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INVESTIGANDO OS EFEITOS DO EXTRATO AQUOSO DE CASCA DE SALGUEIRO (*SALICACEAE*) EM PARÂMETROS URINÁRIOS, PESO RELATIVO, FÍGADO E ÍNDICES DE FUNÇÃO RENAL EM RATOS

INVESTIGATING THE EFFECTS OF AQUEOUS EXTRACT OF WILLOW BARK (*SALICACEAE*) ON URINARY PARAMETERS, RELATIVE WEIGHT, LIVER AND RENAL FUNCTION INDICES IN RAT

بررسي اثرات عصاره آبي پوست بيد (*Salicaceae*) بر پارامترهاي ادراري، وزن نسبي، شاخص هاي عملكرد كبدي و عملكرد كليه اي در موش صحرايي

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RESUMO

A decocção da casca do caule de Salix alba (EASA) é tradicionalmente usada no Irã contra dores de cabeça, lombalgia e para tratar pilhas. O presente trabalho foi realizado para avaliar a segurança da decocção do extrato de casca de caule de Salix alba após administração oral aguda e subcrônica em roedores machos. Para o estudo agudo, uma única administração de (EASA) foi dada oralmente a ratos machos em doses de 2, 4, 8, 12,16 e 20 g/kg. No estudo de toxicidade subcrônica, o extrato de casca de Salix alba foi administrado por via oral em uma única dose a ratos Wistar machos nas doses de 200, 400, 800 e 1600 mg/kg/dia por 28 dias. O peso corporal dos animais foi registrado durante todo o período experimental, enquanto os parâmetros hematológicos e bioquímicos de sangue e urina, bem como o peso dos órgãos, foram avaliados. O peso relativo dos órgãos foi significativamente afetado pelo tratamento nas doses de 800 e 1600 mg/kg entre o fígado e o rim que estavam relacionados com a dose. Alterações hematológicas foram observadas nas doses 400, 800 e 1600 mg/kg nos parâmetros de RBC, Hb, HCT, PLT e WBC. AST sérica, ALT, ALP e bilirrubina total aumentaram significativamente enquanto a proteína total e triglicerídeos diminuíram significativamente. O ensaio de índices de função renal no sangue mostrou uma modificação significativa nos grupos 800 e 1600 mg/kg nos parâmetros de creatinina, ácido úrico e potássio em comparação ao controle, enquanto na urina, proteína e cálcio aumentaram marcadamente nas doses de 1600 mg/kg. Estes resultados mostraram que há uma ampla margem de segurança para o uso terapêutico da EASA e que o extrato aquoso da casca do caule de Salix alba não teve toxicidade na administração oral aguda de dose alta e toxicidade muito baixa na administração subcrônica oral.

Palavras-chave: *Salix alba, casca de caule, extrato aquoso, toxicidade aguda e subcrônica.*

ABSTRACT

The decoction of the stem bark of *Salix alba* (EASA) is traditionally used in Iran against headache, low back pain and to treat piles. The present investigation was carried out to evaluate the safety of decoction of *Salix alba* stem bark extract after acute and sub-chronic oral administration in male rodents. For the acute study, a single administration of (EASA) was given orally to male mice at doses of 2 ,4, 8, 12,16 and 20 g/kg. In the sub-chronic toxicity study, *Salix alba* bark extract was administered orally as a single administration to male Wistar rats at doses of 200, 400, 800 and 1600 mg/kg/day for 28 days. Animal body weight was recorded throughout the experimental period while hematological and biochemical parameters of blood and urine, as well as the weight of organs, were evaluated. The relative weight of organs was affected significantly by the treatment at the doses of 800 and 1600 mg/kg between liver and kidney that was dose-related. Hematological changes were observed at the doses 400, 800 and 1600 mg/kg at the parameters of RBC, Hgb, HCT, PLT, and WBC. Serum AST, ALT, ALP, and total bilirubin increased significantly while total protein and triglycerides

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significantly decreased. Renal function indices assay in blood showed a significant modification in the 800 and 1600 mg/kg groups at the parameters of creatinine, uric acid and potassium compared to control while, in urine, protein, and calcium markedly increased at the doses of 1600 mg/kg. These results showed that there is a wide margin of safety for the therapeutic use of EASA and that the aqueous stem bark extract of *Salix alba* had no toxicity in oral acute high dose administration and very low toxicity in oral sub-chronic administration.

