Ispitivanje hematotoksičnosti ekstrakta lišća *Albizia chevalieri* (Leguminosae)

Hematotoxicity study of the leaf extract of *Albizia chevalieri* harms (Leguminosae)

Yusuf Saidu[1], Lawal Suleman Bilbis[1], Mansur Lawal[1], Simeon Alabi Isezuo[2], Rabiu Aliyu Umar[1]

[1]Odsjek za biokemiju, Sveučilište Usmanu Danfodiyo, Sokoto, Nigeria
[1]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Odsjek za medicinu, Sveučilište Usmanu Danfodiyo, Sokoto, Nigeria
[2]Department of Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria

Sažetak

Uvod: Postoje izvješća o značajnom hipoglikemijskom učinku vodenog ekstrakta lišća biljke *Albizia chevalieri* kod dijabetičnih štakora s šećernom bolesti izazvane aloksanom.

Materijali i metode: Učinak vodenog ekstrakta lišća *Albizia chevalieri* na hematološke varijable i patohistološku analizu ispitan je kod štakora kojima su davane akutne i subkronične doze ovoga pripravka. Štakori na akutnim dozama primili su između 0 i 3000 mg/kg tjelesne težine ekstrakta oralno u jednoj dozi, dok su oni na subkroničnim dozama primali između 0 i 1500 mg/kg tjelesne težine na dan kroz 28 dana. Testirani parametri analizirali su se u uzorcima krvi i tkiva na kraju razdoblja promatranja.

Rezultati: Ekstrakt nije imao značajnog učinka (P>0,05) na koncentraciju hemoglobina, crvenu krvnu sliku, volumen koncentriranih stanica (engl. *packed cell volume*, PCV) (hematokrit), bijelu krvnu sliku i diferencijalnu krvnu sliku, te broj trombocita u testu akutne toksičnosti. Nije bilo značajnog učinka na srednji stanični volumen (engl. *mean cell volume*, MCV), srednju vrijednost staničnog hemoglobina (engl. *mean cell hemoglobin*, MCH) i srednju koncentraciju staničnog hemoglobina (engl. *mean cell hemoglobin concentration*, MCHC). kod štakora na akutnim i subkroničnim dozama ekstrakta (P>0,05). U studiji subkronične toksičnosti, PCV, bijela krvna slika i diferencijalna slika (P<0,05) pokazali su značajne i od dozi neovisne razlike od kontrolnih vrijednosti. Patohistološka analiza tkiva jetre, bubrega i srca štakora pokazala je normalne nalaze kako nakon akutnog tako i nakon subkroničnog davanja ekstrakta.

Zaključak: Vodeni ekstrakt lišća *Albizia chevalieri,* za koji je objavljeno da ima značajno hipoglikemijsko djelovanje kod štakora sa šećernom bolesti izazvane aloksanom, mogao bi biti siguran za primjenu u ispitivanim dozama.

Ključne riječi: hematologija, patohistologija, *Albizia chevalieri*, akutna i subkronična toksičnost

Abstract

Background: Aqueous leaf extract of *Albizia chevalieri* has been reported to have a significant hypoglycemic effect in alloxan induced diabetic rats.

Materials and methods: The effects of the aqueous leaf extract on hematologic variables and histopathologic analyses were assessed in rats treated with acute and sub-chronic doses. Rats treated with acute doses received between 0 and 3000 mg/kg body weight of the extract orally in a single dose, whereas those treated with sub-chronic doses received between 0 and 1500 mg/kg body weight *per* day for 28 days. Blood and tissue samples were analyzed for the tested parameters at the end of the observation period.

Results: The extract had no significant (P>0.05) effect on hemoglobin concentration, red blood cell count, packed cell volume, white blood cell and differential counts, and platelets count in the acute toxicity test. The mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) of rats treated with acute and sub-chronic doses of the extract were not significantly (P>0.05) affected. Packed cell volume (PCV), white blood cell (WBC) and differential counts were significantly (P<0.05) different from the control in a non-dose dependent fashion in the sub-chronic toxicity study. Rat liver, kidney and heart tissues analyzed histopathologically were normal upon both acute and sub-chronic administration of the extract.

