Original Articles
Toxicology International, *May-Aug 2015 / Vol-22 / Issue-2, 58-64*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2015/v22/i2/137657

Acute Oral Toxicity Study of Ethanolic Extract of *Acacia catechu* Willd. Seed using Hematological and Biochemical Parameters in Wistar Albino Rat

Lakshmi Thangavelu[1*], R. Vijayaragavan[2], P. Selvanadhan[3] and D. Ezhilarasan[1]

[1]Department of Pharmacology, Saveetha Dental College and Hospitals, Chennai - 600077, Tamil Nadu, India; lakshmi085@gmail.com
[2]Department of Research, Saveetha University, Chennai - 600077, Tamil Nadu, India
[3]BRULAC, Saveetha Dental College and Hospitals, Chennai - 600077, Tamil Nadu, India

Abstract

Acacia catechu Willd. is widely used traditionally as an herbal remedy for many ailments. This study is aimed to assess the safety of its seed extract using hematological and biochemical parameters. Rats were given p.o., vehicle (control), 800, 1600, and 3200 mg/kg (n = 4 per group) of alcoholic extract of *A. catechu* seed made into a suspension with 1% hydroxyl propyl cellulose and observed for mortality for 14 days. Hematological and biochemical parameters were evaluated after that. No mortality was observed in any of the groups and gross observation showed no abnormality. There was no change in the body weight and organ to body weight ratio as compared to the control group. A significant increase in the hemoglobin and packed cell volume was observed in all the groups as compared to the control. No significant change was observed in glucose, cholesterol, bilirubin, total protein, serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transaminase, lactic dehydrogenase, urea, and creatinine. The uric acid level of 3200 mg/kg dose was significantly lower than the control group. This study shows that ethanolic extract of *A. catechu* Willd. the seed has very low mammalian toxicity.

Keywords: Acacia catechu Willd Seed, Acute Oral Toxicity, Biochemical Parameter, Ethanolic Extract, Hematological, Rat
Received: 13-06-2015 **Revised:** 04-08-2015 **Accepted:** 06-08-2015

1. Introduction

Acacia catechu Willd. (Mimosaceae) is commonly called as khair or black cutch. It is known as khadira in Sanskrit and Karungali in Tamil. People in Kerala drink the boiled juice for digestive disorders. In India, there are the three varieties of *A. catechu*, namely Catechu, Catechuoides, and Sundra. Catechu (heartwood) is commercially used to obtain Katha (a concentrated filtered extract) in North India.[1]

A. catechu has been in use in Indian traditional system for curing various ailments.[2] All parts of the plant, namely bark, heartwood, leaf, and roots possess high medicinal value. The plant is used in the treatment of asthma, bronchitis, angina pectoris, sores in the mouth, and abdominal colic. The leaves are used in dysentery, the bark is used for chronic skin infections and scabies, the heartwood is used for nose bleeding and roots are used for rheumatism and toothache.[2,3] Main chemical constituents of *A. catechu* Willd. are catechin, (-) epicatechin, epigallocatechin, epicatechin gallate, phloroglucin, protocatechuic acid, quercetin, poriferasterol glucosides, poriferasterol acyl glucosides, lupenone, kaempferol, cyanidol, dihydrokaempferol, taxifolin, (+)- afzelechin gum, and minerals.[4-7]

The extracts of various parts of *A. catechu* exhibit variety of pharmacological effects such as analgesic, antipyretic, anti-inflammatory, antidiarrheal, hypoglycemic, hepatoprotective, gastroprotective, hypotensive, anticancer, antioxidant, and antimicrobial activities.[8-10] However, no report has been published

* *Author for correspondence*

about the seed extract of *A. catechu* Willd. other than hypoglycemic activity.[11]

Hence, the acute oral toxicity of ethanolic extract of *A. catechu* Willd. the seed was carried out in rats to establish its safety to explore for further pharmacological actions.

2. Materials and Methods

2.1 Plant Material Collection and Extraction

A. catechu seeds were collected from fields and was authenticated by Green Chem Laboratory (Bengaluru). Seeds were shade dried for a week and made into a fine powder in a mixer grinder. The powder was passed through 100 mesh sieve and stored in sealed polythene bags. About 2.5 kg of *A. catechu* seed powder was mixed with 10 L of ethanol in a 20 L round bottom flask attached with Graham condenser and heated for 1 h at 65°C. The condenser was cooled with circulating chilled water. After 1 h of extraction, the flask was cooled to room temp, and the extract was filtered through Whatman 1 filter paper, and the filtrate was collected. The residue was extracted repeatedly with 10 L of ethanol, twice. All the filtrates were pooled. The pooled extract was evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 65°C. About 150 g of dry powder was obtained per batch (6% yield).