Keywords: *Salix alba, Stem bark, Aqueous extract, Acute, and sub-chronic toxicity.*

چکیده

عصاره پوست ساقه Salix alba (EASA)به طور سنتي در ايران براي مقابله با سردرد، كمردرد و سم زدايي استفاده مي شود. پژوهش حاضر به منظور بررسي ايمني جوشانده از جنس Salix alba و ساقه عصاره پوست درخت بيد پس از تجويز خوراكي حاد و تحت مزمن در جوندگان نر بالغ انجام شد. براي مطالعه حاد، يک بار عصاره (EASAبه صورت خوراكي به موشهاي نر در دوزهاي 2، 4،8، 12،16 و 20 گرم در كيلوگرم داده شد. در مطالعه سميت مزمن، عصاره پوست Salix alba به صورت خوراكي به صورت يک بار در موشهاي صحرايي نر ويستار در دوزهاي 200، 400، 800 و 1600 ميلي گرم بر كيلوگرم در روز به مدت 28 روز تزريق شد. وزن حيوانات در طي دوره آزمايشي ثبت شد، در حاليكه پارامترهاي خونشناسي و بيوشيميايي خون و ادرار و نيز وزن اندام مورد ارزيابي قرار گرفت. وزن نسبي اندام به ميزان قابل توجهي باتيمار در دوزهاي 800 و 1600 ميلي گرم در كيلوگرم بين كبد و كليه متاثر شد. تغييرات هماتولوژي در دوزهاي 400، 800 و 1600 ميلي گرم در كيلوگرم در پارامترهاي RBC، Hgb، HCT، PLT و WBC مشاهده شد. AST سرم، ALT، ALP، و بيلي روبين تام بطور قابل توجهي افزايش، در حالي كه پروتئين تام و تري گليسريد به طور قابل توجهي كاهش يافته است. آزمايشات شاخص عملكردي كليه در خون تغييرات قابل توجهي در گروه هاي 800 و 1600 ميلي گرم در كيلوگرم را در پارامترهاي كراتينين، اسيد اوريک و پتاسيم در مقايسه با كنترل نشان داد، در حالي كه در ادرار، پروتئين و كلسيم به ميزان قابل توجهي در دوزهاي 1600 ميلي گرم / كيلوگرم افزايش يافت. اين نتايج نشان داد كه دامنه گسترده اي براي استفاده درماني از EASA وجود دارد و عصاره پوست ساقه از جنس Salix alba بدون سميت حاد در دز بالاي دهاني بود و سميت بسيار پايين در خوراندن تحت مزمن دهاني داشتند.

كلمات كليدی: ساليكس آلبا، پوست ساقه، عصاره آبي، سميت حاد و تحت مزمن.

1. INTRODUCTION

The history of plants being used for the medicinal purpose is probably as old as the history of mankind [1]. Medicinal plants are popular remedies used by a vast majority of the world's population. In fact, the efficacy of medicinal plant in the management of diseases is nowadays indubitable, and the World Health Organisation has even recognized its use in primary health care delivery system [2]. Willow bark (Salix species) has been used all over the world as an anti-inflammatory and pain-relieving. Its active components, salicin, and its derivates were widely used by 19th-century medical practitioners to treat rheumatic fever, different kinds of pain, back pain, toothache, and headache [3]. Salicylates (salicylic acid and acetylsalicylic acid (ASA, Aspirin) are a class of compounds which have been used throughout the world for centuries as an analgesic, antipyretic, and anti-inflammatory drugs [4]. Their anti-inflammatory function is thought to arise through the inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) leading to the inhibition of prostaglandin (PG) synthesis [5]. In recent years there has been increasing consumer demand for health-related food products which has led to the development of novel functional beverages [6]. White willow (Salix alba) is a medicinal plant indigenous to Europe. It has been traditionally used to treat various ailments due to its antipyretic analgesic and anti-inflammatory properties [7]. However, it was suggested that the pharmacological activity of willow bark could depend on the presence of other compounds as well [8]. The use of complementary traditional medicine which includes herbal medicines to the treatment of various diseases in both developed and developing countries [9]. Ethnobotanically, rheumatic pains, affecting mainly the elderly, can be relieved by a decoction or infusion of *Salix alba* bark [10]. The effect of *Salix alba* leaves, along with clove bud and Nigella, in the treatment of common wart has been reported [11]. Tanacetum parthenium and *Salix alba* combination in migraine prophylaxis have already been reported [12]. The increasing use of herbs, therefore, makes it pertinent that pre-clinical toxicological studies be carried out on these natural products. Very few investigations have been conducted on the tree *Salix alba* in the scientific literature about pariparoba extract toxicity. Therefore, the purpose of this study was to screen the plant *Salix alba* for its oral toxicity

after acute and sub-chronic oral administration in male mice and rats, respectively. By using the aqueous extract, the traditional decoction dosage form of *Salix alba* stem bark could be mimicked.

2. MATERIALS AND METHODS

2.1 Animals and housing condition

All male rats and mice were obtained from the animal house of the laboratory of the pharmacology of the Pastor's Institute in Tehran. The acute toxicity test was carried out on three months old mice of male sex weighing between 18 and 30 g and Wistar rats of male sex aged 6 weeks and weighing 80–130 g at the beginning of the experiment were used for the subchronic toxicity assessment. The animals were allowed to acclimatize for1week before initiation of the experiments. Rats and mice were segregated according to types and were kept in plastic cages (five per cage) under standardized animal house conditions (temperature: 23–30 °C, photoperiod: approximately 12 h natural light per day, relative humidity: 50–55% with frequent air change) with free access to pelleted food and tap water. This study was conducted in accordance with Good Laboratory Practices (GLP) as defined in 40 CFR 792: US EPA Good Laboratory Practice Standards: TSCA; 21 CFR 58: US FDA and with Health Effects Test Guidelines, OPPTS 870.1100 (1998).