Conclusion: Aqueous leaf extract of *A. chevalieri,* which has been reported to have a significant hypoglycemic effect in alloxan diabetic rats, might be safe in the tested doses.

Key words: hematology, histopathology, *Albizia chevalieri*, acute and sub-chronic toxicities

Pristiglo: 19. lipnja 2007. *Received: June 19, 2007*
Prihvaćeno: 9. rujna 2007. *Accepted: September 9, 2007*

Uvod

Albizia je velik rod drveća, porodica graha (*Fabaceae*), udomaćen u toplim krajevima Staroga svijeta. Listovi su perasti i složeni u redovima. *Albizia (A.) chevalieri* je drvo tipa akacije udomaćeno u tropskim i suptropskim krajevima uključujući Nigeriju i Republiku Niger, sa slobodno visećim kuglicama bjelkastih mirisnih cvjetova i ravnim smeđim mahunama. Cvjetići oblikuju okrugle ili prstolike snopiće. Plod je velika remenasta mahuna. Nekoliko drugih vrsta roda *Albizia* rastu kao ukrasno drveće (1).
Ekstrakt lista *A. chevalieri* rabi se kao hladni vodeni uvarak ili se osušeni, samljeveni i prosijani list miješa s kašom za liječenje šećerne bolesti, što se prakticira u tradicionalnoj medicini u nekim dijelovima Republike Niger i u Sokotu, Nigerija. Postoje izvješća o tome da ovaj ekstrakt ima značajan hipoglikemijski učinak (2).
Moguće je da se hipoglikemijska svojstva ove biljke, zapravo ljekovita svojstva biljaka koje se upotrebljavaju u tradicionalnoj medicini zasnivaju na jednom ili nekoliko od mnoštva kemijskih sastojaka biljnog materijala. Ove fitokemikalije, među inima, uključuju složene ugljikohidrate, alkaloide, glikopeptide, terpenoide, tanine, cijanogene, peptide i amine, steroide, flavonoide, lipide, kumarine, sumporne spojeve i anorganske ione. Smatra se da je hipoglikemijski agens ekstrakta *A. chevalieri* fenolni spoj (2). Neki od tih spojeva mogu biti toksični, pa bi stoga biljke koje ih sadrže mogle izazvati toksičnost različite razine u osobe koja ih unosi u organizam. Tako bi neke biljke mogle same po sebi biti opasne, jer sadrže prirodne toksine, često s citotoksičnim, karcinogenim učinkom ili nekim drugim toksičnim svojstvima (3). Na primjer, za poznatu hipoglikemijsku biljku *Carica papaya* (4,5) izvještava se kako izaziva sterilnost kod eksperimentalnih životinja (5,6). Patohistološke promjene zapažene u organima štakora koji su dobivali sirove larve *Cirina forda* (Westwood) također ukazuju na to da je ta biljka toksična (7). Prema literaturnim izvješćima, vodeni ekstrast lišća *A. chevalieri* ima LD_{50} >3000 mg/kg tjelesne težine i ne utječe na većinu biokemijskih serumskih parametara jetrene i bubrežne funkcije kod albino štakora (8).
U ovom su članku opisani akutni i subkronični *in vivo* učinci krutog vodenog ekstrakta lišća *A. chevalieri* na hematološke varijable i patohistološke nalaze albino štakora.

Materijali i metode

Kemikalije i reagencije

Sve kemikalije i reagencije upotrebljene u ovom radu bile su analitičkog stupnja.

