be safe individually and in combination in doses employed in this study in the rabbits. The present study provides a scientific rationale for the traditional use of these herbal drugs in the management of different disorders. However, further pre-clinical studies and studies on human volunteers and patients are needed to establish the safety of these herbal drugs in varied formulations.

REFERENCES

1. Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine (IUPAC Technical Report). Pure Appl Chem 2008; 80: 2195-230.
2. Yu YB, Dosanjh L, Lao L, Tan M., Shim BS, Luo Y. Cinnamomum cassia bark in two herbal formulas increases life span in Caenorhabditis elegans via insulin signaling and stress response pathways. PLoS One. 2010; 5: e9339.
3. Dennehy CE, Tsourouni C. Dietary supplements and herbal medications. In: Basic and Clinical Pharmacology, 12th ed. Katzung BG ed. 2012; p. 1125-37.
4. Feroz Z, Khan RA, Amber, Mahayrookh. Impact of herbal drug combination on lipid profile, renal and cardiac parameters. Pak J Pharm Sci 2013; 26: 819-22.
5. Suresh S, Prithiviraj E, Prakash S. Dose- and time-dependent effects of ethanolic extract of Mucuna pruriens Linn. seed on sexual behaviour of normal male rats. J Ethnopharmacol 2009; 122: 497-501.
6. Ram A, Lauria P, Gupta R, Sharma VN. Hypolipidaemic effect of Myristica fragrans fruit extract in rabbits. J Ethnopharmacol 1996; 55: 49-53.
7. Ngatchic JT, Sokeng SD, Njintang NY, Maoundombaye T, Oben J, Mbofung CM. Evaluation of some selected blood parameters and histopathology of liver and kidney of rats fed protein-substituted mucuna flour and derived protein rich product. Food Chem. Toxicol 2013; 57: 46-53.
8. Chukwudi NH, Simeon O, Chinyere AJ. Analysis of some biochemical and haematological parameters for Mucuna pruriens (DC) seed powder in male rats. Pak J Pharm Sci 2011; 24: 523-6.
9. Rajeshwar Y, Kumar GPS, Gupta M, Mazumder UK. Studies on in vitro antioxidant activities of methanol extract of Mucuna pruriens (Fabaceae) seeds. Eur Bull Drug Res 2005; 13: 31-9.
10. Fung SY, Tan NH, Liew SH, Sim SM, Aguiyi JC. The protective effects of Mucuna pruriens seed extract against histopathological changes induced by Malayan cobra (Naja sputatrix) venom in rats. Trop Biomed 2009; 26: 80-4.
11. Subash, Babu P, Prabuseenivasan S, Ignacimuthu, S. Cinnamaldehyde – a potential antidiabetic agent. Phytomedicine 2007; 14: 15-22.
12. Moselhy SS, Ali HK. Hepatoprotective effect of cinnamon extracts against carbon tetrachloride induced oxidative stress and liver injury in rats. Biol Res 2009; 42: 93-8.
13. Morita T, Jinno K, Kawagishi H, Arimoto Y, Suganuma H, Inakuma T, et al. Hepatoprotective effect of myristicin from nutmeg (Myristica fragrans) on lipopolysaccharide/d-galactosamine-induced liver injury. J Agric Food Chem 2003; 51: 1560-5.
14. Sohn JH, Han KL, Kim JH, Rukayadi Y, Hwang JK. Protective effects of macelignan on cisplatin-induced hepatotoxicity is associated with JNK activation. Biol Pharm Bull 2008; 31: 273-7.
15. Kareem MA, Krushna GS, Hussain SA, Devi KL. Effect of aqueous extract of nutmeg on hyperglycaemia, hyperlipidaemia and cardiac histology associated with isoproterenol-induced myocardial infarction in rats. Trop J Pharm Res 2009; 8: 337-44.

CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.