2.2 Plant material and preparation of the aqueous extract

Salix alba stem bark was collected from a mature tree in Hamedan (West province of Iran) in September 2011 and was identified by Dr. Mansour Ranjbar, University of Hamedan. A voucher specimen (33037) is deposited at the Herbarium of the Department of Botany, University of Bu-Ali Sina, Iran. The pieces of stem bark were washed under running water, shade dried and cut into small pieces and ground into a fine powder. Five hundred grams of the obtained dry powder was macerated in 3 L of distilled water in a round bottom flask and boiled at100 °C for 45 min. The brownish red solution was filtered, and after filtration, the water was evaporated in an oven at a temperature of 40 °C for 24 h to a constant weight of 25 g giving a 5% yield. This extract was stored at –2 °C until used and was dissolved in saline (NaCl 0.9%) upon administration [2].

2.3 Acute toxicity test in mice

For the acute toxicity study Mice were randomly assigned to each of seven groups of 5 male mice. The animals were fasted overnight (12 h) prior to conducting the experiment and treated as follow: the first group (control group) was treated with distilled water while the remaining groups (2–7) received orally in a single administration, EASA at the doses of 2, 4, 8, 12, 16 and 20 g/kg respectively. Animals were monitored continuously for general behavioral changes for 24 h after the treatment and mortality for a period of 14 days post-treatment [26].

2.4 Sub-chronic oral toxicity study in rats

A 28-day subchronic toxicity study of EASA on rats was carried out. Healthy Wistar rats were randomly divided into 5 groups(I–V) of 5 male sex, and their weights were recorded prior to administration. The extract, dissolved in saline and control rats (group I) received distilled water, while groups II–V received EASA at the dose of 200, 400, 800 and 1600 mg/kg respectively. The doses were chosen from literature, and the results of the acute study in mice and human dose based on the effective doses applied mainly on the hepatoprotective activity[27]. All treatments were given by oral route once a day for 28 days. All animals were observed daily prior to and following administration for mortality and clinical signs of toxicity. Individual body weight was measured at the initiation of treatment and once a week during the treatment period thereafter. On the last day of treatment, animals were placed in individual metabolic cages for 24 h, and excreted urine was collected and then kept at –20 °C for ion and biochemical analysis. At the end of the treatment, animals fasted overnight but allowed free access to water. They were then anesthetized with chloroform and blood collected with and without anticoagulant (ethylenediamine tetraacetate) by retro-orbital puncture [28]. After blood collection, rats were sacrificed by decapitation. Organ samples such as heart, liver, lung, kidney, and spleen were collected, blotted dry, and the relative organ weight (weight of organ as a proportion of the total body weight of each rat) was calculated and compared with the value of control [2].

2.5 Measurement of hematological and biochemical parameters in rats

Hematological parameters were

determined by pathobiology laboratory using an automatic hematological analyzer (Sysmex KX-21N). The parameters included: red blood cell (RBC) count, leukocyte (WBC) count, hemoglobin (Hgb), hematocrit (HCt), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet-large cell ratio (P-LCR), mean platelet volume (MPV), platelet distribution width (PDW), platelet count, lymphocyte, monocyte, and neutrophil counts [29]. For biochemical analysis, blood and urine were centrifuged at 3000rpm for 10 min. Serum was separated and stored at –20 °C until determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP) and albumin (ALB). Renal function indices assay in blood was assessed by determining the concentration of creatinine, urea, uric acid, Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+} and inorganic phosphorus. Urine was also assessed for Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, creatinine, urea and protein. The assays were made using automatic biochemical analyzer (Selectra- E2 Germany).

2.6 Statistical analysis

Statistical calculations were carried out with the SPSS 19.0 for Windows software package (Statistica). Comparisons among different groups were performed by analysis of variance (ANOVA). A significant difference between control and experimental groups were assessed by Tukey's multiple comparison tests or Dunkan's using the software Microsoft office Excel 2007. All data are expressed as mean±standard error of the mean (SEM); P values less than 0.05 were considered to be significant.