2.2 Chemicals

Hydroxy propyl cellulose and isoflurane were from Sigma-Aldrich (USA), 70% ethanol was purchased from Hi-Media (Mumbai). All the other chemicals used were of analytical grade.

2.3 Animals

Wistar female rats (150–250 g) that were randomly bred and maintained in the Animal Facility of the Biomedical Research Unit and Laboratory Animal Centre (BRULAC, Saveetha University) were used for the study. The animals were housed in polypropylene cages (4 per cage) containing sterilized paddy husk as bedding material and were provided with pellet diet (commercial feed) and water *ad libitum*. Animals were cared for and maintained as per the approved guidelines of the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA, India). This study was conducted according to the OECD guidelines, and the protocol was approved by the Institutional Animal Ethical Committee (SU/BRULAC /RD/001-2015).

2.4 Experimental Design

The following groups were used:

- Group 1: Control - Hydroxy propyl cellulose p.o
- Group 2: *A. catechu* seed powder (ethanolic extract) 800 mg/kg, p.o
- Group 3: *A. catechu* seed powder (ethanolic extract) 1600 mg/kg, p.o
- Group 4: *A. catechu* seed powder (ethanolic extract) 3200 mg/kg, p.o.

2.5 Acute Toxicity Study

The animals were fasted for 2 h prior to oral administration. 1% hydroxyl propyl cellulose solution was prepared in distilled water. Various concentrations of the seed powder were suspended in the solution and warmed to about 40°C and vortexed. The suspension was administered to the rat using a 16 gauge oral feeding cannula. Initially, a dose of 800 mg/kg was given to four rats. Once the dose was tolerated further doses of 1600 and 3200 mg/kg were given (logarithmic dose). All doses were given once daily. The control group received hydroxyl propyl cellulose. The animals were weighed daily. General behavior and food and water consumption were monitored for a period of 14 days as per OECD guidelines. At the end of the experiment, the animals were anesthetized with isoflurane and blood was withdrawn from the orbital plexus for various hematological and biochemical analysis in a vacutainer with and without anticoagulant (K_2 ethylenediaminetetraacetic acid). They were sacrificed by cervical dislocation and vital organs were removed, freed from extraneous tissues, observed for any gross pathology, weighed, and preserved in 10% formalin.

2.6 Hematological Analysis

Whole blood was subjected to various hematological analysis using Beckman Coulter Cell Counter (UK). The following parameters were determined: Red blood cell count, white blood cell count, hemoglobin (Hb) concentration, Packed Cell Volume (PCV), and Platelet Count (PLT).

2.7 Biochemical Analysis

For clinical biochemistry analyses, serum was used and the estimations were carried out using Roche Modular EVO 9000 auto analyzer (USA). The following analyses were

carried out - glucose, cholesterol, bilirubin, total protein, urea, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP), gamma glutamyl transaminase, lactic dehydrogenase, urea, and creatinine. The uric acid prepared from nonheparinized blood (5 mL).

2.8 Organ to Body Weight Index

The organ to body weight index was calculated as a ratio of organ weight divided by body weight and multiplied by 100.

2.9 Statistical Analysis

The data were expressed as a mean ± standard error. The results were analyzed by one-way analysis of variance followed by Dunnett's test. A probability of 0.05 or less was considered as statistically significant. The statistical analysis and the graph plotting were carried out using SigmaPlot 12 (Systat Software Inc., USA). Four animals per group with three logarithmic doses were used for the estimation of LD_{50} using Gad and Weil moving average method.

3. Results

The body weight change following oral administration of ethanolic extract of *A. catechu* seed powder is shown in Table 1. There was no significant difference in the percent body weight in any of the groups on all the days as compared to the control group, though the significant difference was observed among the groups. The organ to body weight ratio of all the groups is also shown in Table 1. No significant difference was observed in any of the organs as compared to the control group.

The hematological variables following oral administration of ethanolic extract of *A. catechu* seed powder are shown in Table 2. There was a significant increase in the Hb level and also PCV as compared to the control ($P < 0.01$). A significant decrease in the PLT was observed at 3200 mg/kg dose as compared to the control.