Biljni materijal

A. chevalieri je nabavljena iz sela Sanyinna, koje se nalazi oko 50 km južno od grada Sokoto, Nigerija. Biljni materijal

Introduction

Albizia is a large genus of trees, of the pea family (*Fabaceae*), native to warm regions of the Old World. The alternate, compound leaves are bipinnate. *Albizia (A.) chevalieri* is a tree of acacia type native to tropical and subtropical regions including Nigeria and Niger Republic, with loose balls of whitish fragrant flowers and flat brown pods. The small flowers are borne in globular or finger-shaped clusters. The fruit is a large, strap-shaped pod. Several other species of the *Albizia* exist and are grown as ornamentals (1).
The leaf extract of *A. chevalieri* is used either as cold water decoction or dried, ground and sieved leaf mixed with pap, for the management of diabetes mellitus by traditional medical practitioners in some parts of Niger Republic and Sokoto, Nigeria. The extract has been reported to have a significant hypoglycemic effect (2).
The hypoglycemic properties of the plant and indeed the medicinal properties of plants used by traditional medical practitioners may be due to one or more of the many arrays of chemical constituents of the plant material. These phytochemicals include complex carbohydrates, alkaloids, glycopeptides, terpenoids, tannins, cyanogens, peptides and amines, steroids, flavonoids, lipids, coumarins, sulfur compounds and inorganic ions, among numerous others. The hypoglycemic agent of the *A. chevalieri* extract is thought to be a phenolic compound (2). Some of these compounds may be toxic, and thus the plants containing them, when consumed could confer varied levels of toxicity to the individual. Some plants may therefore be inherently dangerous, containing naturally occurring toxins, often with cytotoxic, carcinogenic effects, or some other toxic properties (3). *Carica papaya*, which is a known hypoglycemic plant (4,5), for example, is reported to induce sterility in experimental animals (5,6). The histopathologic changes observed in the organs of rats treated with raw larva of *Cirina forda* (Westwood) have also suggested that the plant is toxic (7). It has been reported that aqueous leaf extract of *A. chevalieri* has an LD_{50} of >3000 mg/kg body weight and has no effect on most serum liver and kidney function biochemical indices of albino rats (8).
In the present study, the *in vivo* acute and sub-chronic effects of the crude aqueous leaf extract of *A. chevalieri* on hematologic variables and histopathologic findings of albino rats were assessed.

Materials and methods

Chemicals and reagents

All chemicals and reagents used in the study were of analytical grade.

Plant materials

A. chevalieri was obtained from Sanyinna village, about 50 km south of Sokoto, Nigeria. A taxonomist from the Bota-

je identificirao taksonomist iz Odjela za botaniku Katedre za biološke znanosti, Sveučilište Usmanu Danfodiyo iz Sokota. Referentni uzorak (UDUS/VS/o4/09) je pripravljen i pohranjen u Herbarij Katedre. Lišće je osušeno na suncu, usitnjeno laboratorijskim batićem u mužaru, te prosijano kroza sito od 1 mm^2. Tako dobiveni prah od lišća spremljen je u plastične vrećice u desikatoru do uporabe.

Priprava krutog ekstrakta

Biljni materijal usitnjen u prah namočen je u hladnu destiliranu vodu u omjeru od 20% (w/v) kroz 24 sata. Ekstrakt je filtriran kroz višestruko presavijen muselin kako bi se uklonile otpadne čestice. Potom je tako dobiveni filtrat protjeran kroz filtar papir Whatman br. 1 i otparen u uređaju za sušenje na 40 °C (9). Tako dobiveni ostatak iznosio je 18,7% (w/w). Tada je upareni ekstrakt rekonstituiran u destiliranoj vodi u omjeru od 30% (w/v) i pohranjen u male plastične spremnike sa zatvaračem na +4 °C do uporabe. Ovaj postupak je primijenjen za testove akutne i subkronične toksičnosti. Doza ekstrakta koja se preporuča u nigerijskoj tradicionalnoj medicini je oko 130 mg/kg tjelesne težine. Ranije je opisano kako je doza od 100 mg/kg tjelesne težine učinkovito snizila razinu glikemije kod Wistar štakora (2).

