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Acute Toxicity Profiling of Combined Aqueous Extracts of *Ficus racemosa* and *Azadirachta indica*

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Abstract

Ficus racemosa and Azadirachta indica have been recognized for their various curative properties and used in traditional medicines from time immemorial. The stem bark extract of Ficus racemosa is known to be a potent anti-inflammatory, memory-enhancing, anti-diabetic and anti-diuretic agent. Azadirachta indica leaf extract has been used extensively in the treatment of leprosy, epistaxis, intestinal worms, biliousness, eye problems and skin ulcers. Though the toxicity profiling of these individual plants have been studied, little is known about their toxicity when used in combination. Therefore, we aimed to study the acute toxicity of a combination of these plant extracts in female albino Wistar rats at a limit test of 2 and 5 g/kg body weight (b. wt.) according to the OECD (Organization for Economic Cooperation and Development) guidelines. Administration was restricted to a single oral dose. Behavioral changes, body and organ weights, hematological and biochemical parameters were analyzed. Results showed normal behavior and normal gain in body and organ weights in all the groups. Total WBC count was significantly reduced in both the treated groups. Blood chemistry was unchanged. No mortality was observed in any of the treated groups indicating that the LD_{50} value of the herbal mixture is above 5 g/kg b. wt.

Keywords: Acute toxicity, *Ficus racemosa, Azadirachta indica,* Limit test, OECD

Introduction

Modern medicine is widely dependent on synthetic compounds which invariably cause undesirable side effects[1]. Using plant sources for development of drugs in these modern times can prove to be more safe, accessible and affordable to the common man[2]. Humankind has been actively employing plants and plant products as a remedy for various ailments since ancient times. Interestingly over time, he realized that individual herbs were not capable of meeting the pharmacological requirement. Therefore, he began to use these medicinal plants as a concoction. *Ficus racemosa* (Moraceae) is a deciduous tree distributed throughout the Indian sub-continent, especially in the moist tropical areas. The aqueous stem bark extract derived from *Ficus racemosa* is known to improve memory and enhance Acetyl Choline levels in rats[3]. It is also known to exhibit potent hypoglycemic effect in rats[4] and individuals with type 2 diabetes[5]. Cold and hot aqueous extracts derived from *Ficus racemosa* stem bark showed a dose dependent anti-cholinesterase activity against acetyl cholinesterase of rat brain *in vitro*[6].

The decoction of stem bark of *Ficus racemosa* is a proven anti-diuretic[7]. Stem bark extracts obtained from *Ficus racemosa* have been proven to potently inhibit COX-1[8] and stimulate glucose uptake by skeletal muscles[9]. *Azadirachta indica* (Meliaceae) is an evergreen tree cultivated throughout India. It is widely used in Ayurvedic medicine, homeopathy and Unani. The aqueous extract of *Azadirachta indica* leaves possesses anti-inflammatory properties[10] and is known to induce apoptosis in ovulated oocytes in rats[11]. The crude methanol extract of *Azadirachta indica* leaves exhibited acaricidal properties against Rhipicephalus (Boophilus) microplus *in vitro*[12]. Sulfonoquinovosyldiacylglyceride, a water-soluble metabolite derived from *Azadirachta indica* leaves proved to possess anti-bacterial and anti-herpes properties[13]. Ethanol extracts of *Azadirachta indica* leaves exhibit anti-hyperglycemic, anti-dyslipidemic potential[14] and anti-filarial properties[15]. Oil procured from *Azadirachta indica* is taken orally for worms and massaged to relieve rheumatic pain[16].Neem leaves are also used to treat fever and stomach pain[17].Neem is also an effective biopesticide[18].

The aqueous extract derived from *Ficus racemosa* bark was studied for its acute toxicity in albino mice at three dose levels of 100, 300 and 1000 mg/100 g b. wt. It was observed that the extract did not cause any lethality at levels 100 times that of therapeutic doses. However, fatty changes in the liver and hyaline degenerative changes in the kidneys were observed and SGPT levels were markedly increased[19]. LD_{50} value of more than 12 g/kg b. wt. was observed for methanol extract of *Azadirachta indica* flowers when studied for their acute toxicity[20].

The main objective of this study was to determine the acute toxicity (LD_{50}) value of the herbal mixture comprising of aqueous extracts derived from *Ficus racemosa* stem bark and *Azadirachta indica* leaves in albino Wistar rats.

Material and Methods

Plant Extracts: 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.

