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Research Article

SUB-ACUTE TOXICITY OF A STEROIDAL GLYCOSIDE FROM THE FLOWERS OF *ALANGIUM SALVIIFOLIUM* WANG

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ABSTRACT

The current study was carried out to investigate the sub-acute toxicity of 3-O-β-D-glucopyranosyl-(24β)-ethylcholesta-5,22,25-triene, a steroidal glycoside isolated from the flowers of *Alangium salviifolium* Wang on Long Evan's rat. After intra-peritoneal administration of the compound at a dose of 300 µg/rat/day for 14 consecutive days, no mortality or significant changes in body weight or behavior were observed. The blood samples of the rats were examined for hematological and biochemical parameters which were statistically insignificant when compared to that of the control group. All the vital organs showed normal histopathological architecture (heart, lungs, liver and kidney) in comparison to the control group. This preliminary investigation demonstrate that the compound is safe at dose of 300 µg/rat/day for 14 consecutive days. But acute, sub-chronic and chronic toxicity evaluations as well as clinical trials need to be done.

KEYWORDS: *Alangium salviifolium*, steroidal glycoside, hematological parameters, biochemical parameters, histopathological study, sub-acute toxicity

INTRODUCTION

Medicines derived from plants always played a significant role in health care system all over the world and have been used in nearly all civilizations and cultures. The native mode of herbal treatment is one of the most dominant method of healing therapy and also part of the culture in many developing countries. As these therapies are the only available source in most cases with a considerable extent of effectiveness, they are socially accepted and are also economically cost effective[1,2]. In traditional preparations generally, a particular part of the plant (fruit, flower, leaves, root, stem bark, seeds or even whole plant) is used. In some cases, pure active constituent is formulated into a desirable preparation. To date most of the medicines used are of herbal origin and near about 25% prescription drugs contain at least one active ingredient derived from plant part[3,4]. Thus, modern scientific approaches have been used to study various medicinal plants for discovering new drugs. Due to the presence of various bioactive compounds, medicinal plants have a wide range of properties and can be used in the treatment of various kinds of ailments[5]. For the toxicity profile of medicinal plants and their extracts more experimental data is needed to increase human safety and their use in the development of pharmaceuticals[6]. Hence, the evaluation of potential toxicity of medicinal plants is a necessary step for the validation of their regular therapeutic use[7]. Thus, toxicological study is an obligatory experiment to establish the safety and efficacy of a new drug. The experiment is first conducted using animals like mice, rat, guinea pigs, dog, rabbit, monkey etc. under various condition of the drug. Clinical trial and as well as toxicological studies is mandatory before any drug is used clinically[8]. Toxicological studies are of three types depending on the period of time of drug exposure to animals (*viz.* acute, sub-acute and chronic toxicological studies)[9-11]. To ascertain the immediate toxic effect of a drug a single dose is given in large quantity, this type of toxicity study is known as acute toxicity study and in most cases is used to determine the median lethal dose (LD_{50}) of drugs. In case of sub-acute toxicity study, instead of single large dose, repeated doses of drug are given over a period of 14 to 21 days in a sub-lethal dose. This type of toxicity study is used to find out the histopathological changes and to determine the effect of the drug on biochemical and hematological parameters of blood of the test animals. Chronic toxicity study is used to establish the carcinogenic and mutagenic potential of drug. In this study, drug is given for a period of 90 days to over a year in different doses[12].

The plant genus, *Alangium* is a monogeneric plant of the family Alangiaceae consisting one genus with twenty two species, out of which *Alangium salviifolium* Wangerin (Synonym *A. lamarkii*) locally known as ankor or ankor kanta is a deciduous shrub or a small tree ranging from 3 feet to 12 feet in height[13,14]. It is the only species used medicinally in Bangladesh, India, China and Phillipines[15] and is native to Western Africa, Madagascar, Southern and Eastern Asia (China, Malaysia, Indonesia, India, and Philippines), tropical Australia, the western Pacific Ocean islands and New Caledonia[16] and distributed throughout India, Bangladesh and other parts of Southeast Asia[17]. The plant is also widely cultivated in India. Its dried leaves have traditionally been used to treat various ailments in Asia[18]. It is a popular folk medicine and has been studied for its antifertility[19], anti-inflammatory[20], antimicrobial[21], antioxidant[22], antitumor[23] and anti-ulceric[24] activities.