No significant change was observed in the levels of glucose, cholesterol, bilirubin, and total protein in any of the groups when compared with the control [Figure 1]. Urea and creatinine levels were also not significant when compared with the control group [Figure 2]. However, the uric acid level was significantly lower in 3200 mg/kg dose when compared with the control ($P < 0.05$). The enzyme levels aspartate aminotransferase, alanine aminotransferase, ALP, gamma glutamyl transaminase, and lactic dehydrogenase did not show any significant difference when compared with the control [Figures 3 and 4].

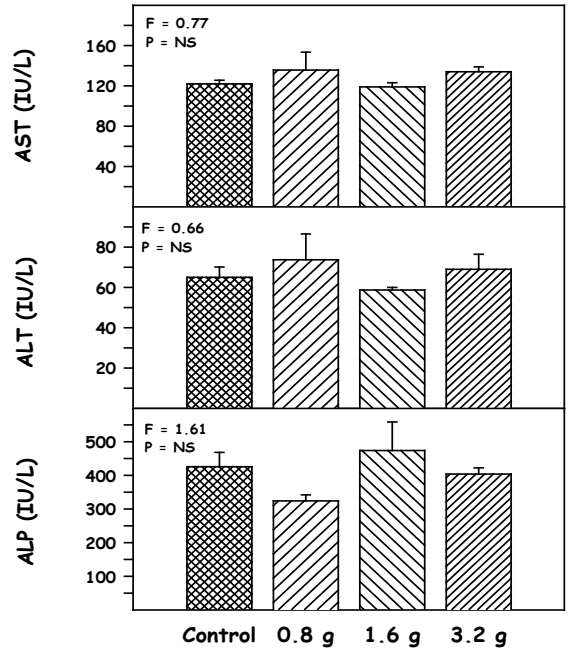


Figure 1. AST, ALT and ALP levels after oral administration of dry ethanolic extract of Acacia catechu seed. Values are mean + SE (n = 4), 14 days after p.o. administration.*Statistically significant from control by one way ANOVA with Dunett's test.

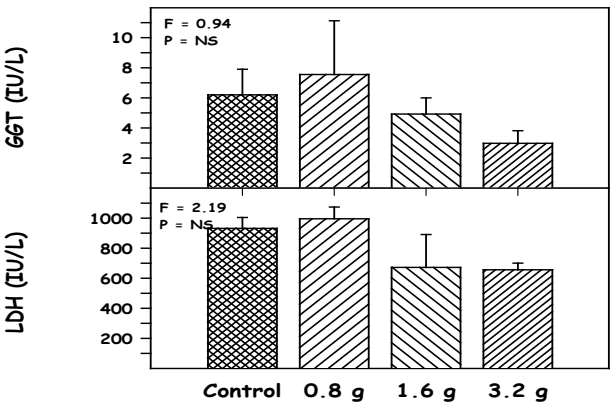


Figure 2. GGT and LDH levels after oral administration of dry ethanolic extract of Acacia catechu seed. Values are mean + SE (n = 4), 14 days after p.o. administration.*Statistically significant from control by one way ANOVA with Dunett's test.

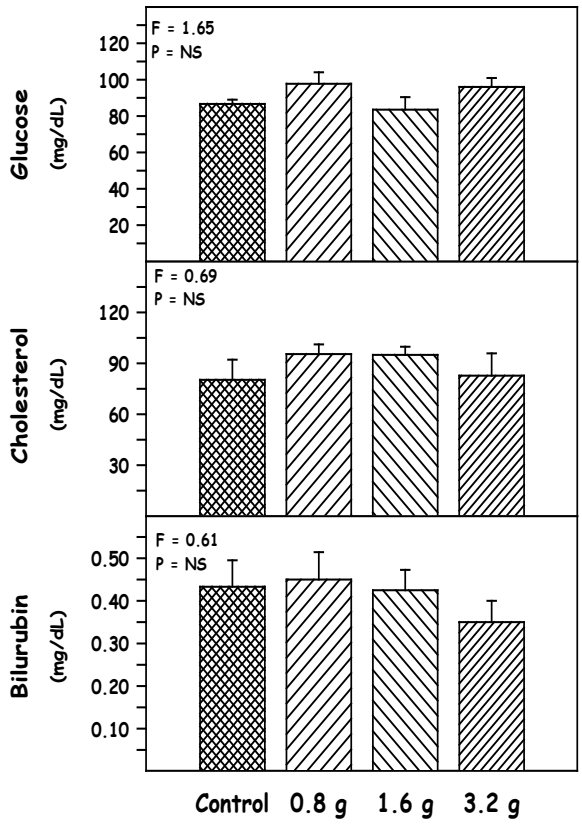


Figure 3. Glucose, cholesterol and bilurubin levels after oral administration of dry ethanolic extract of Acacia catechu seed. Values are mean + SE (n = 4), 14 days after p.o. administration.*Statistically significant from control by one way ANOVA with Dunett's test.