Animals: Fifteen healthy adult female Wistar rats, weighing 170-260 g at the commencement of the study were acquired from the animal house of K. S. Hegde Medical Academy, Nitte University. All the rats were nulliparous and non-pregnant. They were separated into 3 groups of 5 each and housed in clean polypropylene cages for 5 days before dosing to ensure their acclimatization to the laboratory conditions. The temperature was maintained at 25°C (± 3°C); with a light period of 12 h. The rats were provided clean paddy husk bedding. They were fed with commercially available standard pellet chow and unlimited supply of filtered drinking water.

Acute Toxicity Profiling (Limit test at 2 and 5 g/kg b. wt.): Before administration of the extract, the rats were fasted overnight. After the period of fasting, the rats were weighed and the dose was calculated in accordance with their body weights. The volume given did not exceed 2 ml/100 g b. wt. A single dose of the herbal mixture (1:1) was given orally. Food was withheld for 3-4 hours after dosing. Control animals (Group I) were given distilled water. Limit test at 2 g/kg b. wt. (Group II) and 5 g/kg b. wt. (Group III) was performed as per Paragraph 33(a) of OECD guidelines 425[21].

Clinical Observations: Behavior: The rats were observed constantly during the first half an hour after dosing and observed intermittently for the next 24 hours and then daily thereafter, for 14 days. All findings were scientifically documented with individual records being maintained for each rat. Observations comprised of changes in fur and skin, mucous membranes and eyes and behavioral pattern. Special consideration was given for observations of salivation, sleep, lethargy, tremors, convulsions, diarrhea, coma and mortality.

Body weight: Body weight of each rat was noted before dosing on the 1st day and thereafter on the 7th and 14th day of the study before withdrawal of blood. Changes in the weight were compared with that of the control rats.

Hematology: On the 15th day blood samples were drawn and the animals were sacrificed. Total RBCs and total WBCs were counted using a hemocytometer.

Blood chemistry: The biochemical parameters were analyzed by 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-PAP method) using a semi-auto analyzer. While MDA was estimated by the method of Beuege and Aust[22]using a UV-Vis spectrophotometer.

Organ Weights: Liver, kidneys and brain from each rat were isolated after their thorough profusion with neutral saline, and pressed with a tissue paper to remove any moisture. The organs were observed for their morphology and weighed individually. The results were compared with that of the controls.

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

Administration of the herbal combination (1:1) at doses of 2 and 5 g/kg b.wt. which were approximately equivalent to 100 and 200 times of human use, caused no lethality or signs and symptoms of toxicity indicating that the LD_{50} value is greater than 5 g/kg b. wt.

Behavior: Fur, skin, mucous membrane, eyes, salivation, behavioral pattern and sleep of Group II and III were found to be normal. Lethargy, diarrhea, tremors, and coma were not observed. The observations reiterated the results obtained from the acute toxicity studies of the individual plant extracts[19,20].

Body weight: Normal body weight gains were observed in all the treated groups (figure-1). The increase in body weight of test rats indicated that dosing with the herbal mixture did not affect their growth.

Organ weights: Observations of morphology of vital organs such as brain, kidneys and liver indicated no signs of inflammation or toxicity in both the treated groups. Also, no significant difference in organ weights was observed in either of the treated groups when compared with the control group (figure-2).

Hematology: Total RBCs and total WBCs were estimated on the 15th day. A statistically significant reduction in total WBC count was observed in group II ($P<0.05$) and group III ($P<0.01$) in comparison with the control group (table-1). This result was in concordance with a study done by Jayakaran *et al*[19]. No significant difference in total RBC count was observed.

Biochemical analysis: Total proteins, albumin and ALT levels did not show any significant difference when compared to the control group, indicating normal functioning of the liver (table-2). ALP levels did not show any variation. No difference in urea levels were observed in both the treated groups in comparison with the control group, indicating normal kidney function. Total cholesterol showed no variation which was in agreement with a previous study[19]. MDA levels also did not vary with treatment with a single oral dose of the herbal mixture.