Eksperimentalne životinje

Zdravi muški Wistar štakori, težine 163±11 g, nabavljeni su od Državnog instituta za istraživanje tripanosomijaze, Vom, blizu Jos Plateaua, Nigerija. Životinje su ostavljene kroz jedan tjedan da se prilagode laboratorijskoj okolini i za to vrijeme su imale slobodan pristup čistoj vodi i hrani.

Ispitivanje akutne toksičnosti

Životinje su podijeljene u šest skupina od po sedam životinja, označene A_C, B_C, C_C, D_C, E_C i F_C. Kruti vodeni ekstrakt davao se u pojedinačnim oralnim dozama od 500, 1000, 1500, 2200 i 3000 mg/kg tjelesne težine skupinama B_C, C_C, D_C, E_C odnosno F_C. Životinje u skupini A_C dobile su 0,5 mL destilirane vode istim putem i služile su kao kontrole. Životinje su izvagane prije i 72 sata nakon davanja lijeka, te su promatrane kroz 72 sata zbog toksičnih simptoma kao što su slabost i agresivnost, odbijanje hrane, gubitak težine, iscjedak iz očiju i ušiju, šumovi pri disanju i smrtnost (10,11).

Ispitivanje subkronične toksičnosti

Životinje su podijeljene u 6 skupina od po 7 životinja, označenih kao S_C, T_C, V_C, X_C, Y_C i Z_C. Kruti vodeni ekstrakt davao se oralno u 5 gradacija od 150, 300, 500, 1000 i 150 mg/kg tjelesne težine na dan kroz 28 dana skupinama S_C, T_C, V_C, X_C, Y_C odnosno Z_C. Životinje u kontrolnoj skupini S_C primale su 0,5 mL destilirane vode pod sličnim eksperimentalnim uvjetima, istim putem i kroz isti broj dana. Promjene tjelesne težine promatrale su se tjedno tijekom

ny Unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, identified the plant material. A voucher specimen (UDUS/VS/04/09) was prepared and deposited in the Department Herbarium. The leaves were sun dried, ground using laboratory pestle and mortar, and sieved through a 1-mm^2 sieve. The powdered leaves were kept in plastic bags in desiccators until required.

Preparation of crude extracts

The powdered plant material was soaked in cold distilled water at 20% (w/v) for 24 h. The extract was filtered through several folded clean white muslin cloth to remove debris. The filtrate was then filtered through a Whatman no. 1 filter paper and evaporated in a drying cabinet set at 40 °C (9). The residue obtained was 18.75% (w/w). The evaporated extract was then reconstituted in distilled water at 30% (w/v) and stored in small-capped plastic containers at +4 °C until required. This was used for both acute and sub-chronic toxicity tests. The recommended dose of the extract in Nigerian traditional medicine is about 130 mg/kg body weight. It has been reported earlier that a dose of 100 mg/kg body weight is effective in reducing glycemia levels in Wistar rats (2).

Animals

Apparently healthy male Wistar rats weighing 163±11 g were purchased from National Institute for Trypanosomiasis Research (NITR) Vom, near Jos Plateau State Nigeria. The rats were allowed to acclimatize to the laboratory environment for a week during which they were allowed free access to clean water and food.

Acute toxicity study

The animals were divided into six groups of seven each and labeled A_C, B_C, C_C, D_C, E_C and F_C. The crude aqueous extract was administered in single oral doses of 500, 1000, 1500, 2200 and 3000 mg/kg body weight in groups B_C, C_C, D_C, E_C and F_C, respectively. Group A_C animals were administered 0.5 mL of distilled water by the same route and served as control. The animals were weighed before and 72 hours after the administration of the drug, and were observed for toxic symptoms such as weakness or aggressiveness, food refusal, weight loss, diarrhea, discharge from eyes and ears, noisy breathing and mortality for 72 hours (10,11).

