WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 1019-1026. Research Article ISSN 2277– 7105

SAFETY EVALUATION OF A HERBAL MIXTURE OF AQUEOUS EXTRACTS OF *FICUS RACEMOSA* AND *AZADIRACHTA INDICA*: ASSESSMENT OF SUB-CHRONIC TOXICITY IN RATS

Pearl Andrea Dias[1], Lathika Shetty[2] and Suchetha Kumari Nalilu[2]*

[1]Dept. of Biochemistry, K. S. Hegde Medical Academy, Nitte University, Deralakatte, Mangalore - 575018

[2]Dept. of Radiology, K. S. Hegde Medical Academy, Nitte University, Deralakatte, Mangalore - 575018

[2]*Dept. of Biochemistry, K. S. Hegde Medical Academy, Nitte University, Deralakatte, Mangalore - 575018

Article Received on
07 October 2014,

Revised on 30 Oct 2014,
Accepted on 25 Nov 2014

***Correspondence for Author**
Dr. Suchetha Kumari Nalilu
Department of Biochemistry, K. S. Hegde Medical Academy, Nitte University, Mangalore – 575018, Karnataka

ABSTRACT

Ficus racemosa has been used in traditional medicine for the treatment of diabetes, dysentery, diarrhea, bilious affections, menorrhage, haemoptysis, piles, stomach ache and as a carminative and astringent. While *Azadirachta indica* has been used extensively in the management of jaundice, eczema, cough, vomiting, malaria and diabetes mellitus. Reports of the safety evaluation of each of these plants are available in literature. However, toxicity profiling of these plant extracts when used in combination is lacking. Hence, the aim of the current study was to assess the sub-chronic toxicity of a 1:1 combination of the aqueous extracts of *Ficus racemosa* stem bark and *Azadirachta indica* leaves. Three groups of female albino Wistar rats received the herbal mixture orally at dose levels of 100, 200 and 1,000 mg/kg body weight (b. wt.) for 28 consecutive days, respectively. The study was carried out as per Organization for Economic Cooperation and Development (OECD) guidelines. Behavioral changes, body and organ weights, hematological and biochemical parameters were analyzed. No concrete evidences of toxicities attributable to treatment with the herbal mixture were observed on behavioral pattern and body weight analysis. Significant reduction in organ weights was observed in the treated groups. Changes in hematology and blood biochemistry were significant.

KEYWORDS: Sub-chronic toxicity, *Ficus racemosa*, *Azadirachta indica*, OECD.

INTRODUCTION

Utilization of herbs and plants is known to humankind since ancient times. Traditional forms of medicine like Ayurveda, Unani and homeopathy make use of plant extracts extensively. However, the safety of these products has been questioned time and again by the modern systems of evidence-based medicine. Therefore, it is essential to carry out the safety evaluation of these plant products before deeming them 'safe to use' for the common man. *Ficus racemosa* Linn (Moraceae) is a moderate-sized deciduous tree found throughout India in moist localities. Its leaves, fruits, bark, latex and sap are all useful in preparation of medications. The bark is used as a mouth wash in spongy gum because of its astringent properties. It is used internally in menorrhagia, dysentery, and haemoptysis. [1] *F. racemosa* possesses hepatoprotective [2], chemopreventive [3], anti-diabetic [4], anti-inflammatory [5], anti-pyretic [6], anti-tussive [7] and anti-diuretic [8] properties. The bark was studied for its cytotoxic effects using 1BR3, HepG2, HL-60 cell lines and found to be safe and less toxic than aspirin. [9] *Azadirachta indica* is the most versatile medicinal plants having a wide spectrum of biological activities. Neem oil and the bark and leaf extracts have been therapeutically used to control leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter. [10] It is also used in the treatment of rheumatism, chronic syphilitic sores and indolent ulcers. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis. [11]

The sub-chronic toxicity profiles of these plants have been evaluated individually. However, such information with regard to their combination is lacking. Therefore, the objective of this study was to evaluate the safety of the herbal mixture comprising of aqueous extracts of *Ficus racemosa* stem bark and *Azadirachta indica* leaves in Wistar rats.

MATERIALS AND METHODS

Plant material: The aqueous extracts of *Ficus racemosa* stem bark and *Azadirachta indica* leaves were purchased from Amsar Pvt. Ltd., Indore.

Ethical clearance: The study was approved by the Animal Ethics Committee, K. S. Hegde Medical Academy, Nitte University. Reference code KSHEMA/AEC/17/2010.