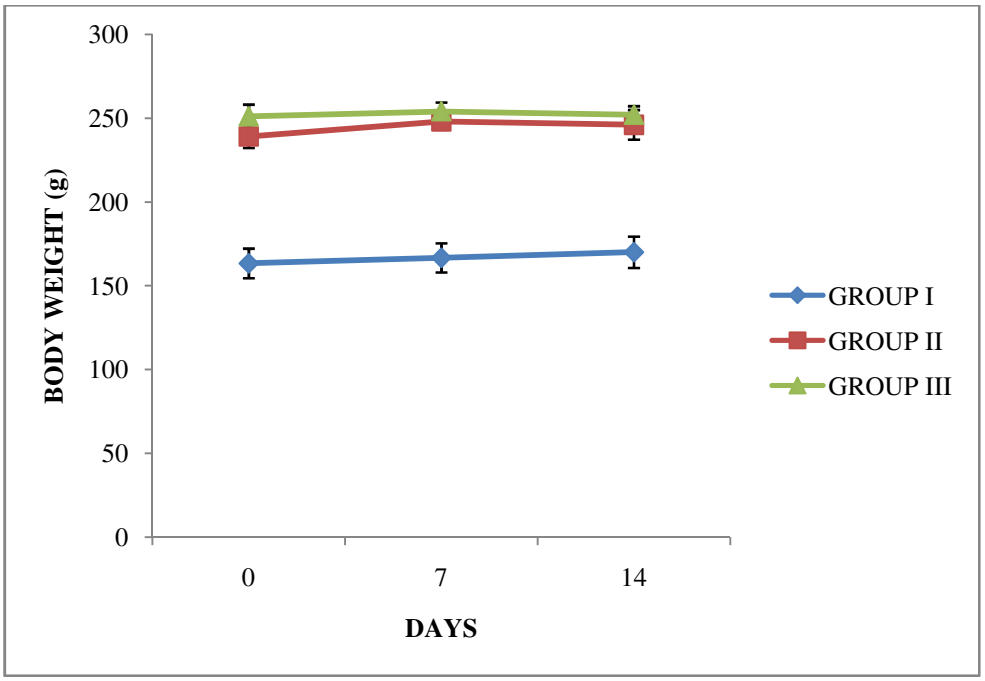

Figure-1
Changes in body weight during a two-week acute toxicity study. Values are mean ± SEM. Group I (Control), Group II (2 g/kg b. wt. herbal mixture) and Group III (5 g/kg b. wt. herbal mixture)

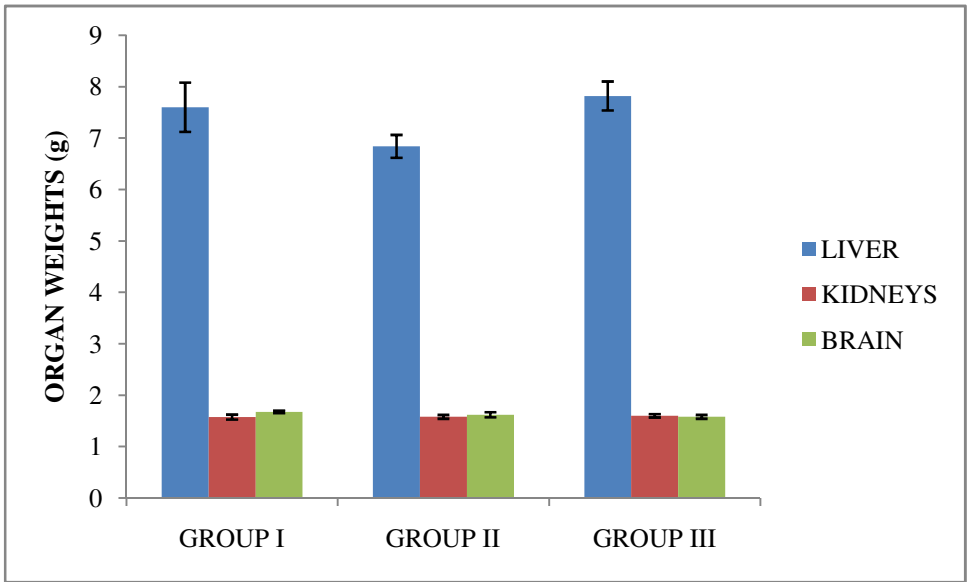

Figure-2
Absolute organ weights measured on day 15 on necropsy. Values are mean ± SEM. Group I (Control), Group II (2 g/kg b. wt. herbal mixture) and Group III (5 g/kg b. wt. herbal mixture)

Table-1
Hematological parameters after a two-week acute toxicity study. Values are mean ± SEM. Significant differences from Group I[*] (*P*<0.05), [†] (*P*<0.01). Group I (Control), Group II (2 g/kg b. wt. herbal mixture) and Group III (5 g/kg b. wt. herbal mixture)

Parameters	Group I	Group II	Group II
Total RBC (Million/cc)	9.15±0.38	6.80± 0.92	10.04± 1.08
Total WBC (Thousand/cc)	5.71±0.61	3.26±0.52[*]	2.59±0.33[†]

Table-2
Serum biochemical parameters after a two-week acute toxicity study. Values are mean ± SEM. Group I (Control), Group II (2 g/kg b. wt. herbal mixture) and Group III (5 g/kg b. wt. herbal mixture)