Sub-chronic toxicity study

There were 6 groups of 7 animals each labeled S_C, T_C, V_C, X_C, Y_C and Z_C. The crude water extract was administered orally in 5 gradations of 150, 300, 500, 1000 and 1500 mg/kg body weight *per* day for 28 days to groups T_C, V_C, X_C, Y_C and Z_C animals, respectively. The control group (group S_C) received 0.5 mL of distilled water, under similar experimental conditions and by the same route and for the sa-

čitavog razdoblja eksperimenta. Životinje su također promatrane zbog manifestacije toksičnosti i smrtnosti kao i u dijelu studije u kojem se ispitivala akutna toksičnost.
Na kraju razdoblja promatranja (u ispitivanjima subkroničnе i akutne toksičnosti) životinje su anestetizirane pomoću para kloroforma. Uzeti su uzorci krvi srčanom punkcijom i prenešeni u označene plastične bočice koje su sadržavale EDTA (disodium etilenediamin tetraacetat) u količini od 1,5 mg/mL krvi (12).

Hematološke pretrage

Sadržaj hemoglobina (Hb) u uzorcima krvi određen je spektrofotometrijskom metodom (12). Crvena krvna slika (engl. *red blood cell*, RBC) određena je metodom vizualnog brojenja (12). Volumen koncentriranih stanica (engl. *packed cell volume*, PCV), bijela krvna slika (engl. *white blood cell*, WBC), diferencijalna krvna slika i broj trombocita (Plt): pomoću mehaničkog širenja i optičkog uvećanja, pojačano supravitalnim bojenjem stanica, sustav QBCII izdaje broj trombocita, bijelu krvnu sliku i broj dviju glavnih podskupina bijele krvne slike iz linearnih mjerenja slojeva koncentriranih stanica u sloju leukocita i trombocita. Također se mjeri PCV, kao i kod konvencionalnih postupaka mikrocentrifugiranja (13). Epruveta s krvlju napuni se antikoaguliranom krvlju pomoću uređaja za pipetiranje. Vrh epruvete očisti se od krvi papirnim rupčićem. Epruveta se čvrsto zatvori i lagano protrlja među prstima kroz 5 sekunda. Nezatvoreni kraj epruvete prevuče se preko vrha prethodno postavljenog plovka i gurne tako da plovak uđe što dublje u epruvetu. Tada se epruveta s krvlju centrifugira na rotoru centrifuge QBCII kroz 5 minuta i očita na čitaču QBCII. Izračunati su srednji stanični volumen (engl. *mean cell volume*, MCV), srednji stanični hemoglobin (engl. *mean cell haemoglobin*, MCH) i srednja koncentracija staničnog hemoglobina (engl. *mean cell hemoglobin concentration*, MCHC) (12).

Patohistološke pretrage

Iz svake su skupine nasumce odabrane po tri životinje, anestetizirane parama kloroforma i secirane kroz središnji rez na trbuhu. Uzeti uzorci bubrega, srca i jetre i odmah fiksirani u 10%-noj fiziološkoj otopini-formalinu u označene plastične bočice za uzorke. Tkiva su dehidrirana u gradiranim koncentracijama ksilena, uklopljena u rastaljeni parafinski vosak i izrezana u komadiće od 5 μ. Tako izrezana tkiva su fiksirana na bezmasna predmetna stakalca i obojena hematoksilinom i eozinom za pregled pod svjetlosnim mikroskopom uz povećanje 400 puta (14). Napravljeni su fotomikrogrami nekih tkiva pomoću mikroskopa s fotografskim aparatom i razvijeni rutinski u laboratoriju za fotografiju u boji.

Statistička analiza

Rezultati su prikazani kao srednja vrijednost ± standardna devijacija. Rezultati hematološke analize obrađeni su

me number of days. Body weight changes were monitored throughout the experimental period on a weekly basis. The animals were also observed for the manifestation of toxicity and mortality as in acute toxicity test.
At the end of the observation periods (both the sub-chronic and acute toxicity tests), the animals were anesthetized using chloroform vapor. Blood samples were then collected by cardiac puncture and transferred into labeled plastic sample bottles containing EDTA (disodium ethylenediamine tetraacetate) at 1.5 mg/mL of blood (12).

