Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2014; 6(4): 80-85

ISSN: 0975-5160

Research Article

Toxicological Evaluation of the Aqueous Fraction of *ficus racemosa* and *Bauhinia variegate* Bark in Rats.

*Solanki ND[1], Bhavsar SK[2]

[1]*Assistant Professor, Department of Pharmacology, Ramanbhai Patel College of Pharmacy, CHARUSAT, TA-Petlad, Dist. Anand, Changa – 388421, India*
[2]*Professor & Head, Department of Pharmacology and Toxicology, College of Veterinary Science & Animal Husbandry, Navsari Agricultural University, Navsari-396 450, India*

Available Online: 22nd November, 2014

ABSTRACT
Ficus Racemosa (FR) grows in the green tropical regions in India. *Bauhinia Variegate (BV)* is distributed in Himalayan region and widely planted in tropic regions of the world. To validate its use in traditional medicine, it is important to evaluate its toxicity. The aim of the study was to evaluate toxicity of the aqueous extracts of *Ficus racemosa* and *Bauhinia variegate* bark in rats. Acute toxicity test was conducted by single high dose of 2000 mg/kg body weight of the rats. Delayed effects of aqueous extracts of FR & BV on haematological, renal, and hepatic markers were analyzed. No mortality & signs of neurological and behavioural changes were noticed with in 72 h when treated with FR & BV. Delayed effects of extracts were observed and found no significant change in body weight as compare to normal animals; while significant changes were observed of liver parameters by use of FR & BV. No any significant change found in creatinine & total protein level, while haematological parameters were not altered during toxicity study compare to normal animals. Histopathology revealed no specific structural changes in the heart, kidney, liver & nerve tissues when treated with FR & BV compare to control animals.

Key word: *Ficus Racemosa*, *Bauhinia Variegate*, haematological, renal & hepatic marker

INTRODUCTION

Since ancient times, plants have commonly been used in folk medicine for the treatment of various ailments. The rationale for the utilization of medicinal plants has rested largely on the long-term clinical experience with little or no scientific data on their efficacy and safety. However, in the recent past, pharmacological and toxicological effects of these plants have begun to receive attention from scientists for the verification of their claimed pharmacological and therapeutic properties [1]. *Ficus Racemosa Linn*. (FR) belongs to the family Moraceae which is a subdivision of Urticaceae. The family consisting of 116 Genera and 1632 species. It Consists of dried bark of *FR* of deciduous tree distributed all over India & grows in ever green forest, moist localities and in tropical regions. The roots, bark-skin, fruits, latex and leaves have great medicinal value. It is a one of the herbs mentioned in all ancient scriptures of Ayurveda called as Udumbara, which is considered sacred to God Dattaguru. The stem bark of Ficus racemosa contains tannin, wax, saponingluanol acetate, β-sitosterol, leucocyanidin- 3 – O – β – D - glucopyrancoside, leucopelargonidin – 3 – O – β – D - glucopyranoside, leucopelargonidin – 3 – O – α – L - rhamnopyranoside, lupeol, cerylbehenate, lupeol acetate, α-amyrin acetate, leucoanthocyanidin, and leucoanthocyanin from trunk bark, lupeol, β-sitosterol and stigmasterol are isolated [2]. *Bauhinia Variegate* (BV) belongs to family Laguminosae. It is distributed in high altitudes of Himalaya. It is widely planted in tropic and warm regions of the world. It belongs to more than 200 species in the genus Bauhinia [29]. Stem bark of BV contain 5,7dihydroxy and 5,7 dimethoxy flavanone-4-O-a-L rhamnopyrosyl-ß-D-glycopyranosides, Kaempferol-3-glucoside, lupeol, and betasitosterol [3].

Maharishi Charka has categorized udumbara as mutrasangrahaniya anti-udumbara as mutrasangrahaniya – antidiuretic herb. Susruta has described the properties of the plant, like astringent, promotes callus healing in fractures (bhagnasandhaniya), alleviates Rakta pitta, burning sensation and obesity, and useful in vaginal disorders. The Plant FR have hypoglycaemic [5], Hypolipidemic [6], renal anticarcinogenic [7], wound healing [8], antioxidant [9] potential. The roots and bark are astringent, acrid, cooling, constipating, depurative, anthelmintic in nature. BV have Antioxidant [10], Anti-inflammatory [11], Hypoglycaemic & antidiabetic [12] activity. In spite of the wide use of FR & BV in traditional medicine, data on the systematic evaluation of its toxic effects is lacking. Therefore, the aim of the present study was to investigate the toxic effects of aqueous extracts of FR & BV bark in rats.