Parameters	Group I	Group II	Group III
Total Proteins (g/dL)	7.98±0.61	6.27±0.28	6.69±0.39
Albumin (g/dL)	3.02±0.31	2.40±0.07	2.38±0.28
ALP (IU/L)	224.24±36.56	197.38±14.62	203.14±25.92
ALT (IU/L)	89.09±10.99	63.32±11.40	64.48±6.76
Urea (mg/dL)	31.94±3.62	31.98±0.76	41.12±1.97
Total Cholesterol (mg/dL)	76.54±9.09	56.90±3.00	65.48±15.85
Malondialdehyde (µM/L)	1.82±0.30	1.53±0.34	2.81±0.02

A limit test was done since we had suitable information from literature indicating that the individual plant extracts were most likely to be non-toxic. Female rats were used for the current since they are slightly more sensitive in nature. A limitation of the study is the difference in mean body weights between the groups.

Our results are compatible with Okapanyi *et al*[23] who observed that LD_{50} value of neem leaves and bark ethanol extract in mice was about 13 g/kg b. wt. The neem leaves aqueous extract was not toxic to mice up to the dose of 1g/kg b. wt[24]. The neem leaf ethanol extract when injected subcutaneously at a dose of 10 g/kg b. wt. caused no toxicity in mice[25]. However, Chattopadhyay[26] reported the LD_{50} value of ethanol extract of neem leaf in male rats to be 4.57 g/kg b. wt. Lupeol, β-sitosterol and stigmasterol have been isolated from the stem bark of *Ficus racemosa*[27]. Azadirachtin is the active compound found in all parts of the neem[28]. The acute oral toxicity in rats fed with azadirachtin was found to be greater than 5 g/kg b. wt[29, 30].

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. It is well evident from the results that the LD_{50} of the herbal mixture (1:1) of *Ficus racemosa* and *Azadirachta indica* is above 5 g/kg b. wt. which is about 100 times that of human use.

References

1. Jain S. K., Rajvaidya S., Singh G.K., Jain P. and Nagori B.P.,Investigation of Herbal Extract as Hepatoprotective, *Res. J. Pharmaceutical Sci.,***1(3)**, 16-18 **(2012)**

2. Velayudam, Arul A. and Ilavarasan, Hepatoprotective Activity of Kadukkai Maathirai (A Siddha Polyherbal Formulation) Against Carbon Tetrachloride Induced Liver Damage in Rat, *Res. J. Pharmaceutical Sci.,* **1(4)**, 17-21 **(2012)**

3. Ahmed F., Narendra J.N., Chandra S. and Manjunath S., Acetylcholine and memory-enhancing activity of Ficus racemosa bark, *Pharmacogn. Res*., **3(4)**, 246—249 **(2011)**

4. Sachan N. K., Kumar Y., Pushkar S., Thakur R. N., Gangwar S. S. and Kalaichelvan V. K., Anti-diabetic potential of alcoholic and aqueous extracts of Ficus racemosa Linn. Bark in normal and alloxan induced diabetic rat, *Int. J. Pharm. Sci. Drug Res.*,**1 (1)**, 24-27**(2009)**

5. Ahmed F., Hudeda S. and Urooj A., Antihyperglycemic activity of Ficus racemosa bark extract in type 2 diabetic individuals, *Journal of Diabetes*, **3**, 318–319 **(2011)**

6. Ahmed F. and Urooj A., Anticholin esterase activities of cold and hot aqueous extracts of F. racemosastem bark, *Pharmacogn. Mag*., **6(22)**, 142–144**(2010)**

7. Ratnasooriya W.D., Jayakody J.R. and Nadarajah T., Antidiuretic activity of aqueous bark extract of Sri Lankan Ficus racemosa in rats, *ActaBiol Hung*., **54(3-4)**, 357-63 **(2003)**

8. Li R.W., Myers S.P., Leach D.N., Lin G.D. and Leach G., A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants, *J. Ethnopharmacol.,* **85**, 25–32 **(2003)**

9. Ramya E., Veeresh P. V., Prabhakar K. R. and Unnikrishnan M. K., Effect of *Ficus racemosa*stem bark extract in type 2 diabetes induced by high-fat diet and low dose stzin rats: A mechanistic approach, *Indian J. Pharmacol.*, **40(2)**, 160 **(2008)**

10. Syed A. M., Mosaddek and Rashid M. M. U., A comparative study of the anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone, *Bangladesh J. Pharmacol*., **3**, 44-47**(2008)**