3. RESULTS AND DISCUSSION

Oral administration of EASA at doses lower than 8 g/kg produced no apparent toxicity. However, from 8 g/kg EASA caused slow movement of animal, somnolence, decrease in aggressiveness, touch and pain sensibility but did not cause grossly negative behavioral changes such as excitement, respiratory distress, convulsions or coma. All the observed signs appeared at least 2 h post administration and disappeared depending on the dose of extract. No mortality was observed up to 14 days. The median lethal dose (LD_{50}) of the EASA was then greater than 20 g/kg body weight (Table 1). Control and animals treated with EASA presented a constant increase in body weight (Figure 1). The animal receiving EASA at the dose of 800 mg/kg showed decreased body weight at the third week of treatment, but there was not significantly different, as compared to control rats. Macroscopic analysis of target organs of treated animals (liver, heart, lung, kidney, and spleen) did not show significant changes in color and texture when compared with the control group. Throughout the experimental period, no deaths or visible clinical signs were found in any group. Blood samples were collected, and hematological parameters of both experimental and control rats were measured. Relative weights of liver and kidney were significantly affected by EASA treatment as there were significantly decreased at the doses of 800 and 1600 mg/kg in livers weight and at the dose of 800 mg/kg in kidneys weigh (Table 2). The results are presented in Table 3. Compared with the control rats, all hematological parameters measured (WBC, HGB, PLT, HcT, MCV, McH, McHc, PDW, MPV, P-LCR% lymphocytes, %neutrophils, %Monocyte), were not significantly different, except for a significant decrease in the RBC count in treated males (800 mg/kg/day) and of hemoglobin and % Hematocrit at the doses of 800 and 1600 mg/kg/day ($p < 0.01$), and an increase in the counts of platelets at the doses of 400 or 800 or 1600 mg/kg/day ($p < 0.01$), and of WBC count at the dose of 1600 mg/kg/day ($p < 0.01$) in treated males at the end of experiment. Subchronic administration of EASA did not cause any significant changes in albumin(ALB), total cholesterol (TC), low-density lipoprotein(LDL), high-density lipoprotein (HDL) at the end of the test. The values of the biochemical parameters in treated and control rats are shown in Table. 4. However, liver enzymes (AST, ALT, and ALP), total serum bilirubin significantly increase in a dose-depend manner. Also, there was a significant decrease in total protein (800 mg/kg) and triglyceride at doses 400 and 800 mg/kg. Blood concentration of creatinine, uric acid, and K^+ significantly decreased in EASA treated animals ($P < 0.05$) as compared to control (Table. **5**). As shown in Table 6, there were no significant changes in the daily urine excretion of creatinine, Na^+, K^+, Cl^-, Mg^{2+},and inorganic phosphorus while at the dose of 1600 mg/kg, the urine content of protein and Ca^{2+} significantly increased ($P < 0.001$). Complementary and alternative

medicines (CAMs) such as herbal remedies require an important and deep evaluation of their efficacies and safety due to their growing use all over the world [13]. The increasing use of CAM is due to a variety of factors, including limitations of current therapy and adverse effects of conventional drugs. Traditional herbal medicine is the most important part of CAM and has been practiced for thousands of years. Although there are many traditional herbal medicines available, and some have been verified by clinical trials, their efficacy and safety are still questioned by consumers [14]. *Salix alba* is a plant used worldwide in traditional medicine for the treatment of various ailments. The present work evaluates the acute and subchronic toxicity of the aqueous extract of its stem bark. In the acute toxicity study, no adverse effect was observed up to the dose 5 g/kg of EASA. Mice receiving this extract at the doses comprised between comprised between 8 g/kg and 20 g/kg showed hypoactivity marked by a reduction in Food consumption, locomotion, and aggressiveness. It can be thought that this extract possesses a depressive effect on the nervous system. Ancient civilizations used willow tree bark extracts to treat pain, inflammation, and musculoskeletal conditions [4]. This may justify the reduction in pain sensibility observed in EASA treated animals. All Animals treated with the EASA survived beyond the 14 days observation period. The median acute toxicity value (LD_{50}) of EASA is then up to 20 g/kg body weight. Materials that showLD_{50} higher than 5.0 g/kg by oral route can be considered almost non-toxic [15]. Hence, it can be suggested that EASA is vacuous of acute oral toxicity. This means that the EASA can be rapidly absorbed at the level of the gastrointestinal tract. The sub-chronic test showed that 28 days treatment of EASA did not produce any death or clinical signs of toxicity. There was a decrease in the relative body weight of rats treated by EASA at the doses of 800 mg/kg in the third week of the treatment while no significant difference was observed in all groups along the four weeks of treatment. Analysis of blood parameters is relevant to risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity when the data are translated from animal studies [16]. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals [17]. Increase in white blood cells indicates the strengthening of the organism defense [18]. Increase in total leucocyte count states that EASA contains biologically active principles that have the ability to advance the immune system by increasing the population of defensive white blood cells. In this study, a significant rise in total bilirubin was also observed, and it is obviously known that increase in bilirubin levels suggests an increase in hemolysis [19]. Thus, the increase in bilirubin level may indicate an elevated level of hemolysis. This may be easily understood and not indicating any toxic effect. Whereas, factors like (MCV, MCH, and MCHC have not changed significantly and just number of RBC count, hematocrit and hemoglobin decreased significantly, so the results explain anemia in animals, and the anemia is the type of normochromic normocyte [20,21]. The increased levels of AST and ALT in the blood are associated with damage of hepatic cells [22]. Also, have reported that enhancement in the level of serum proteins is an indication of tissue injury and reflection of hepatic toxicity [23]. Sub-chronic administration of EASA caused a significant decrease in the levels of total protein. These observations of a significant decrease in the levels of serum proteins may indicate that EASA has hepatoprotective effects and increase in AST, ALT and ALP could have resulted in an increase of administration of doses. Also, these results are in accordance with those obtained by Udem *et al*. [24, 2] when studying the acute and chronic toxicity of chloroform stem bark extract of *Erythrina senegalensis* in mice. Kidney functions were evaluated by means of serum urea, creatinine, and electrolytes in both blood and urine. Increase blood creatinine is a good indicator of a negative impact on kidney functions [25]. In the present study, blood creatinine, uric acid, and an electrolyte such as K^+ decrease significantly, while urinary excretion of electrolytes was not affected by EASA treatment except that there was a significant increase in protein and Ca^{2+} that this increase can be considered as a contrast of *Salix alba* extract against some partly adverse agents, and the only effect was anemia. However, the significant increase in liver enzymes as observed in this study need further investigations.