A steroidal glycoside, 3-O-β-D-glucopyranosyl-(24β)-ethylcholesta-5,22,25 -triene (**1**) was isolated from the flowers of this plant[25]. As part of our continuing studies on the isolated

compounds from the medicinal plants of Bangladesh specially the *Alangium* species, we previously reported the antibacterial activity of compound **1**[26]. But its toxicological evaluation has not been explored yet. Therefore, in the present investigation an effort was made to evaluate the sub-acute toxicities of compound **1** at a dose of 300 µg/rat/day on Long Evan's rat for consecutive14 days through intra-peritoneal injection.

MATERIALS AND METHODS

Isolation of Compound 1

The collected flowers (676 g) were air dried and pulverized. Extraction was done with ethanol (3.5 L) at room temperature, filtered and concentrated under reduced pressure to obtain a brownish mass (27.6 g). Solvent-solvent partitioning of the mother extract was done with petroleum ether, chloroform, ethyl acetate and finally methanol to yield 9.3 g, 10.7 g, 2.3 g and 4.1 g, respectively. Column chromatography of the chloroform soluble fraction and preparative thin layer chromatography of the column fraction yielded Compound **1** as an amourphous solid (47 mg). The structure was confirmed on the basis various spectral data and their comparison with published spectral data[27]. Compound **1** was kept in a refrigerator at 4 ℃ until further use and was dissolved in distilled water with the help of Tween-80 as co-solvent.

Experimental Animals and Their Collection

8 Long Evan's male rat[28] (6 months old) were used for this experiment. They were collected from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDRB, B)'s Animal Branch.

Maintenance of Rats

During the entire experiment all the rats were individually and hygienically housed in properly numbered iron cages under constant conditions of temperature, humidity and light[29]. The animals were provided with standard rodent pellet diet according to the criteria given by ICDDR, B. The test animals were well-kept in this condition for 15 days before the experiment to adjust with food and environment.

Grouping of Rats

The rats were individually weighed and the randomly grouped (four in each group) into two groups, control group (A) and experimental group (B). Average body weight of Group A and B was 103.75 ± 3.96 gm and 103.63 ± 3.86 gm, respectively. According to the current guidelines[30] for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals all the experiments were carried out in the morning.

Sample Administration

The test sample (Compound **1**) was dissolved in such a manner with distilled water and Tween-80 so that each 0.3 ml of the final solution contained 300 µg of the compound[8]. For 14 consecutive days daily each rat for control group A was injected with the vehicle only (0.3ml isotonic solution) and for the experimental group B each rat was injected with 0.3 ml sample solution (containing 300 µg compound). In both the groups A & B the route of administration was the intraperitoneal route.

Gross General Observation

The behavior of all the rats in both the groups A & B, along with their CNS excitation and depression, muscular weakness, reflexes, intake of food, salivation and diarrhoea were observed daily during the entire period of the experiment. The weight of the rats was also measured before administration of the test sample 1 and also before sacrificing the animals.

Investigation of Hematological Parameters

Blood was withdrawn from the tail veins of both groups A & B on the 15th day after the completion of the treatment and blood smears were made on glass slides followed by staining with Leisman reagent[31] to study the hematological parameters like Total count (TC) of RBC and WBC, Differential count (DC) of WBC, hemoglobin percentage and platelet count. Capillary tube was used to withdraw blood from each rat for estimating the hemoglobin percentage by Van Kampen-Ziftra's method[31].