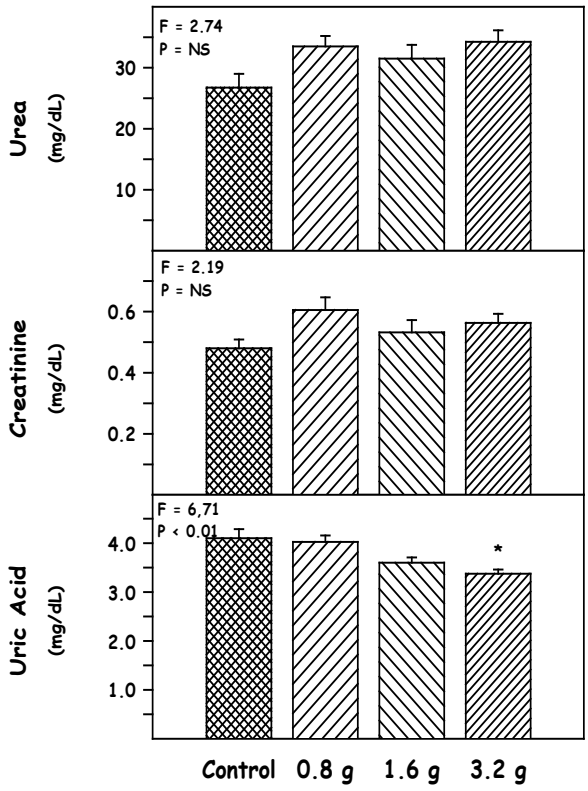


Figure 4. Urea, creatinine and uric acid levels after oral administration of dry ethanolic extract of Acacia catechu seed. Values are mean + SE (n = 4), 14 days after p.o. administration.*Statistically significant from control by one way ANOVA with Dunett's test.

Table 1. Body weight and Organ to body weight ratio after oral administration of dry ethanolic extract of *Acacia catechuseed*

Variables	Control	Acacia catechu dose (p.o) g/kg			F	P
		0.8	1.6	3.2		
% Body Weight 3 days	100 + 1	103 + 3	106 + 1	95 + 1	7.33	< 0.01
% Body Weight 7 days	103 + 3	107 + 3	111 + 1	106 + 1	2.18	NS
% Body Weight 14 days	110 + 3	106 + 3	110 + 1	103 + 1	2.32	NS
% Lung Weight	0.492 0.042	0.615 0.054	0.603 0.025	0.759 0.120	2.44	< 0.05
% Liver Weight	3.165 9.200	3.226 0.095	3.508 0.198	3.943 0.237	3.486	NS
% Kidney Weight	0.814 0.028	0.836 0.057	0.845 0.018	0.975 0.056	2.83	NS
% Heart Weight	0.320 0.024	0.364 0.018	0.335 0.011	0.372 0.017	1.86	NS
% Spleen Weight	0.227 0.010	0.234 0.016	0.286 0.040	0.299 0.020	2.24	NS

Values are mean ± SE (n = 4), 14 days after p.o. administration.

*Statistically significant from control by one way ANOVA with Dunett's test.

Table 2. Haematological variables after oral administration of dry ethanolic extract of *Acacia catechuseed.*

Variables	Control	Acacia catechu dose (p.o) g/kg			F	P
		0.8	1.6	3.2		
Haemoglobin (g/dL)	12.1+ 0.3	13.4* + 0.3	13.9* + 0.3	13.7* + 0.3	8.83	< 0.01
WBC X $10^3/cm^3$	11.1 + 0.5	8.8 + 1.6	8.0 + 0.5	7.8 + 1.2	2.15	NS
RBCX $10^6/cm^3$	4.1 + 0.1	4.4 + 0.1	4.7 + 0.2	4.6 + 0.1	3.19	NS
Platellets X $10^6/cm^3$	967 + 59	804 + 62	797 + 50	505* + 167	3.93	< 0.05
Packed Cell Volume (%)	37.3 + 0.6	42.6* + 0.9	43.7* + 1.3	42.8* + 0.8	9.59	< 0.01

Values are mean ± SE (n = 4), 14 days after p.o. administration.