4. CONCLUSION

In conclusion, the present study showed that the aqueous extract from the stem bark of *Salix alba* might be considered as relatively safe of toxicity, as it did not cause any lethality nor produced any remarkable hematological, biochemical and structural adverse effects both in acute and sub-chronic toxicity studies in rodents.

5. REFERENCES

1. Islam M S , Zahan R, Nahar L, Alam M B, Naznin M, Sarkar G C, Mosaddik M A, Haque M Ekramul. Antibacterial, insecticidal and in vivo cytotoxicity activities of Salix tetrasperma. International Journal of Pharmaceutical Sciences and Research 2011; 2: 2103-2108.
2. Atsamo AD, Nguelefack TB, Datté JY, Kamanyi A. Acute and subchronic oral toxicity assessment of the aqueous extract from the stem bark of *Erythrina senegalensis* DC (Fabaceae) in rodents. Journal of Ethnopharmacology2011; 134: 697–702.
3. Hedner T, Everts B. The early clinical history of salicylates in rheumatology and pain. Clin Rheumatol 1998; 17:17–25.
4. Mahdi JG, Mahdi AJ, Bowen ID. The historical analysis of aspirin discovery, its relation to the willow tree and antiproliferative and anticancer potential. Cell Proliferation 2006; 39: 147–155.
5. Yu H G, Huang J A, Yang Y N, Huang H, Luo H S, Yu J P. The effects of acetylsalicylic acid on proliferation, apoptosis, and invasion of cyclooxygenase-2 negative colon cancer cells. European Journal of Clinical Investigation 2002; 32: 838–846.
6. Katan M B, De Roos N M. Promises and problems of functional foods. Critical Reviews in Food Science and Nutrition 2004;44: 369–377.
7. Bruneton J. Pharmacognosy, phytochemistry, medicinal plants. Paris: Lavoisier Press; 1995: 221–223.
8. Fiebich B L, Chrubasik S. Effects of an ethanolic Salix extract on the release of selected inflammatory mediators in vitro. phytomedicine, 2004;11: 135–138.
9. Salawu OA, Chindo BA, Tijani AY, Obidike IC, Salawu TA, and Akingbasote AJ. African Journal of Pharmacy and Pharmacology 2009;3: 621 – 626.
10. Leporatti ML, Impieri M. Ethnobotanical notes about some uses of medicinal plants in Alto Tirreno Cosentino area (Calabria, Southern Italy). J. Ethnobiol. Ethnomed 2007; 3: 34.
11. Rezaei K, Jebraeili R, Delfan B, Noorytajer M, Meshkat MH, and Maturianpour H. The effect of clove bud, Nigella and *Salix alba* on wart and comparison with conventional therapy. Eur. J. Sci. Res 2008; 21:444-450.
12. Shrivastava R, Pechadre JC, and John GW. Tanacetum parthenium and *Salix alba* (mig-RL®) combination in migraine prophylaxis: A prospective, open-label study. Clin. Drug Invest 2006; 26: 287-296.
13. Firenzuoli F, Gori L, Herbal medicine today: clinical and research issues. Evidence-Based Complement and Alternative Medicine 2007; 4: 37–40.
14. Cheng C W, Bian Z X, Wu T X. Systematic review of Chinese herbal medicine for functional constipation. World Journal Gastroenterology 2009;15:4886–4895.
15. Kennedy GL, Ferenz, Burgess BA. Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD50. Journal of Applied Toxicology 1986;6: 145–148.
16. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Spes G, Bracken W, Dorato M, Deun KV, Smith P, Berger B, Heller A. Concordance of toxicology of pharmaceuticals in humans and animals. Regulatory Toxicology and Pharmacology 2000;32: 56–67.
17. Acute Toxicity Study On Citrullus Colocynthis Fruit .., http://www.japsonline.com/admin/php/uploads/929_pdf.pdf (accessed February 14, 2019).
18. Chang-Gue S, Seung-Hyun H, Jung-Hyo C, Jang-Woo S, Chin-Ho C, Yeon-Weol L, Chong-Kwan C. Induction of hemopoiesis by saenghyuldan, a mixture of Ginseng radix, Paeoniae radix, and Hominis placenta. Acta Pharmacologica Sinica 2003; 24: 120–126.
19. Orish EO, Johnson OJ, Chude MA, Obi E, Dioka CE. Sub-chronic toxicity studies of the aqueous extract of Boerhavia diffusa leaves. Journal of Health Science 2003;49: 444–447.
20. Braunwald E, Fauci A. Kasper D.Harrison's Principles of Internal Medicine.15th ed. McGraw Hill. USA; 2001:348-52, 457, 1711.
21. Robbins SI, Kumar V, Collins T. Robbins Pathologic Basis of Disease,6th edition. W B Saunders: UK;1999: 26, 55, 102-106, 851-852.
22. Witthawaskul P, Panthong A, Kanjanapothi D, Taesothikul T, Lertprasertsuke N . Acute and subacute toxicities of saponin mixture isolated from Scheffera leucantha Viguier. Journal of Ethnopharmacology 2003; 89: 115–121.
23. Emerson FS, Shadara AC, Devi PU. Toxic effects of crude extract of Plumbago rosea (Rokta chitraka). Journal of Ethnopharmacology 1993;38: 79–84.
24. Udem SC, Obidoa O, Asuzu IU. Acute and chronic toxicity studies of *Erythrina senegalensis* DC stem bark extract in mice. Comparative Clinical Pathology2009; 19: 275–282.