MATERIAL AND METHODS

Plant material & extraction: Pharmacognostic identified stem bark of *Ficus racemosa (H1) and Bauhinia variegate*

*Author for correspondence

Table 1: Sign of toxicity and mortality results of the acute toxicity study of Aqueous extract of H1 & H2

Groups	Dose (mg/kg)	Sign of toxicity (ST/NB)	Mortality (D/S)
AH1, AH2	2000	0/5	0/5

ST: Sign of toxicity; NB: Normal behaviour; D: Died; S: Survived. Values are expressed as animal number

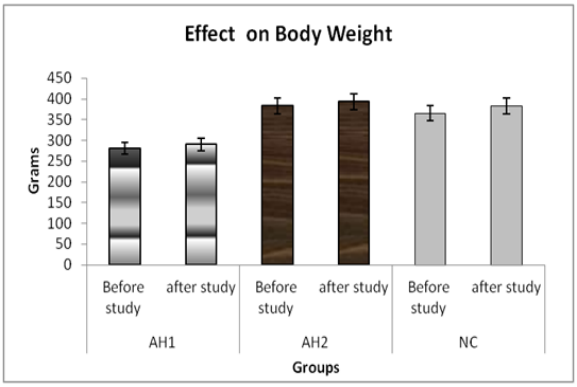

Fig. 1: Effect of AH1 & AH2 on body weight changes in the acute toxicity study. Each bar represents mean±SEM (n=5)