Animals: Totally 20 healthy adult nulliparous and non-pregnant female Albino Wistar rats, weighing140-200 g at the start of the experiment procured from the animal house of K. S. Hegde Medical Academy, Nitte University were used. Animals were divided into 4 groups of 5 animals each and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Room temperature was 25^{o}C (± 3^{o}C), with a light period of 12 h. Clean paddy husk bedding was provided to the animals. The animals were fed with commercially available standard pellet chow and unlimited supply of drinking water.

Administration of Herbal Mixture of Aqueous Extracts of *Ficus racemosa* Bark and *Azadirachta indica* Leaves: (100, 200 and 1,000 mg/kg b. wt.)

Prior to dosing, the animals were subjected to overnight fasting. Following the period of fasting, the body weight of each animal was determined and the dose was calculated according to the body weight. The volume given was not more than 2 ml/100 g b. wt. Control animals (Group I) was given distilled water. Groups II, III and IV were given 100, 200 and 1000 mg/kg b. wt. of the herbal mixture (1:1) for 28 consecutive days, respectively. It was orally administered in a single dose by using a gavage. After the herbal mixture was administered, food was withheld for a further 3-4 hours. The test was performed as per OECD. [12]

Clinical Observations

Behavioral analysis

Animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, during the 28 day dosing period. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality.

Body weight analysis

Individual weights of animals were recorded before the administration of drug on 1st day of the study and thereafter on the 7th, 14th, 21st and 28th day of the experiment. Changes in the weight were calculated and compared with the control group.

Hematological analysis

On the 29th day blood samples were collected and the animals were sacrificed. Total RBCs and total WBCs were counted using a hemocytometer.

Biochemical analysis

The samples were analyzed for the biochemical parameters using standard procedures (Total protein by Biuret Method, Albumin by BCG Method, ALP by PNPP method, ALT by UV-Kinetic Method, urea by UV kinetic/GLDH method, total cholesterol by CHOD-POD method) using semi-auto analyzer. While MDA was estimated using a UV-Vis spectrophotometer. [13]

Organ weight analysis

Vital organs like liver, kidneys and brain from each animal were isolated after thorough profusion of the organs with neutral saline, and pressed with help of tissue paper to remove any moisture. The isolated organs were observed for their morphology such as presence of any kind of lesions, etc., and the individual organ from each animal was weighed.

Statistical Analysis: The results were statistically analyzed by one-way ANOVA followed by Tukey's test to analyze intergroup variation at $P<0.05$ using Prism version 3.0.

RESULTS AND DISCUSSION

A repeated dose 28 day oral toxicity study of the herbal mixture (1:1) of the aqueous extracts of *Ficus racemosa* stem bark and *Azadirachta indica* leaves at three levels 100, 200 and 1,000 mg/kg b. wt. approximately equivalent to 7, 13 and 67 times of human use did not cause mortality in any of the treated rats. No external signs and symptoms of toxicity were observed.

Behavioral observations

Skin, fur, eyes, mucous membrane, behavioral pattern, salivation and sleep were found to be normal. Tremors, lethargy, diarrhea and coma were not observed.

Body weight

A significant increase in body weight was observed in Group II from day 14 to day 21 ($^{*}P<0.05$) and from day 21 to day 28 ($^{**}P<0.001$) indicating that the herbal mixture did not affect the growth of the animals at a dose of 100 mg/kg b. wt. Groups III and IV showed a reduction in body weight from day 7 to day 14 and an increase thereafter. However, it was not

statistically significant (Figure I). This observation was in agreement with a 90-day sub-chronic toxicity study of methanol extract of neem flowers wherein doses of 150, 750 and 1,500 mg/kg b. wt. did not have any effect on the growth rate of female rats. [14]