*Statistically significant from control by one way ANOVA with Dunett's test.

4. Discussion

Natural products have a broad spectrum of therapeutic effect and are widely used in many disease conditions.[12-15] Based on the therapeutic application, the natural products are used for thousands of years.[16] However, these herbal products do not have a history of scientific development, and hence, information and data are required on the safety of herbal preparations.[17,18] Without undergoing toxicity testing and several herbal medicines are given for human consumption.[19] Recently, adverse effects of herbal medicines have been reported.[20,21]

Ephedrine which is used as a nasal decongestant and weight loss product has been reported to cause kidney stones when consumed.[22-24] Drug interactions associated with the use of few herbal drugs such as kava, ginseng, St. Johns wort have been reported. Spontaneous hyphema and bilateral subdural hematomas were associated with the consumption of ginkgo biloba.

Several plants have beneficial properties in which the leaf, stem, root, fruit, and seed have medicinal values. Traditional herbs such as Neem, *Aloe vera*, Palm, *Alibiza lebbek*, *Anacardium occidentale*, *Areca catechu*, *Averrhoa bilimbi*, *Bixa orellana*, *Bauhinia variegata*, *Cajanus indicus*, *Carica papaya*, *Cassia fistula*, *Hibiscus sabdariffa*, *Magnifera indica, Tamarindus indica, Andrographis paniculata, Bacopa monnieri* possess high nutritional and medicinal value.[25]

Several parts of *A. catechu* has medicinal value, namely the leaf, bark, and root.[26-30] The medicinal properties of the seed have not been reported. Several plant seeds have nutritional as medicinal value. However, there are few plants such as ricin from *Ricinus communis*,[31] abrin from *Abrus precatorius* seed, morin from Jatropha[32,33] in which the seeds are highly toxic due to the ribosomal inhibiting proteins.

Hence, this study was undertaken to evaluate the safety of the *A. catechu* seeds, and the ethanolic extract was chosen so that hydrophilic and lipophilic materials can be extracted. In the present study, the highest dose of 3200 mg/kg also did not show any mortality. Since the material was a crude ethanolic extract for range finding toxicity more than 2000 mg/kg was also selected. Presuming that all animals died in the next logarithmic dose of 6400 mg/kg the estimated LD_{50} will be more than 4500 mg/kg by the moving average method of Gad and Weil.[34]

All the acute doses studied did not show any significant change in the body weight, organ to body weight ratio, and general biochemical markers. Synthetic drugs and chemicals such as erythromycin, roxithromycin, azithromycin, and aminoglycoside antibiotics that inhibit protein synthesis and ciprofloxacin and other fluoroquinolones, nitroimidazole compounds like metronidazole that damage DNA are expected to significantly change these parameters. Natural products such as berberine, piperine, warfarin from coumarin inhibits DNA synthesis. Ellagitanin will bind to the protein.[35-38]

All the animals appeared healthy without any apparent symptoms suggesting that the compounds are well absorbed in the digestive system. Evaluation of various hematological parameters did not reveal any toxicity of the seed extract except a decrease in platelet count at 3200 mg/kg dose. Interestingly, in the present study, an increase in hemoglobin and PCV was observed in all the doses showing that the ethanolic extract of the seed may have some beneficial effect. Hepatotoxicity, cardiotoxicity, and cell damage are generally assessed by the estimation of enzyme levels, namely aspartate aminotransferase, alanine aminotransferase, ALP, gamma glutamyl transaminase, and lactic dehydrogenase. The above marker enzymes were evaluated in serum at the end

of 14 days for a high dose of *A. catechu* seed extract, and no change was observed showing that the seed extract has no delayed toxicity. Natural products are known to cause cell damaging effect for example *Fagonia cretica* induces DNA Damage, Cell Cycle Arrest, and Apoptosis in Breast Cancer Cells through FOXO3a and p53. Expression and hot water extracts of *Chlorella vulgaris* showed anti-proliferative and apoptotic effects on hepatoma cell line HepG2.[39,40]

Biomarkers of kidney damage such as urea and creatinine did not show any significant change. Natural products are known to cause nephrotoxicity. High oxalate contents in star fruit, triptolide from *Tripterygium wilfordii* hook, cranberry juice, Guaifenesin from *Guaiacum officinale* (stone formation), Peruvian cats claw, willow bark induce nephrotoxicity by induction of apoptosis.[41] Interestingly, in the present study, the uric acid level was lower than the control and at 3200 mg/kg it was statistically significant. A possible reason may be due to decreased catabolic effect in the system and whose significance will be difficult to predict in the acute dose study.