25. Hassan S W, Ladan M J, Dogondaji R A, Umar R A, Bilbis L S, Hassan L G, Ebbo A A, Matazu I K. Phytochemical and toxicological studies of aqueous leaves extracts of Erythrophleum africanum. Pakistan Journal of Biological Sciences 2007;10: 3815–3821.
26. Costa-Silva J H, Lima C R, Silva E J R, Araujo AV, Fraga M C C A, Ribeiro A, Arruda A C, Lafayette S S L, Wanderley A G. Acute and subacute toxicity of the Carapa guianensis Aublet (Meliceae) seed oil. Journal of Ethnopharmacology 2008; 116: 495–500.
27. Chrubasik S. , Eisenberg E. , Balan E., Weinberger T. , Luzzati R. , Conradt C. Treatment of low back pain exacerbations with willow bark extract. Am J Med 2000 ; 1: 9-14 , 109.
28. Waynforth BH. Injection techniques. In: Experimental and Surgical Techniques in the Rat. London/NY, USA: Academic Press; 1980: 30–61.
29. Silva EJR, Goncalves ES, Aguiar FJS, Evencio LB, Lyra MMA, Coelho MCOC, Fraga MCCA, Wanderley AG. Toxicological studies on hydroalcohol extract of Calendula officinalis L. Phytotherapy Research 2007; 21: 332–336.

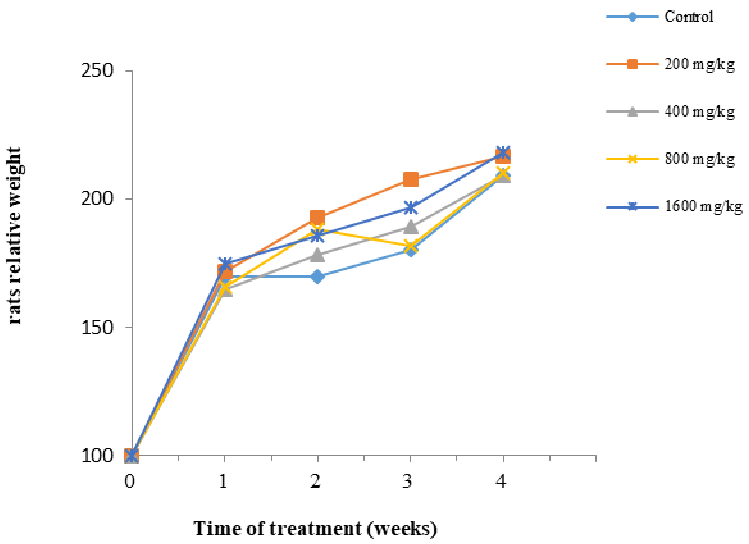

Figure 1. Relative weight of rats treated orally with *Salix alba* stem bark aqueous extract (200–1600 mg/kg/day) for four weeks. Each point represents the mean±SEM, N= 5 (male sex) Data compared to the control group (two way ANOVA followed by Tuky's test).

Table 1. Acute toxicity of the stem bark aqueous extract of *Salix alba* administrated orally to mice.