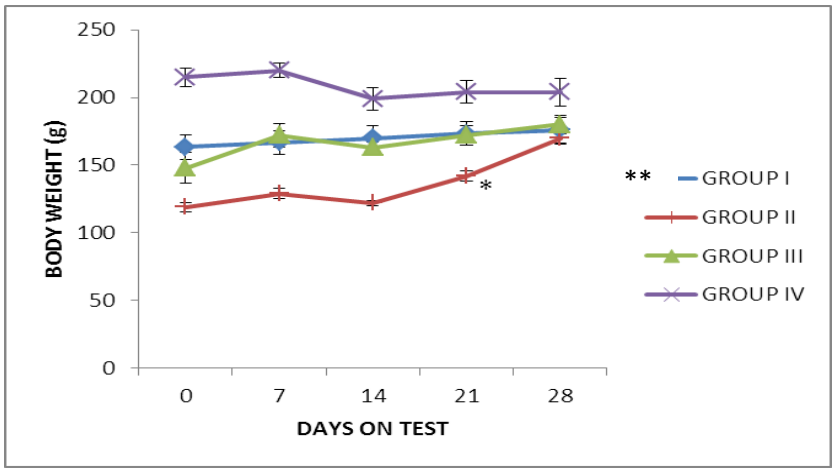

Figure I: Changes in body weight during a 28-day sub-chronic toxicity study. Values are mean ± SEM. A significant increase in body weights was observed in group II when a comparison was made between days 14 and 21 ($^{*}p<0.05$) and days 21 and 28 ($^{**}p<0.001$).

Organ weight analysis

Morphological observations of vital organs such as brain, kidneys and liver indicated that there were no signs of any inflammation or toxicity in the animals. Liver and kidneys weights were significantly lower in Group III ($^{\#}P<0.01$) when compared with the control group (Figure II). Also, brain weight of Group II and IV ($^{*}P<0.05$), were significantly lower than the control.

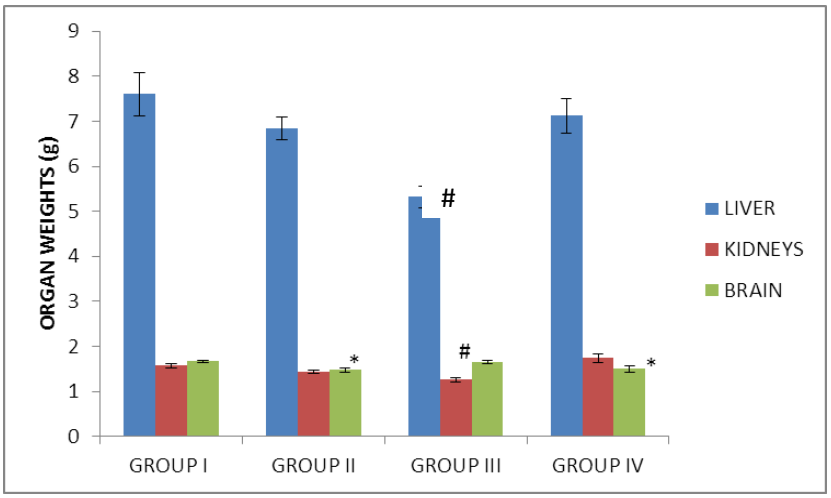

Figure II: Absolute organ weights measured on day 29 on necropsy. Values are mean ± SEM. Significant differences from the control (I) group $^{*}P<0.05$, $^{\#}P<0.01$

Hematological parameters

Hematological parameters such as total RBC and total WBC count were estimated on the 29th day (Table I). Total RBC count in Group IV was found be significantly lower in comparison with the control group. No statistically significant variation in total WBC count was observed.

Table I: Hematological parameters after a four-week sub-chronic toxicity study. Values are mean ± SEM. Significant differences from Control (I) group ($^{*}P<0.05$).

Parameters	Group I	Group II	Group III	Group IV
Total RBC (Million/cc)	9.15±0.38	8.00±0.45	7.22±0.97	6.36±0.68*
Total WBC (Thousand/cc)	5.71±0.61	9.12±1.50	5.23±1.45	3.19±0.37

Biochemical analysis: Total proteins and albumin levels did not show any variation when compared with the treated groups (Table II). Serum ALP levels were significantly reduced in Group III ($^{\dagger}P<0.01$). Serum ALT levels were reduced significantly in all the treated groups. This observation was contrary to a sub-chronic toxicity study of the aqueous extract of *Ficus racemosa* stem bark done on Sprague Dawley rats which showed definitive liver damage. However, the hepatic damage appeared to be reversed during the recovery period. [15] No variation in urea levels were observed in any of the treated groups. These results indicate that the herbal mixture did not affect the functioning of the kidneys. However, Arvind et al observed significant increase in blood urea levels. Total cholesterol levels were lowered in Group II ($^{*}P<0.05$). MDA levels were significantly reduced in both Groups II and IV ($^{*}P<0.05$). Hence, the herbal mixture played a beneficial role in lowering the total cholesterol levels and MDA levels which is a decomposition product of lipid peroxidation.

Table II: Serum biochemical parameters after a two-week acute toxicity study. Values are mean ± SEM. Significant differences from Control (I) group ($^{*}P<0.05$, $^{\dagger}P<0.01$).