Analysis of Biochemical Parameters

After 14 days of sample application to the rats of both groups A & B, blood samples were collected from the throat veins while they were being sacrificed by decapitation[32]. Then SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase), SALP (Serum Alkaline Phosphatase), urea, serum bilirubin and serum creatinine were determined using the methodology and chemicals as described in Englehringer Mannheim GmbH Diagnostica[33,34].

Histopathological studies

The tissue samples of the liver, kidney, heart and lungs were collected separately, sliced into pieces and fixed with 10% buffered formalin for twenty-four hours. The formalin fixed organ samples were then stained with hematoxylin-eosin reagent and mounted with diphenyl xylene to analyse the histopathological architecture under photo microscope at the Department of Pathology, Rajshahi Medical College, Bangladesh.

Statistical Analysis

Four replicates of each sample were used for statistical analysis and the values were reported as mean±SD.

RESULTS AND DISCUSSION

Toxicological evaluation of the steroidal glycoside, 3-O-β-D-glucopyranosyl- (24β)-ethylcholesta-5,22,25-triene (compound **1**) (Figure-1) was carried out by sub-acute toxicity study for 14 days. After 14 days all the rats of both the groups showed no signs of paroxysm, quivering, muscular abnormalities or reflex abnormalities. Also, salivation and food intake were the same with no sign of diarrhoea.

Changes in body weight and relative weight vital organs are indicators of the effect of an administered substance[35]. 14 days of treatment with compound **1** isolated from the flowers of *A. salviifolium*, has shown no remarkable difference in body weight of the rats compared to the control (Table 1).

The evaluation of the hematological parameters is very important in the determination of the anomalies induced by a plant extract[36]. After intraperitoneal administration of compound **1** the hematological profiles of the experimental and control group rats were determined before treatment, at 7th day of treatment and on the 14th day prior to sacrificing of the animals and compared to check the hematological disorders. Very insignificant change in the values of RBC count, WBC count, platelet count, differential WBC count, ESR and haemoglobin percentage of experimental rats were observed when compared to that of control group rats (Table 2). The differences obtained do not show a hematological change, since they are in the normal range of health for this animal species[37].

The study of biochemical parameters are indicators of toxicity, raising the effectiveness or the installation of a toxicity on the vital organs. In this study, parameters like SGOT, SGPT, SALP, urea, serum bilirubin and serum creatinine were studied (Table 3). It was found that most of the parameters were slightly increased

with respect to control animals but remained within normal range. From the Table 3, it was found that in every instance of calculated $|t|$ value was smaller than the $|t|$ value at 5% level of significance. This indicates that the changes are also statistically insignificant in this species[37]. Thus, the results showed that the compound **1** have no adverse effects on liver and kidney functions.

After 14 days of drug treatment and observation, the animals of both control and experimental groups were sacrificed and the organs such as heart, liver, lungs, and kidney were isolated and histopathological architecture was examined under a photo microscope. No detectable abnormalities (Figure 2) were observed which indicates that the compound **1** have no effect on cellular structures, i.e. they do not cause degeneration of the cells of these organs.

Table 1: Effect of compound 1 on body weight after intraperitoneal administration

Group	Body weight(gm) Before drug administration	Body weight(gm) After drug administration	% of change	Calculated "t" value	"t" value at 5% significance
A	103.75± 3.96	109.75±3.56	+5.78	+2.26	2.447
B	103.63±3.86	108.88±4.33	+5.37	+1.81	2.447