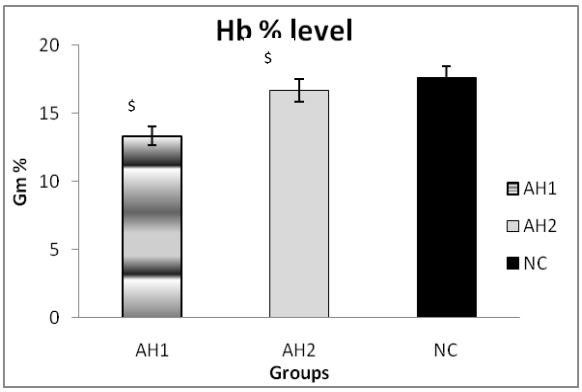

Fig. 2: Effect of AH1 & AH2 on Hb % level (n=3), $ - Non significant

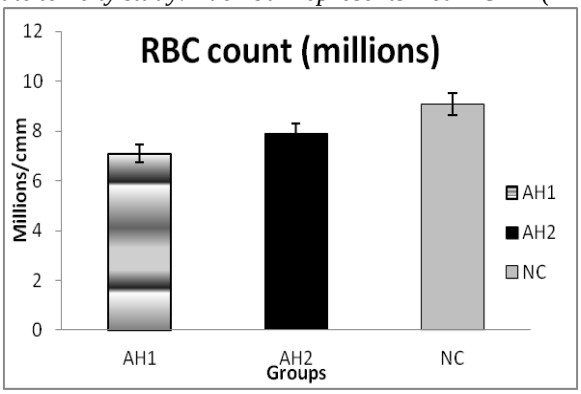

Fig. 3: Effect of AH1 & AH2 on RBC count (n=3), $ - Non significant

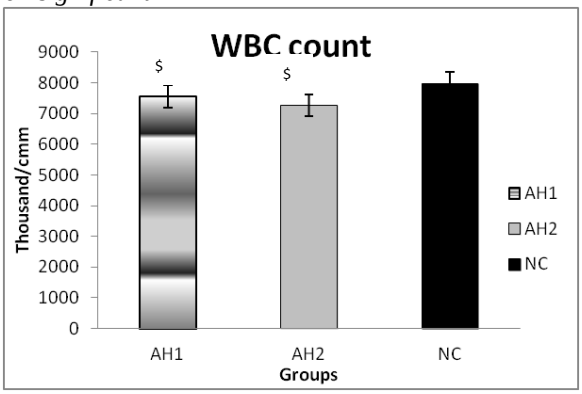

Fig. 4: Effect of AH1 & AH2 on WBC count (n=3), $ - Non significant

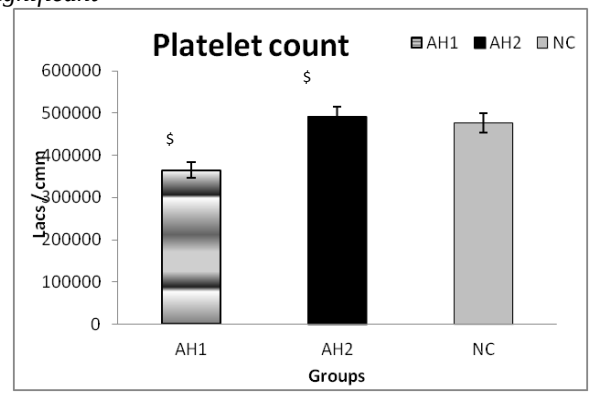

Fig. 5: Effect of AH1 & AH2 on Platelet count (n=3), $ - Non significant

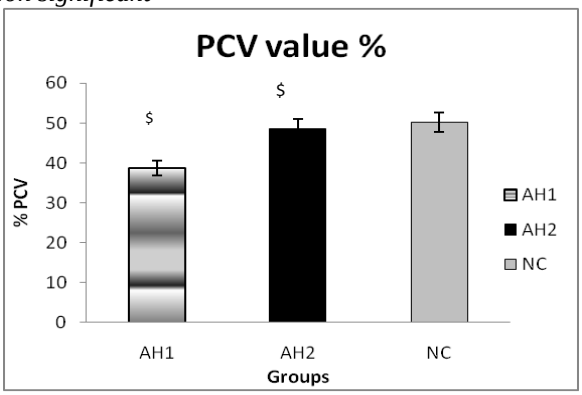

Fig. 6: Effect of AH1 & AH2 on PCV % value (n=3), $ - Non significant

(H2) were collected & authenticated from Directorate of Medicinal and Aromatic Plants Research (DMAPR) at Boriavi, Gujarat, India. A voucher specimen of the barks are deposited in the department for future reference. The plant material was then shade dried at a temperature of 30°C±3°C for a period of 15 days and ground to get coarse powder. Crude powder of H1 & H2 were heated in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heatstable constituents. The starting ratio of crude drug to water is 1:4, the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. The extract was dried in a oven and refrigerated until used. The extractive values of H1 & H2 were found to be 18.4 and 15.2 gram respectively. The extract for administration was prepared with distilled water in the dose of 2000 mg/kg once a day. Experimental animals: Wistar albino rats were provided from Zydus research center, Ahmedabad, India. All animals were maintained in an air-conditioned room at

Table-2 Effect of Aqueous extracts of H1 & H2 on biochemical estimation of SGOT, SGPT, Creatinine,

Biochemical parameter	AH1	AH2	Normal control group
SGOT	23.68±3.2 $	16.03±2.73 **	31.33±0.81
SGPT	9.17±6.55 **	10.95±1.24 **	41.47±11.26
Creatinine	2.13±0.41 $	1.78±0.21$	2.20±0.26
Total Protein	4.55±0.70 $	4.18±0.165 $	4.86±0.36 $

*Total protein in toxicity study All the values were expressed as Mean ± SEM, n=5; Significant Difference observed at * P<0.05, ** P<0.001 compared to normal control group, $ P>0.05- non significant difference*

22°C±2°C, with a relative humidity of 75%±5%, and a 12-h light/dark cycle. A basal diet (Pranav agro, India) and water were provided *ad libitum*. A single dose of 2000 mg/kg of ingested orally to Male rats by stratified random sampling based on body weight in the different group of animals. The experiment was approved by the Institutional Animal Ethics Committee (approval no. RPCP/IAEC/2012-13/R-13) constituted as per the norms of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Toxicity study: Acute toxicity study was conducted as per OECD 423 guidelines in wistar rats (OECD) [13]. A single dose level was selected as 2000 mg/kg of body weight. Each group contains 5 animals; total 3 groups were there AH1, AH2, and NC. After depriving the animals food overnight, the normal control group (NC) received 1 ml of 0.9 % saline solution orally, while each treated groups (AH1, AH2) received aqueous extract orally once. Aqueous extract was dissolved in 2 % tween 20 solution.