The present study shows that ethanolic extract of *A. catechu* Willd. the seed has very low mammalian toxicity. Since the highest dose of 3200 mg/kg was also tolerated by the animals, a dose of 1000 mg/kg can be given orally to identify various beneficial pharmacological actions of ethanolic extract of *A. catechu* seed.

5. Acknowledgements

The authors are thankful to Saveetha University for providing all the facilities for carrying out this study, Dr. Ezhilarasan Devaraj, Lecturer, Department of Pharmacology, Saveetha dental College and Hospitals, and Mr. R. Rajendran, Green Chem Herbal Extracts and Formulations, Bangalore, India are acknowledged for their support.

6. References

1. Singh KN, Lal B. Note on traditional uses of Khair (Acacia catechu Willd.) by inhabitants of shivalik range of western Himalaya. Ethnobotanical Leaf. 2006; 10:109–12.
2. Anonymous. The Wealth of India, Raw Material. New Delhi: CSIR. 2004; 1:11.
3. Qadry JS. Shah's and Qadry's Pharmacognosy. 12th ed. Ahmedabad: B.S Shah Prakashan. 2008; 302–3.
4. Lakshmi T, Magesh A, Rajendran R. Estimation of biomarker epicatechin in ethanolic bark extract of Acacia catechu Willd by HPLC method. J Pharm Sci Res. 2012; 4:1764–7.
5. Sharma P, Dayal R, Ayyer KS. Acylglucosterole from Acacia catechu. J Med Aromat Plants Sci. 1999; 21:1002–5.
6. Jain R, Patni V, Arora DK. Isolation and identification of flavonoid "Quercetin" from Acacia catechu (L.F.) Willd – A katha yielding plant. J Phytol Res. 2007; 20:43–5.
7. Lakshmi T, Rajendran R, Madhusudhanan N. Chromatographic fingerprint analysis of Acacia catechu Ethanolic leaf extract by HPTLC technique. Int J Drug Dev Res. 2012; 4:180–5.
8. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK et al. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry. 2003; 63:97–104.
9. Negi BS, Dave BP. In vitro antimicrobial activity of Acacia catechu and its phytochemical analysis. Indian J Microbiol. 2010; 50:369–74.
10. Ghate NB, Hazra B, Sarkar R, Mandal N. Heartwood extract of Acacia catechu induces apoptosis in human breast carcinoma by altering bax/bcl-2 ratio. Pharmacogn Mag. 2014; 10:27–33.
11. Singh KN, Mittal RK, Barthwal KC. Hypoglycaemic activity of Acacia catechu, Acacia suma, and Albizzia odoratissima seed diets in normal albino rats. Indian J Med Res. 1976; 64:754–7.
12. Leung PC. A practical way of research in Chinese medicine. Ann Acad Med Singapore. 2006; 35:770–2.
13. Bent S. Herbal medicine in the United States: Review of efficacy, safety, and regulation: Grand rounds at University of California, San Francisco Medical Center. J Gen Intern Med. 2008; 23:854–9.
14. Pratt DS, Kaplan MM. Evaluation of liver function. In: Longo DS, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J editors. Harrison's Principles of Internal Medicine. 18th ed. New York, USA: McGraw Hill Medical. 2012; 2527–31.
15. Harris RC, Neilson EG. Adaptation of the kidney to renal injury. In: Longo DS, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J editors. Harrison's Principles of Internal Medicine. 18th ed. New York, USA: McGraw Hill Medical. 2012; 2527–31.
16. Awang DV. Quality control and good manufacturing practices: Safety and efficacy of commercial herbals. Food Drug Law J. 1997; 52:341–4.
17. Blumenthal M, Busse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW et al. The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines. Austin, TX/Boston, MA, American Botanical Council/Integrative Medicine Communications. 1998.
18. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res. 2000; 33:179–89.
19. Shaw D. Risks or remedies? Safety aspects of herbal remedies. J R Soc Med. 1998; 91:294–6.