Parameters	Group I	Group II	Group III	Group IV
Total Proteins (g/dL)	7.98±0.61	6.26±0.09	7.27±1.48	6.51±0.39
Albumin (g/dL)	3.02±0.31	2.05±0.05	3.78±0.42	2.35±0.10
ALP (IU/L)	224.24±36.56	144.46±12.73	73.40±19.63†	153.07±19.01
ALT (IU/L)	89.09±10.99	46.25±2.24*	36.17±9.25†	53.46±5.59*
Urea (mg/dL)	31.94±3.63	27.51±2.92	39.57±7.13	30.85±2.27
Total Cholesterol	76.54±9.10	38.98±5.87*	59.52±13.60	70.42±2.72
Malondialdehyde (µM/L)	1.82±0.30	0.64±0.05*	1.41±0.31	0.83±0.06*

CONCLUSION

The use of plant resources in development of suitable treatment for ailments is widely prevalent even today in spite of the advancement in modern medicine. However, the need for evidence based medicine cannot be denied. While, the toxicity profiles of individual herbs have been greatly studied, such information on a mixture of herbal extracts so commonly used in traditional medicine is scarcely available. Therefore, it was imperative to carry out this study. The results of the repeated-dose 28-day sub-chronic toxicity of the herbal mixture (1:1) of *Ficus racemosa* and *Azadirachta indica* showed no evidence of toxicities with regard to the behavioral pattern and body weight analysis. However a significant reduction in the weights of vital organs was observed. Variations in the hematological and biochemical parameters were seen. Reduction in total cholesterol and MDA levels were reflective of the anti-dyslipidemic nature of the extracts.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Naveen P. for his timely help during the course of this study.

REFERENCES

1. Chopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous Drugs of India. 2nd edn. Academic Publisher, Calcutta, 1958; 508-674.
2. Mandal SC, Tapan K, Maity J, Das M, Pal M, Saha BP. Hepatoprotective activity of Ficus racemosa leaf extract on liver damage caused by carbon tetrachloride in rats. Phytother. Res., 1999; 13(5):430-432.
3. Khan N, Sultana S. Chemomodulatory effect of Ficus racemosa extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats. Life Sci., 2005; 29:1194-1210.
4. Rao BR, Anipama K, Swaroop A, Murugesan T, Pal M, Mandal SC. Evaluation of anti-pyretic potential of Ficus racemosa bark. Phytomedicine, 2002; 9:731-733.
5. Mandal SC, Saha BP, Pal M. Studies on bacterial activity of Ficus racemosa leaf extract. Phytother Res., 2000; 14(4):278-280.
6. Rao BR, Murugesan T, Sinha S, Saha BP, Pal M, Mandal SC. Glucose lowering efficacy of Ficus racemosa bark extract in normal and alloxan diabetic rats. Phytother. Res., 2002; 16:590-592.
7. Rao BR, Murugesan T, Pal M, Saha BP, Mandal SC. Antitussive potential of methanol extract of stem bark of Ficus racemosa Linn. Phytother.Res., 2003; 17:1117-1118.

8. Ratnasooriya WD, Jayakody JR, Nadarajah T. Antidiuretic activity of aqueous bark extract of Sri Lankan Ficus racemosa in rats. Acta Biol Hung., 2003; 54(3-4):357-63.
9. Li RW, Leach DN, Myers SP, Lin GD, Leach GJ, Waterman PG. A new anti-inflammatory glucoside from Ficus racemosa L. Planta Med., 2004; 70:421-426.
10. Kirtikar KR, Basu BD. Indian Medicinal Plants, Lalitha Mohan Basu, Allahabad, 2nd edn. 1935; 536.
11. Mitra CR. Neem. Dr M. S. Patel, Indian Central Oilseeds Committee, Hyderabad. 1963; 69–94.
12. Repeated Dose 28-Day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals. 2008; 407:1-13.
13. Beuege JA, Aust SD. Microsomal lipid peroxidation. Method Enzymol, 1978; 30:302-10.
14. Piengchai K, Anong T, Nopsarun T, Nuntana M, Sirirat T, Warayupa T, Yaninee J, Wannee RK. Toxicity Testing of Flowers of Neem Tree (Azadirachta indica A. Juss). Thai J. Vet. Med., 2010; 40(1):47-55.
15. Arvind P, Jaykaran, Nilesh C, Manoj S, Preeti Y. Subacute toxicity study of an aqueous extract of Ficus racemosa Linn. bark in rats. Journal of Pharmacy Research, 2010; 3(4):814-817.