Observations: The animals were observed continuously for the first 4 h and then each hour for the next 24 h, 48 h & there after once in a day for 7 days after administering of the diff. extracts once, to observe any death or changes in general behaviour and other physiological activities as stated in literature [14,15]. 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 behavioural pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and mortality. Changes in wellness parameters were compared with that of control animals.

Haematological & Biochemical analysis: At the end of the study blood samples were collected through retro orbital route from rats & measure Haemoglobin content, Red Blood Cells, White Blood Cells, Platelet count, PCV (%) were determined using an automatic hematological analyzer; while serum were separated from whole blood by use of cooling centrifuge at 5000 RPM for 10 min and analyzed for SGOT, SGPT, Creatinine & total protein.

Histopathological evaluation: The organs were stained with the hematoxylin–eosin (H and E) stain of the AH1 & AH2-treated and control groups, following fixation with 10% formalin and embedding in paraffin wax. A histopathology was performed of the tissues/organs like heart, kidney, lever & sciatic nerve.

Statistical analysis: The differences among treated and control groups for serum biochemistry parameters, haematological parameters were determined using the Microsoft excel. Comparisons among different groups were performed by analysis of variance using the one way ANOVA test. All data are expressed as mean±standard error of mean (SEM); *P* values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Acute toxicity study: No lethal effect or mortality was observed in animals throughout the test period following single oral administration at all selected dose level of AH1 & AH2.

The animals did not show any changes in the general appearance during the observation period. Morphological characteristics (fur, skin, eyes, and nose) were unchanged. The treated animals did not show any tremors, convulsion, salivation, diarrhea, lethargy, or unusual behaviors such as self-mutilation, walking backward etc. There was no significant difference in body weights before and after the study period (fig-1). Generally, the decrease in the body weight gain is a simple and sensitive index of toxicity after exposure to potentially toxic substances. [16-18]

At the end of the study blood samples were collected & blood indices were analyzed in all group of animals. In AH1 (13.33 ± 1.65) & AH2 (16.23 ± 0.73) showed no significant alteration in Haemoglobin (gm %) level (n=3) as compare to normal control rats (17.6 ± 1.06) (Fig-2). RBC count in AH1 (7.1±1.1) & AH2 (7.9±1.00) treated group showed no significant alteration compared to normal control group of animals (n=3) (9.07±0.22) (Fig- 3). There were no significant alteration observed in total WBC count (thousand/cmm) in treated grps AH1, AH2 (7553±380, 7266±754) which was similar to the normal control group of animals (7966±410) (Fig–4). Platelet count (lacs/cmm) in treated groups AH1 & AH2 were found to be (364666.7± 81482.16, 491333±39794.47) respectively which were non-significantly different to normal control group of animals (476666±48761) (Fig-5). So, From the present study it was found that no significant changes for H1 & H2 plant treated animals were observed in Blood indices compared to Normal animals. Blood parameters analysis is relevant to risk evaluation as the haematological system has a higher predictive value for toxicity in humans (91%) when assay involves rodents [19]. Blood forms the main medium of transport for many drugs and xenobiotics in the body and for that matter components of the blood such as red blood cells, white blood cells, haemoglobin and platelets are at least initially exposed to significant concentrations of toxic compounds. The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status for both animals and humans [20]. There were no treatment-related changes in the different hematological parameters between the control and treatment group after