Dose (g/kg)	D/T		
		Latency	Symptoms
0	0/5	-	None
2	0/5	-	None
4	0/5	-	None
8	0/5	> 2 h < 4 h	Somnolence, Locomotion↓
12	0/5	> 2 h < 8 h	Somnolence, Locomotion↓
16	0/5	> 2 h < 10 h	Somnolence, Locomotion↓, Food consumption ↓,
20	0/5	> 2 h < 24 h	aggressiveness↓ High Somnolence, Locomotion↓, squirt and puke

The stem bark aqueous extract of Salix alba, dissolved in distilled water, was administrated orally; each dose was administrated to a group of 5 mice (5 males). All the treated animals were examined for 14 days for any signs of toxicity (behavioral changes and mortality). D/T: dead/treated mice; none: no toxic symptoms were seen during the observation period; latency: time at which appear toxic signs after the dose; ↓ decrease.

Table 2. Effect of various doses of *Salix alba* stem bark aqueous extract (EASA) on relative weights (g/100 g body weight) of organs in albinos Wistar rats treated for 28 consecutive days.

Organs	Control	Treatment (mg/kg)			
		200	400	800	1600
Heart	0.36 ± 0.01	0.38 ± 0.01	0.33 ± 0.02	0.38 ± 0.02	0.38 ± 0.02
Liver	2.99 ± 0.07	2.94 ± 0.04	2.65 ± 0.11	2.57 ± 0.08 **	2.58 ± 0.11**
Lung	0.57 ± 0.03	0.53 ± 0.01	0.48 ± 0.02	0.48 ± 0.03	0.46 ± 0.04
Spleen	0.28 ± 0.02	0.30 ± 0.07	0.24 ± 0.01	0.26 ± 0.03	0.23 ± 0.01
Kidney	0.38 ± 0.05	0.36 ± 0.08	0.35 ± 0.01	0.32 ± 0.01**	0.33 ± 0.01

Values are expressed as mean ± SEM of 5 animals (male).** $P < 0.01$ significantly different from the control using one way ANOVA, followed by Duncan's multiple comparison test.

Table 3. Effect of oral administration of the aqueous extract (EASA) of the stem bark of *Salix alba* (200–1600 mg/kg/day) on hematological parameters in Wistar rats treated for 28 consecutive days.

Parameters	Control	Treatment (mg/kg)			
		200	400	800	1600
RBC ($\times 10^6$ /µL)	10.12±0.21	9.44±0.49	9.07±0.26	8.07±0.31 **	9.05±0.14
WBC($\times 10^3$/µL)	4.32±1.39	6.64±1.40	7.22±0.60	6.62±0.20	7.64±0.43 **
Haemoglobin(g/dL)	19.22±0.38	18.54±0.37	18.30±0.16	17.52±0.30 **	16.62±0.20 **
Haematocrit (%)	58.34±1.8	52.88±2.20	55.78±0.30	49.74±0.87 **	52.68±0.49 **
MCV(fL)	58.14±0.50	57.60±0.56	58.41±0.47	58.66±0.28	58.08±0.24
McH(pg)	19.88±0.91	19.72±0.99	20.50±0.20	20.12±0.33	19.74±0.29
McHc(g/dL)	32.56±0.25	33.04±0.76	31.92±1.7	34.16±0.17	33.78±0.49
Platelets (10^3/µL)	538.2±3.27	564.4±27	936.4±20**	1044.6±25**	875.8±23**
Neutrophils (%)	42.80±4.90	42.20±1.90	34.80±2.3	33.40±2.37	41.40±2.6
Lymphocytes (%)	64.78±6.70	62.20±8.40	79.44±1.4	72.22±3.9	68.94±0.33
Monocytes (%)	1.80±0.20	1.60±0.24	1.40±0.24	1.80±0.37	1.60±0.24
PDW(fL)	18.44±0.16	8.40±0.89	7.98±0.30	8.14±0.08	8.52±0.02
MPV(fL)	7.48±0.24	7.40±0.20	7.06±0.12	6.96±0.17	7.06±0.13
P-LCR(%)	8.76±0.69	8.60±0.20	8.52±0.17	8.53±0.16	8.54±0.28

Values are mean ± SEM of 5 animals (male).** $P < 0.01$ significantly different from the control using one way ANOVA, followed by Tukey's multiple comparison test.

Table 4. Liver function indices in rats administrated orally with stem bark aqueous extract of *Salix alba* (EASA) for 28 consecutive days.