Table 2: Hematological profile of compound 1 on rats

Hematological parameters		Group A (Control)			Group B (Experimental)		
		Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
RBC (million/mm^3)		4.80 ±0.07	5.25 ±0.11	5.07 ±0.08	4.85 ±0.41	4.95 ±0.22	5.13 ±0.38
WBC (thousand/mm^3)		7.23 ±0.13	7.15 ±0.13	7.33 ±0.13	7.35 ±0.26	7.43 ±0.32	7.48 ±0.13
Differential WBC Count in %	Neutrophil	42.50 ±2.06	44.25 ±1.64	42.63 ±2.00	41.50 ±2.27	42.25 ±2.95	42.00 ±2.73
	Lymphocyte	51.00 ±2.35	51.25 ±0.83	52.25 ±1.79	51.25 ±1.26	52.75 ±2.50	51.25 ±2.22
	Monocyte	5.00 ±1.58	3.25 ±1.29	3.25 ±0.83	5.00 ±0.70	4.00 ±1.58	3.25 ±1.08
	Eosinophil	1.50 ±0.5	1.25 ±0.43	1.75 ±0.43	2.00 ±0.70	2.00 ±0.70	1.50 ±0.50
Platelet (no/mm^3)		346250 ±6496.0	345000 ±3535.5	347500 ±5590.2	346250 ±4145.8	346250 ±4145.8	346250 ±4145.8
Hemoglobin (%)		13.65 ±0.25	13.78 ±0.11	13.32 ±2.21	13.63 ±0.18	13.70 ±0.11	13.33 ±0.18

Table 3: Effect of compound 1 on biochemical parameters on rats

Biochemical parameters	Group A (Control)	Group B (Experimental)	% of change	Calculated "t" value	"t" value at 5% significance
SGOT IU/L	10.825 ±0.72	11.75±1.08	8.50	0.65	2.447
SGPT IU/L	8.50 ±0.50	9.25±0.83	8.82	1.55	2.447
SALP IU/L	44.25 ±2.86	45.00±1.87	1.69	0.44	2.447
Urea mg/dl	18.25 ±0.83	19.25±1.47	5.48	1.18	2.447
Serum creatinine mg/dl	0.85 ±0.09	0.875±0.09	2.94	0.197	2.447
Serum bilirubin mg/dl	0.28 ±0.01	0.2975±0.03	6.25	0.978	2.447