20. WHO. Annex II. Guidelines for the Assessment of Herbal Medicines (WHO Technical Report Series No. 863). Geneva: WHO. 1996.
21. Cupp MJ. Herbal remedies: Adverse effects and drug interactions. Am Fam Physician. 1999; 59:1239–45.
22. Rowin J, Lewis SL. Spontaneous bilateral subdural hematomas associated with chronic Ginkgo biloba ingestion. Neurology. 1996; 46:1775–6.
23. Rosenblatt M, Mindel J. Spontaneous hyphema associated with ingestion of Ginkgo biloba extract. N Engl J Med. 1997; 336:1108.
24. Powell T, Hsu FF, Turk J, Hruska K. Ma-huang strikes again: Ephedrine nephrolithiasis. Am J Kidney Dis. 1998; 32:153–9.
25. Seth SD, Sharma B. Medicinal plants in India. Indian J Med Res. 2004; 120:9–11.
26. Ray D, Sharatchandra KH, Thokchom IS. Antipyretic, antidiarrhoeal, hypoglycaemic and hepatoprotective activities of ethyl acetate extract of Acacia catechu Willd. in albino rats. Indian J Pharmacol. 2006; 38:408–13.
27. Rahmatullah M, Hossain M, Mahmud A, et al. Antihyperglycemic and antinociceptive activity evaluation of khoyer prepared from boiling the wood of Acacia Catechu in Water. Afr J Tradit Complement Altern Med. 2013; 10:1–5.
28. Alam G, Singh MP, Singh A, Patel R. Investigation of anthelmintic, anti-inflammatory activity of leaves extract of Acacia catechu Willd. J Pharm Res. 2012; 5:2587–9.
29. Alambayan J, Vats M, Sardana S, Sehrawat R. Evaluation of antiulcer activity of roots of Acacia catechu Willd. (Mimosoideae). J Pharmacogn Phytochem. 2015; 3:79–84.
30. Sham JS, Chiu KW, Pang PK. Hypotensive action of Acacia catechu. Planta Med. 1984; 50:177–80.
31. Final report on the safety assessment of Ricinus communis (Castor) seed oil, hydrogenated castor oil, glyceryl ricinoleate, glyceryl ricinoleate SE, ricinoleic acid, potassium ricinoleate, sodium ricinoleate, zinc ricinoleate, cetyl ricinoleate, ethyl ricinoleate, glycol ricinoleate, isopropyl ricinoleate, methyl ricinoleate, and octyldodecyl ricinoleate. Int J Toxicol. 2007; 26 Suppl 3:31–77.
32. Dickers KJ, Bradberry SM, Rice P, Griffiths GD, Vale JA. Abrin poisoning. Toxicol Rev. 2003; 22:137–42.
33. Kavitha C, Ramesh M, Kumaran SS, Lakshmi SA. Toxicity of Moringa oleifera seed extract on some hematological and biochemical profiles in a freshwater fish, Cyprinus carpio. Exp Toxicol Pathol. 2012; 64:681–7.
34. Gad SC, Weil CS. Statistics for toxicologists. In: Hayes AW editor. Principles and Methods of Toxicology. 2nd ed. New York: Raven Press. 1989; 463–7.
35. Bose PK. On some biochemical properties of natural coumarins. J Indian Chem Soc. 1958; 58:367–75.
36. Clark AM. Natural products as a resource for new drugs. Pharm Res. 1996; 13:1133–44.
37. Lewis WH, Elvin-Lewis MP. Medicinal plants as sources of new therapeutics. Ann Mo Bot Gard. 1995; 82:16–24.
38. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999; 12:564–82.
39. Lam M, Carmichael AR, Griffiths HR. An aqueous extract of Fagonia cretica induces DNA damage, cell cycle arrest and apoptosis in breast cancer cells via FOXO3a and p53 expression. PLoS One. 2012; 7:e40152.
40. Yusof YA, Saad SM, Makpol S, Shamaan NA, Ngah WZ. Hot water extract of Chlorella vulgaris induced DNA damage and apoptosis. Clinics (Sao Paulo). 2010; 65:1371–7.
41. Allard T, Wenner T, Greten HJ, Efferth T. Mechanisms of herb-induced nephrotoxicity. Curr Med Chem. 2013; 20:2812–9.