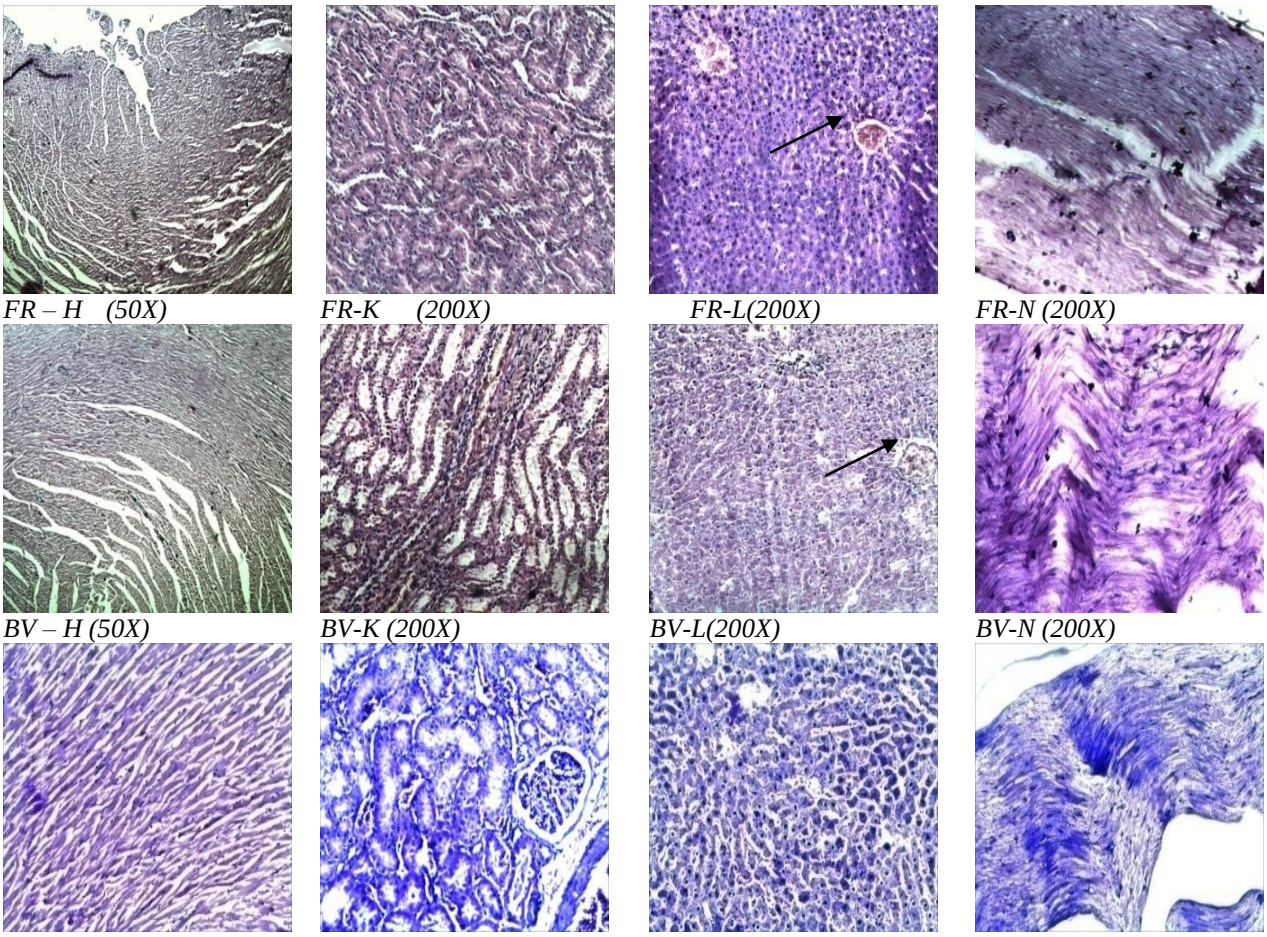

Fig. 7: Histopathology of Major organs as heart (H), Level (L), Kidney (K), Sciatic nerve (N) when treated with FR & BV extracts and Normal control (NC) animals. Slight damage was observed for liver in treated groups marked as when compared to NC animals.

treatment period, with H1 & H2. It indicates that H1 & H2 does not affect haematopoiesis and leucopoiesis in experimental animals. Thus, the orally administrated doses of the extracts (2000 mg/kg) were nontoxic and did not interfere with the production of circulating red blood cells, white blood cells, and platelets.

n the biochemical analysis few significant changes were observed though they were not entirely dose dependent. The biochemical evaluation is important since there are several reports of liver and kidney toxicity related to the use of phytotherapeutic [21,22] products. In preclinical toxicity studies, hepatic & renal changes were particularly liable to occur because of the high doses given and the fact that the kidneys eliminate [23,24] many drugs and their metabolites. In toxicity studies SGOT, SGPT, creatinine, total protein determinations were critical [21,25].