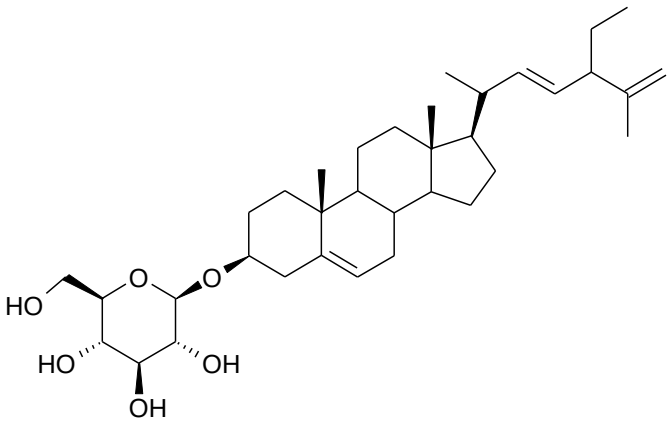
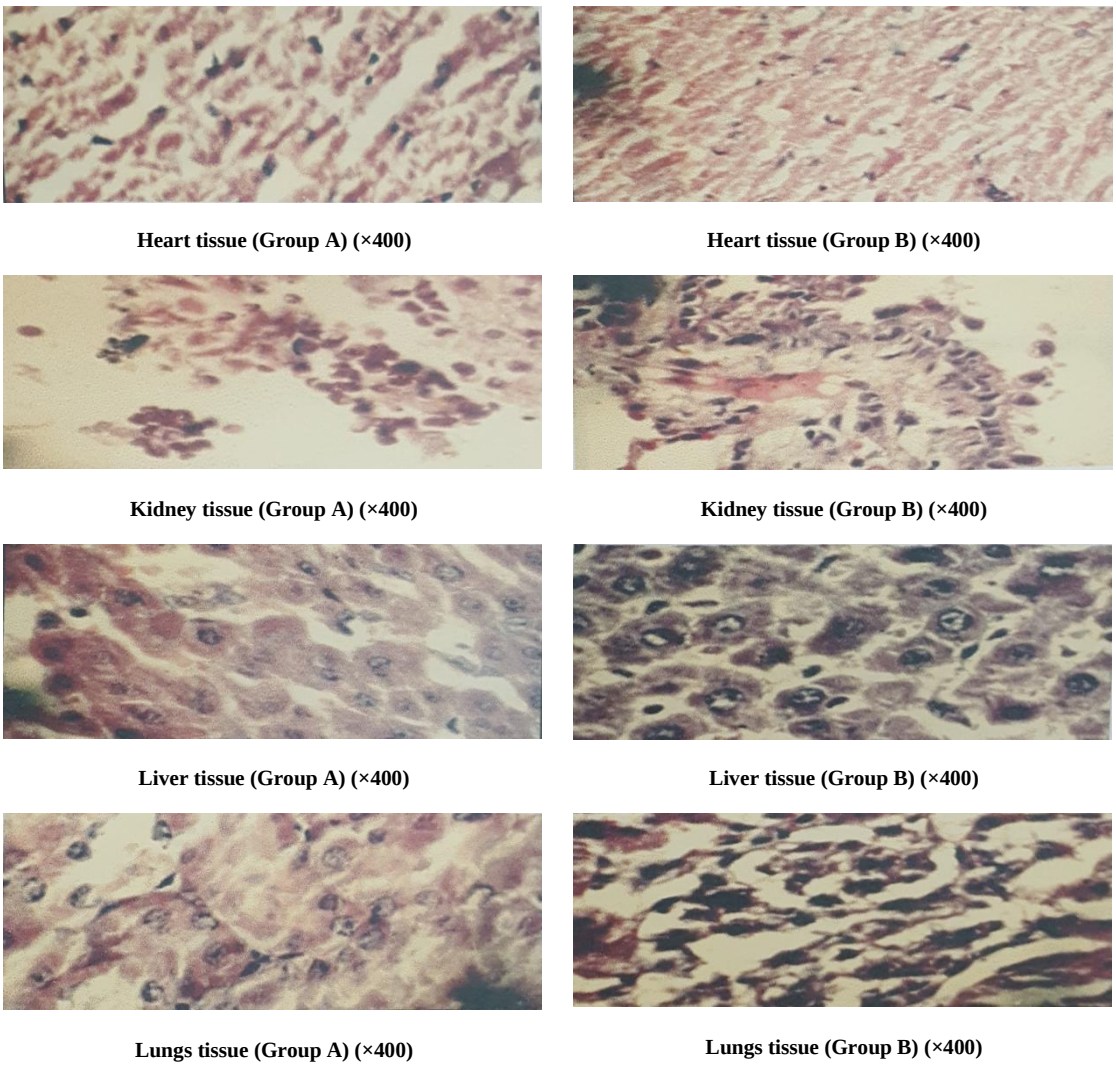


Figure 1: Structure of compound 1



Heart tissue (Group A) (×400) **Heart tissue (Group B) (×400)**

Kidney tissue (Group A) (×400) **Kidney tissue (Group B) (×400)**

Liver tissue (Group A) (×400) **Liver tissue (Group B) (×400)**

Lungs tissue (Group A) (×400) **Lungs tissue (Group B) (×400)**

Figure 2: Histopathological studies of the vital organs with compound 1 at dose of 300µg/rat/day for 14 consecutive days

CONCLUSION

The results of the sub-acute toxicity studies have shown no abnormalities on body weight, hematological and biochemical parameters of blood and on histopathological slides. The biological effect of compound **1** (3-O-β-D-glucopyranosyl-(24β)-ethyl cholesta-5,22,25-triene) and its present toxicological studies suggest that it can be safely subjected to acute, sub-chronic and chronic toxicological studies and also clinical trials.