Hematology

Hemoglobin (Hb) levels in blood samples were determined by the spectrophotometric method (12). Red blood cell (RBC) count was determined by the visual counting method (12). Packed cell volume (PCV), white blood cell (WBC), differential and platelet (Plt) counts: utilizing mechanical expansion and optical magnification, augmented by supravital cell staining, the QBCII system derives platelet count, WBC and counts of the two main WBC subgroups from linear measurements of the packed cell layers in a buffy coat. As in conventional microcentrifugation procedures, the PCV is also measured (13). The blood tube was filled with anticoagulated blood using a pipetter. The tip of the tube was cleaned off the blood with tissue paper. The tube was sealed and gently rolled between fingers for about 5 seconds. The unsealed end of the tube was slid over the tip of the prepositioned float and pushed until the float was inside the tube as far as possible. The blood tube was then centrifuged on the rotor of the QBCII centrifuge for 5 minutes and read on the QBCII reader. MCV, MCH and MCHC were calculated (12).

Histopathology

Three animals were selected randomly from each group, anesthetized with chloroform vapor and dissected through a central abdominal incision. The kidney, heart and liver samples were collected and immediately fixed in 10% saline-formalin in labeled sample plastic bottles. The tissues were dehydrated in graded concentrations of xylene, embedded in molten paraffin wax and sectioned at 5 μ. Tissue sections were fixed on grease-free glass slides and stained with hematoxylin and eosin for light microscopy at 400X (14). Photomicrographs of some of the tissues were taken using a microscope fitted with a camera unit, and processed routinely in a color photo laboratory.

Statistics

The results are presented as mean + standard deviation. The results of hematologic analyses were analyzed using analysis of variance (ANOVA). The post hoc tests multiple comparisons using LSD were utilized to identify differences in means. P values lower than 0.05 were considered

analizom varijance (ANOVA). Za utvrđivanje razlika u srednjim vrijednostima primijenjene su višestruke usporedbe *post hoc* testova pomoću LSD. Vrijednosti P manje od 0,05 smatrale su se statistički značajnima. U analizi je korišten statistički paket SPSS Windows, verzija 10.

Rezultati

Rezultati o učincima različitih akutnih i subkroničnih doza krutog ekstrakta lišća *A. chevalieri* na hematološke varijable prikazani su u tablicama 1. i 2. Rezultati nisu pokazali nikakve značajne razlike (P>0,05) između životinja na različitim dozama ekstrakta ni u jednom modelu.
Rezultati patohistološke analize tkiva štakora koji su dobivali akutne i subkronične doze ekstrakta lišća *A. chevalieri*

statistically significant. Statistic softwarw SPSS Windows, Version 10, was employed for the analysis.

Results

Results on the effects of different acute and sub-chronic doses of the crude leaf extract of *A. chevalieri* on the hematologic variables are presented in Tables 1 and 2, respectively. The results showed no significant difference (P>0.05) between the animals on different doses of the extract for either test model. The results of tissue histopathologic analyses of rats treated with acute and sub-chronic doses of the leaf extract of *A. chevalieri* are scored according to the method of Akinnawo *et al.* (7) and presen-

Tablica 1. Hematološki parametri Wistar štakora koji su dobivali akutne oralne doze vodenog ekstrakta lišća *A. chevalieri*

Table 1. Hematologic parameters of Wistar rats treated with acute oral doses of aqueous leaf extract of *A. chevalieri*