Serum SGOT level were significantly altered in the extract treated groups of AH2 (16.03±2.73) as compared to NC group, while in AH1 treated group showed non-significant change in SGOT level (Table-2). Serum SGPT level were significantly reduced in AH1, AH2 treated group of animals as compared to NC group of animals (Table-2). It is well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion hence it was found necessary to estimate the effects of the extracts on kidney functions. Serum biochemical parameters related to kidney functions viz. creatinine and total protein demonstrated no significant differences with respect to NC animals (Table-2).

In the acute oral toxicity study, a product is considered safe if no death occurs and no clinical signs are observed at doses below 5 g/kg [26]. In present study AH1 & AH2 did not show any toxic reactions at a dose of 2000 mg/kg. Thus, the no-observed adverse effect level of AH1 & AH2 was 2000 mg/kg, but due to ethical reasons a small sample size of animals were utilized & only with one species of test animal, findings of this study cannot be directly extrapolated to humans. The popularity of herbal medicine is increasing in developing countries. It is often believed that such remedies don not have adverse effects, since these treatments are "natural" and commonly used for self medication without supervision. These medicinal plants possess several biological activities in humans but very little is known regarding their potential toxicity [27]. The same is also applicable to *Ficus racemosa* & *Bauhinia variegate*.

For light microscopic investigation, brain & sciatic nerve tissue specimens were fixed in a 10% formaldehyde, dehydrated in alcohol solution, embedded in paraffin and stained by hemotoxilin and eosin (H&E) as per method described by Sudoh *et al.*, 2004 [28] which were used for histopathological examination.

Overall histological examination revealed that no any specific structural changes were seen in the different organs like heart, liver, kidney & nerve when treated with FR & BV, Only few structural alterations were observed in the treated groups (FR & BV) for the hepatocyte cells as compared to normal animals.

CONCLUSION

Observational, biochemical, structural parameters were revealed that oral toxicity of stem bark of *Ficus racemosa* & *Bauhinia variegate* in aqueous extract has not produced any significant toxic reactions to the wistar rats; overall toxicity is very low. However, since this finding cannot be directly extrapolated to humans, caution should be exercised in its use especially at high doses.

ACKNOWLEDGEMENT

Authors are thankful to the Ramanbhai Patel College of Pharmacy, Charotar University of science and technology, Changa, India for providing necessary facility and financial support for the study; authors are also thankful to Pathology department, College of Veterinary Science & Animal Husbandry, AAU, India for providing their support in histopathology.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this work.