Parameters	Control	Treatment (mg/kg)			
		200	400	800	1600
AST(U/L)	208.4±4.2	272.8±8.9	268±19.9	298.2±2.8*	310.4±14*
ALP(U/L)	298.2±2.6	271.4±4.9	313.2±8.3	349±13.6*	379.4±3*
ALT(U/L)	34.20±2.3	45.80±4.45	41.80±4.3	45±5.2	57.40±0.81*
ALB(g/dL)	3.40±0.10	3.62±0.12	3.58±0.08	3.40±0.16	3.48±0.07
TP(mg/dL)	8.62±0.13	8.28±0.04	8.98±0.27	7.92±0.18	8.18±0.35
TG(mg/dL)	47.40±0.81	59.80±7.9	35.80±1.59	37.80±2.2	55.40±5.7
TC(mg/dL)	53.20±0.86	55.80±0.58	65.20±1.15	58.20±2.7	62.60±7.3
HDL(mg/L)	34.20±0.80	30.6±1.2	34.40±1.2	38.80±2.5	35.60±4.7
LDL(mg/L)	18.16±1	16.6±1.3	22±2.2	20.4±0.87	19±2
TB(mg/dL)	0.658±0.04	0.860±0.01*	0.864±0.006*	0.8765±0.002*	0.920±0.002*

Values are mean ± SEM of 5 animals (male).* $P < 0.05$ significantly different from the control by using one way ANOVA, followed by Tukey's multiple comparison test.

Table 5. Renal function indices assay in the blood of rats administrated orally with the stem bark aqueous extract of *Salix alba* (EASA) for 28 consecutive days.

Parameters	Control	Treatment (mg/kg)			
		200	400	800	1600
Creatinine(mg/dL)	0.842 ±	0.64 ± 0.067*	0.68 ± 0.049	0.52 ±	0.48 ± 0.037**
Urea (mg/dL)	0.016	39.2 ± 1.7	38.6 ± 3.5	0.037**	37.8 ± 1.65
Uric acid (mg/dL)	44.6 ± 0.67	3.06 ± 0.22	2.88 ± 0.18	40.2 ± 1.2	2.12 ± 0.16**
Na^+ (mEquiv./L)	3.16 ± 0.05	135.4 ± 1.02	137.4 ± 0.5	2.36 ± 0.32	136.2 ± 1.39
Cl^- (mEquiv./L)	136.6 ± 1.24	96.8 ± 0.86	102.8 ± 4.4	137.8 ± 0.73	99 ± 2.81
K^+ (mEquiv./L)	95 ± 0.31	7.4 ± 0.37	6.66 ± 0.25	96.6 ± 1.5	5.24 ± 0.08**
Ca^{2+} (mg/dL)	7.7 ± 0.13	7.9 ± 0.49	6.6 ± 0.23	6.32 ± 0.49*	6.04 ± 0.65
Mg^{2+} (mg/dL)	8.92 ± 0.10	4.36 ± 0.28	4.12 ± 0.08	9.10 ± 0.27	3.84 ± 0.22
Inorganic phosphorus (mg/dL)	4.76 ± 0.48	7.64 ± 0.35	8.08 ± 0.15	4.04 ± 0.09	8.16 ± 0.29
	7.40 ± 0.08			8.26 ± 0.22	

Values are mean ± SEM of 8 animals (4/sex).
* $P < 0.05$ significantly different from the control by using one way ANOVA followed by Tukey's multiple comparison test.
** $P < 0.01$ significantly different from the control by using one way ANOVA followed by Tukey's multiple comparison test.

Table 6. Urinary parameters in rats administrated with the stem bark aqueous extract of *Salix alba* (EASA) for 28 consecutive days.

Parameters	**Control**	**Treatment (mg/kg)**			
		200	400	800	1600
Creatinine (mg/kg/24 h)	2.59±0.3	2.68 ± 0.19	2.63 ± 0.18	2.66 ± 0.16	2.64 ± 0.34
Protein (mg/kg/24 h)	0.023±0.003	0.024 ± 0.003	0.025 ± 0.002	0.025 ± 0.003	0.027± 0.005***
Urea (mg/kg/24 h)	0.052±0.005	0.053 ± 0.005	0.053 ± 0.005	0.053 ± 0.008	0.052 ± 0.001
Ca^{2+} (mg/kg/24 h)	1.03±0.003	1.03 ± 0.002	1.07 ± 0.02	1.02 ± 0.001	1.12 ± 0.03**
Mg^{2+} (mg/kg/24 h)	0.638±0.007	0.639 ± 0.006	0.64 ± 0.004	0.642 ± 0.01	0.646 ± 0.004
Cl^- (mEquiv./kg/24 h)	0.811±0.01	0.811 ± 0.01	0.829 ± 0.006	0.817 ± 0.01	0.840 ± 0.01
K^+ (mEquiv./kg/24 h)	0.082±0.001	0.085 ± 0.002	0.085 ± 0.002	0.086 ± 0.003	0.09 ± 0.001
Na^+ (mEquiv./kg/24 h)	2.15±0.001	2.15 ± 0.003	2.17 ± 0.02	2.15 ± 0.003	2.15 ± 0.002

Values are mean ± SEM of 5 animals (male).
** $P < 0.01$ significantly different from the control using one way ANOVA, followed by Tukey's multiple comparison test.
*** $P < 0.001$ significantly different from the control by using one-way ANOVA followed by Tukey's multiple comparison test.