Group	Dose (mg/kg)	PCV (%)	Hb (g/dL)	RBC ($x10^{12}$/L)	WBC ($x10^9$/L)	Gr (%)	Ly/Mo (%)	PLT ($x10^9$/L)	MCV (fl)	MCH (pg)	MCHC (g/L)
A_C	0	28.5±7.7	11.48±4.7	4.21±1.1	8.5±2.1	38.3±5.8	61.8±5.8	270.5±21.0	6.77±0.21	2.73±0.16	0.40±0.08
B_C	500	32.3±4.4	12.24±2.3	4.39±0.62	13.8±4.3	46.4±13.3	53.6±13.2	367.6±23.2	6.74± 0./34	2.56±0.18	0.38±0.04
C_C	1000	33.3±2.4	14.04±2.1	4.82±0.34	8.8±2.7	36.8±12.9	63.4±12.8	204.6±94.2	6.91± 0.17	2.91±0.16	0.42±0.05
D_C	1500	30.4±6.8	12.86±3.8	4.3±1.0	12.2±1.4	45.8±34.4	54.4±30.2	360.0±45.6	7.07±0.41	2.99±0.09	0.42±0.03
E_C	2200	35.3±4.2	13.98±2.4	5.01±0.59	10.5±4.5	30.2±14.3	59.1±30.5	313.4±23.7	7.05±0.32	2.79±0.21	0.40±0.04
F_C	3000	35.1±4.7	13.96±2.2	4.98±0.66	16.4±3.3	30.2±13.9	63.8±9.3	345.0±32.4	7.17±0.35	2.80±0.14	0.40±0.03

Values are mean ± standard deviation of 7 animals, observed for 72 hours
Gr = Granulocytes; Ly = Lymphocytes; Mo = Monocytes

Tablica 2. Hematološki parametri Wistar štakora koji su dobivali subkronične oralne doze vodenog ekstrakta *A. chevalieri*

Table 2. Hematologic parameters of Wistar rats treated with sub-chronic oral doses of aqueous leaf extract of *A. chevalieri*

Group	Dose (mg/kg)	PCV (%)	Hb (g/dL)	RBC ($x10^{12}$/L)	WBC ($x10^9$/L)	Gr (%)	Ly/ Mo (%)	PLT ($x10^9$/L)	MCV (fl)	MCH (pg)	MCHC (g/L)
S_C	0	29.1±4.66	12.48±2.29	4.21±1.1	28.6±13.12	47.25±3.62	52.75±3.6	345.5±22.48	6.91±0.54	2.96±0.14	0.43±0.09
T_C	150	22.8±0.8	9.0±1.37	5.01±1.17	32.37±14.69	19.67±2.04	80.33±2.04	431±23.33	4.56±0.92	1.77±0.23	0.39±0.05
V_C	300	28.3±9.08	12.67±5.6	4.32±0.98	17.07±10.22	43.33±2.83	56.67±2.83	372±18.7	6.55±0.71	2.93±0.17	0.45±0.06
X_C	500	21.35±3.93	8.48±1.93	4.59±1.16	30.8±13.62	45.5±2.03	54.5±2.03	184.25±10.8	4.65±1.11	1.85±0.19	0.40±0.07
Y_C	1000	19.23±7.56	8.93±3.8	4.69±1.21	34.53±12.36	15.67±1.13	84.33±1.17	483±34.11	4.10±0.82	1.90±0.27	0.46±0.09
Z_C	1500	28.5±7.7	11.48±4.7	4.48±0.91	8.5±2.1	38.3±5.8	61.8±5.8	270.5±21.0	6.36±0.67	2.56±0.25	0.40±0.06

Values are mean ± standard deviation of 7 animals, treated for 28 days;
PCV: S_c *vs.* Y_c (P = 0.05);
WBC: S_c *vs.* Z_c (P = 0.027), T_c vs. Z_c (P = 0.017), X_c *vs.* Z_c (P = 0.016), Y_c *vs.* Z_c (P = 0.010);
Gr: S_c *vs.* T_c (P = 0.038), S_c *vs.* Y_c (P = 0.041);
Ly/Mo: S_c *vs.* T_c (P = 0.048); S_c *vs.* Y_c (P = 0.043)
Gr = Granulocytes; Ly = Lymphocytes; Mo = Monocytes.

zbrajani su po metodi Akinnawoa i sur. (7), a prikazani u tablicama 3 i 4. Ekstrakt nije imao nikakav učinak na većinu tkiva.