11. Tripathi A., Shrivastav T.G. and Chaube S.K., An increase of granulosa cell apoptosis mediates aqueous neem (Azadirachta indica) leaf extract-induced oocyte apoptosis in rat, *Int J Appl Basic Med Res*., **3(1)**, 27–36 **(2013)**

12. Shyma K. P., Gupta J. P., Ghosh S., Patel K.K. and Singh V., Acaricidal effect of herbal extracts against cattle tick Rhipicephalus (Boophilus) microplus using in vitro studies, *Parasitol Res*., **113(5)**, 1919-26 **(2014)**

13. Bharitkar Y.P., Bathini S., Ojha D., Ghosh S., Mukherjee H., Kuotsu K., Chattopadhyay D. and Mondal N. B., Antibacterial and antiviral evaluation of sulfonoquinovosyldiacylglyceride: a glycolipid isolated from Azadirachta indica leaves, *Lett Appl Microbiol*., **58(2)**, 184-9 **(2014)**

14. Mukherjee N., Mukherjee S., Saini P., Roy P. and Babu S. P. S., Antifilarial effects of polyphenol rich ethanolic extract from the leaves of Azadirachta indica through molecular and biochemical approaches describing reactive oxygen species (ROS) mediated apoptosis of Setariacervi, *Exp Parasitol.*, **136**, 41–58 **(2014)**

15. Bisht S. and Sisodia S.S., Anti-Hyperglycemic and Antidyslipidemic Potential of *Azadirachta indica* Leaf Extract in STZ- Induced Diabetes Mellitus, *J. Pharm. Sci. & Res.*, **2 (10)**,622-627**(2010)**

16. Chunamoni D. and Robindra T., Ethnobotanical notes of the *Rabha*community in Mataikhar reserve forest of Kamrup district, Assam, India, *Res. J. Recent Sci.,* **3(6)**, 26-33 **(2014)**

17. Sharma M., Sharma C.L. and Marak P.N., Indigenous uses of medicinal plants in North Garo Hills, Meghalaya, NE India, *Res. J. Recent Sci.,* **3(ISC-2013)**, 137-146 **(2014)**

18. Mondal D. and Mondal T., A Review on efficacy of *Azadirachta indica* A. Juss based biopesticides: An Indian perspective, *Res. J. Recent Sci.,***1(3)**, 94-99 **(2012)**

19. Jaykaran, Bhardwaj P., Kantharia N., Yadav P. and Panwar A., Acute toxicity study of an aqueous extract of Ficus racemosa Linn. bark in albino mice, *J. Toxicol.*,**6(1)**, **(2008)**

20. Kupradinun P., Tepsuwan A., Tanthasri N., Meesiripan N., Tunsakul S., Tompat W., Jarratwisarutporn Y. and Kusamran W. R.., Toxicity Testing of Flowers of Neem Tree, *Thai J. Vet. Med,* **40(1)**, 47-55 **(2010)**

21. Acute Oral Toxicity – Up-and-Down-Procedure (UDP), *OECD Guidelines for the Testing of Chemicals*, **425**, 1-27**(2008)**

22. Beuege J. A. and Aust S. D., Microsomal lipid peroxidation, *Method Enzymol.*,**30**, 302-10**(1978)**

23. Okpanyi S.N. and Ezeukwu G.C., Anti-inflammatory and antipyretic activities of Azadirachta indica, *Planta Med*., **41**, 34-39 **(1981)**

24. Subapriya R. and Nagini S., Medicinal properties of neem leaves: a review, *Curr. Med. Chem. Anti-Canc. Agents*, **5**, 149-156 **(2005)**

25. Mokkhasmitt M., Swatdimogkol K. and Satrawaha P., Study on toxicity of Thai medicinal plants, *Bull. Dept. Med. Sci.,***12(2/4)**, 36-65 **(1971)**

26. Chattopadhyay R.R., Possible biochemical mode of anti-inflammatory action of Azadirachta indica A. Juss. in rats, *Indian J. Exp. Biol,.***61(1)**, 75 **(1998)**

27. Paarakh P.M., Ficus racemosa Linn. – An overview, *Nat. Prod. Radiance*, **8(1)**, 84-90 **(2009)**

28. Subramanian S.S. and Nair A.G.R., Melicitrin-A new myricetin glycoside from the flowers of Meliazadirachta, *Indian J. Chem.*, **10**, 452 **(1972)**

29. Farm Chemicals Handbook, Meister Publishing Co. Willoughby, OH., Available: http://www.neemuses.com /azadirachtin.php.**(1995)**

30. Thomson, W.T., Agricultural Chemicals. Book I: Insecticides. Thomson Publications, Fresno, CA., Available: http:/www.neemuses.com/azadirachtin.php. **(1992)**