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REFERENCES

1. World Health Organization. Traditional medicine, African Health Monitor. World Health Organization: Regional Office for Africa 2003; 4:1.
2. Argw TM. Approaches towards evaluation of medicinal plants prior to clinical trials. The Foundation for Medical Research 2006; 143.
3. Taylor JLS, Rabe T, McGaw LJ, J¨ager AK, Van Staden J. Towards the scientific validation of traditional medicinal plants. Plant Growth Regulation 2001; 34: 23-37.
4. Bashar S, Omar S. Greco-Arab and Islamic Herbal Medicine: Traditional System, Ethics, Safety, Efficacy, and Regulatory Issues. Wiley-Blackwell John Wiley & Sons Inc., Hoboken, NJ, USA, 2011; 303-339.
5. Piemel CA, Penlap VN, Nkegoum B, Taziebou1 PCL, Tekwu EM, Etoa FX, Ngongang J. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L.) Roxb (Ceasalpiniaceae). African Journal of Biotechnology 2006; 5(3):283-289.
6. Yuet KP, Darah I, Chen Y, Sreeramanan S, Sasidharan S. Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. Biomed Research International 2013; 2013:182064.
7. Kabbaoui ME, Chda A, El-Akhal J, Azdad O, Mejrhit N, Aarab L, *et al*. Acute and subchronic toxicity studies of the aqueous extract from leaves of *Cistus ladaniferus* L. in mice and rats. Journal of Ethnopharmacology 2017. 209:147-156.
8. Alam AHMK, Islam R, Salam KA, Manir MM, Baki MA, Hossain MA, Sadik G. Toxicological studies of N-transferloyl-4methyldopamine isolated from *Achranthes*