REFERENCES

1. Rebecca MA, Ishii-Iwamoto EL, Grespan R, Cuman RK, Caparroz-Assef SM, Mello JC, et al. Toxicological studies on Stryphnodendron adstringens. J Ethnopharmacol 2002;83:101-4.
2. Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z, Singh AK. Dictionary of Indian Medicinal Plants, CIMAP, Lucknow, India, 1992, 546.
3. Yadava RN, Reddy VM. A new flavone glycoside, 5-hydroxy 7,3',4’5'- tetra-methoxy flavone 5-O-ß-D-xylopyranosyl-(1—>2)-a-Lrhamnopyranoside from Bauhinia variegataLinn, J Asian Nat Pro Res., 3, 2001, 341–6.
4. Chopra RN, Chopra IC, Varma BS. Supplement to Glossary of Indian Medicinal Plants. CSIR: New Delhi; 29-30; 1992.
5. Swain LE, Downum KR, Light-activated toxins of the Moraceae.Biochem.Sys.Ecol, 18, 1990, 153-156.
6. Agarwal V and Chauhan BM, A study on composition and hypolipidemic effect of dietry fibre u. 2011, 200-202.
7. NaghmaK, and Sultana S. "Modulatory Effect of Ficusracemosa: Diminution of Potassium Bromate-Induced Renal Oxidative Injury and Cell Proliferation Response." Basic & clinical pharmacology & toxicology 97.5 (2005): 282-288.
8. Biswas, TuhinKanti, and Biswapati Mukherjee. "Plant medicines of Indian origin for wound healing activity: a review." The international journal of lower extremity wounds 2.1 (2003): 25-39.
9. Veerapur, V. P., et al. "Ficusracemosa stem bark extract: a potent antioxidant and a probable natural radioprotector." Evidence-Based Complementary and Alternative Medicine 6.3 (2009): 317-324.
10. Gautam B, Vadivel V, Stuetz W, Biesalski HK, Bioactive compounds extracted from Indian wild legume seeds: antioxidant and type II diabetes related enzyme inhibition properties, International Journal of Food Sciences and Nutrition, 2011, 1-4.
11. Rao YK, Fang SH, Tzeng YM, Antiinflammatory activities of flavonoids and a triterpene caffeate isolated from Bauhinia variegata,Phytother Res., 22, 2008, 957–962.
12. Frankish N, de Souza Menezes F, Mills C, Sheridan H, Enhancement of Insulin Release from the ß-Cell Line INS-1 by an Ethanolic Extract of Buahiniavariegataand Its Major Constituent Roseoside, from Ficusracemosa L. Planta Med., 70, 2004, 421-426.
13. OECD: Acute oral toxicity test method. In: OECD Guidelines for testing of Chemicals. No. 423 Paris, France: Organization for Economic Cooperation and Development; 2001.
14. Shah AMA, Garg SK, Garg KM. Subacute toxicity studies on Pendimethalin in rats. Indian J. Pharm. 1997, 29: 322-324.
15. Bürger C, Fischer DR, Cordenunzzi DA, Batschauer de Borba AP, Filho VC, Soaresdos Santos AR. Acute and subacute toxicity of the hydroalcoholic extract from Wedeliapaludosa in mice. J. Pharm. Sci. 8(2), 2005, 370-373.
16. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D, L methylphenidate in Sprague-Dawley rats. Toxicology 2002;179:183-96.
17. Tofovic SP, Jackson EK. Effect of long-term caffeine consumption on renal function in spontaneously hypertensive heart failure prone rats. J Cardiovasc Pharmacol 1999;33:360-6.
18. Raza M, Al-Shabanah OA, El-Hadiyah TM, Al-Majed AA. Effect of prolonged vigabatrin treatment on haematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Sci Pharm 2002;70:135-45.
19. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of toxicity of pharmaceuticals in humans and in animals. RegulToxicolPharmacol. 2000; 32:56-67.
20. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of Musanga cecropioides in rats. J Ethnopharmacol 2006;105:374-9.
21. Obici S, Otobone FJ, da Silva Sela VR, Ishida K, da Silva JC, Nakamura CV. Preliminary toxicity study of dichloromethane extract of Kielmeyeracoriacea stems in mice and rats. J Ethnopharmacol. 2008;115(1):131-9.
22. Corns CM. Herbal remedies and clinical biochemistry. Ann ClinBiochem. 2003; 40 (Pt 5): 489-507.

23. Greaves P. Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation. Access Online via Elsevier, 2011.
24. Schreiner GE., Maher JF. "Toxic nephropathy." The American Journal of Medicine 38.3 (1965): 409-449.
25. Arneson, W and Brickell J. "Assessment of Renal Function." Clinical Chemistry: A laboratory Perspective. 1st ed. Philadelphia: FA Davis Company (2007): 201-32.
26. Brock WJ, Trochimowicz HJ, Millischer RJ, Farr C, Kawano T, Rusch GM. Acute and subchronic toxicity of 1,1-dichloro-1-fluoroethane (HCFC-141b). Food Chem Toxicol 1995;33:483-90.
27. Rosidah, Yam MF, Sadikun A, Ahmad M, Akowuah GA, Asmawi MZ. Toxicology evaluation of standardized methanol extract of Gynura procumbens. J Ethnopharmacol 2009;123:244-9.
28. Sudoh Y, Desai SP, Haderer AE, Sudoh S, Gerner P, Anthony DC, De Girolami U, Wang GK. Neurologic and histopathologic evaluation after high volume intrathecal amitriptyline. Reg Anesth Pain Med. 2004, 29: 434-440.
29. Ghaisas, M. M., S. A. Shaikh, and A. D. Deshpande. "Evaluation of the immunomodulatory activity of ethanolic extract of the stem bark of Bauhinia variegata Linn." International Journal of Green Pharmacy 3.1 (2009): 70