ferruginea. Pakistan Journal of Biological Sciences 2006. 9: 1052-1055.
9. Klaassen CD. Principle of Toxicology and Treatment of Poisoning. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th edition McGraw-th Hill Companies, United States, America; 1996; 63-74.
10. Katzung BG. Basic and Clinical Pharmacology. 6th edition Prentice-Hall International In., California, 1994; 879-881.
11. Khan A. Phytochemical and Pharmacological investigations on roots of Laportea crenulata Gaud and Rhizome of *Drynaria quercifolia* J. Smith. Master of Pharmacy Thesis Dissertation, Rajshahi University, Bangladesh 2004. 72.
12. Baki MA, Khan A, Al-Bari MAA, Mosaddik A, Sadik G, Mondal KAMSH. Sub-Acute Toxicological Studies of Pongamol Isolated from *Pongamia pinnata.* Research Journal of Medicine and Medical Sciences 2007; 2(2): 53-57.
13. Hooker JD. The Flora of British India. L. Reeve & Co., London; 1875; 741.
14. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd edition Lalit Mohan Basu India; 1994;1236.
15. Jubie SN, Jawahar R, Koshy B, Gowramma V, Murugan B, Suresh, B. Anti-arthritic activity of bark extracts of *Alangium salvifolium* Wang. Rasayan Journal of Chemistry 2008; 1: 433-436.
16. Balakrishnan N, Kumar S, Balasubramaniam A, Sangameswaran B, Chaurey M. Antiepileptic Activity of *Alangium salvifolium* Leaf Extracts. Herbal Tech Industry 2010; 20-23.
17. Yusuf M, Chowdhury JU, Wahab MA, Begum J. Medicinal Plants of Bangladesh. BCSIR Laboratories, Chittagong, Bangladesh 1994; 252.
18. Jain A, Katewa SS, Galav PK, Sharma P. Medicinal plant diversity of Sitamata wildlife sanctuary Rajasthan, India. Journal of Ethnopharmacology 2005; 102(2): 143-157.
19. Murugan V, Shareef H, Ramasharma GVS, Ramanathan M, Suresh B. Anti-fertility activity of the stem bark of *Alangium salvifolium* (Linn.F) Wang in Wistar female rats. Indian Journal of Pharmacology 2000; 32(6): 388-389.
20. Porchezhian E, Ansari SH, Ahmad S. Analgesic and Anti-Inflammatory effects of *Alangium salvifolium*. Pharmaceutical Biology 2001; 39(1): 65-66.
21. Pandian MR, Banu GS, Kumar G. A study of the antimicrobial activity of *Alangium salvifolium*. Indian Journal of Pharmacology. 2006; 38(3): 203-204.
22. Jain VC, Patel NM, Shah DP, Patel PK, Joshi BH. Antioxidant and antimicrobial activities of *Alangium salvifolium* (L.F) Wang Root. Global Journal of Pharmacology 2010; 4(1): 13-18.
23. Nahar L, Ronok Z, Ashik M, Islam S, Haque A, Fazal A, Jesmin M. Antioxidant and antitumor activity of chloroform extract of *Alangium salvifolium* flowers. Phytopharmacology 2012; 2(1): 123-134.
24. Subudhi SK, Prusty KB, Panda PK. Phytochemical and Antiulcer activity of petroleum ether and chloroform extracts of leaves of *Alangium salvifolium* Linn. (Family-Alangiaceae). International Journal of Pharmaceutical Research and BioScience 2012; 1(2): 102-114.
25. Anjum A, Haque ME, M. Rahman MM, Rahman MM, Sarker SD. Alkaloids and steroid from the flowers of *Alangium salviifolium.* Dhaka University Journal of Pharmaceutical Sciences 2003; 2(2): 97-101.
26. Anjum A, Haque ME, Rahman MM, Sarker SD. Antibacterial compounds from the flowers of *Alangium salviifolium* (Alangiaceae). Fitoterapia 2002; 73: 526-528.
27. Leitao SG, Kaplan MAC, Monache FD, Akihisa T, Tamura T. Sterols and sterol glucosides from two *Aegiphilia* species. Phytochemistry 1992; 31: 2813-2818.
28. Ramasamy A. Could anyone explain me what is the criteria to select the gender of the species in In vivo experiments? 2003 Dec 3. Retrieved from: https://www.researchgate.net/post/Could-anyone-explain-me-what-is-the-criteria-to-select-the-gender-of-the-species-in-In-vivo-experiments/529d7a18d3df3e57208b456f/citation/download.
29. Khanna N, Ray A, Alkondon M, Sen P. Effect of â-adrenoceptor antagonists and some related drugs on maximal electroshock seizures in mice. Indian Journal of Experimental Biology 1989; 27: 128-130.
30. Zimmerman M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16:109-110.
31. Ghai CL. A Textbook of Practical Physiology. 6th edition Jaypee Brothers Medical Publishers, New Delhi 2004; 130-175.
32. Naz T, Mosaddik A, Haque ME. Toxicological evaluation of a monoterpene ester, bornyl piperate from *Piper chaba* root. International Research Journal of Pharmacy 2014; 5:12.
33. Reitman S., Frankel, FD. A calorimetric method for the determination of serum glutamate-oxalate and pyruvate transaminases. American Journal of Clinial Pathology 1957; 28: 56-63.
34. Fawcett JK, Scott JF. A new semimicro method for determination of urea. Journal of Clinical Pathology 1960; 13: 156-159.
35. Berenguer-Rivas CA, Castillo AA, Martínez HS, Zapata EP, Hernández JB, Tassé YM. Acute oral toxicity of *Azadirachta indica* (Neem Tree). Revista Cubana de Plantas Medicinales 2013; 18:502-507
36. Khan SA, Epstein JH, Olival KJ, Hassan MM, Hossain MB, Rahman KBMA, *et al*. Hematology and serum chemistry reference values of stray dogs in Bangladesh. Open Veterinary Journal 2011; 1:13-20.
37. Giknis MLA, Clifford CB. Clinical laboratory parameters for Crl:CD (SD) rats Charles River Laboratories, Wilmington, MA, USA 2008; 1-17.

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