Rasprava

Točnim određivanjem hematoloških parametara može se postaviti oko 80% hematoloških dijagnoza, te dobiti potrebne podatke za procjenu stadija određene bolesti ili pak za dijagnosticiranje nekih bolesti koje ne moraju biti izravno povezane s hematopoetskim sustavom (15). Podaci dobiveni iz broja leukocita, a bez diferencijalne krvne slike mogu biti tek djelomični, a u nekim slučajevima mogu čak dovesti do zabune. Stoga se u diferencijalnoj slici procjenjuju podvrste leukocita, kako bi se utvrdila narav infekcije (15). Broj eozinofila povišen je u gotovo svim vrstama alergijskih bolesti, parazitnim infekcijama, te u manjoj mjeri kod zloćudnih bolesti (15). S druge strane, povišen broj monocita nalazi se kod kroničnih upalnih bolesti,

ted in Tables 3 and 4. Most of the tissues were unaffected by the extract.

Discussion

With accurate determination of hematologic parameters, about 80% of hematologic diagnoses can be made and information collected to evaluate the stage of a particular disease or to diagnose some diseases that may not be directly related to the hematopoietic system (15). Information generated from white blood cell count, without differential count may only be partial and in some cases misleading. Thus, granulocytes are estimated in differential count to establish the nature of infection (15). Eosinophils are elevated in allergic disorders of nearly all kinds, parasitic infections, and to a lesser extent in malignancy (15). Monocyte elevation, on the other hand, is seen in chronic inflammatory disorders, whereas lymphocytes are elevated in chronic lymphocytic leukemia, infectious diseases

Tablica 3. Zbirna patologija organa Wistar štakora nakon akutnog oralnog davanja različitih doza vodenog ekstrakta lišća *A. chevalieri* za tri životinje tretirane akutnom dozom

Table 3. Organ pathology scores in Wistar rats after acute oral administration of various doses of aqueous leaf extract of *A. chevalieri* for 3 animals treated with acute dose

	Group (dosage in mg/kg body weight)					
Organ/Lesions#	**A_C (0)**	**B_C (500)**	**C_C (1000)**	**D_C (1500)**	**E_C (2200)**	**F_C (3000)**
Liver						
Congestion	–	–	–	+	–	–
Degeneration	–	–	–	–	–	–
Cellular infiltration	–	–	–	–	–	–
Bile duct hyperplasia	–	–	–	–	–	–
Fibrosis	–	–	–	–	–	–
Perivascular cuff	–	–	–	–	+	+
Kidney						
Congestion	–	+	–	+	–	–
Degeneration and necrosis	–	–	–	–	–	–
Cellular infiltration	–	–	–	–	–	–
Tubular protein cast	+	+	–	+	–	–
Heart						
Congestion and hemorrhage	–	–	–	–	–	–
Myofibril degeneration and necrosis	–	–	–	–	–	–
Hyalinized vessels	–	–	–	–	–	–

– = not observed; + = mild or absent; + = moderate; ++ = severe;

Tablica 4. Zbirna patologija organa Wistar štakora nakon subkroničnog oralnog davanja različitih doza vodenog ekstrakta lišća A. chevalieri za tri životinje tretirane subkroničnom dozom (28 dana)

Table 4. Organ pathology scores in Wistar rats after sub-chronic oral administration of various doses of aqueous leaf extract of A. chevalieri for 3 animals treated with sub-chronic (28 days) doses

	Group (dosage in mg/kg body weight)					
Organ/Lesions	**S_c(0)**	**T_c(150)**	**V_c(300)**	**X_c(500)**	**Y_c(1000)**	**Z_c(1500)**
Liver						
Congestion	–	–	–	–	–	–
Degeneration	–	–	–	–	–	–
Cellular infiltration	–	–	–	–	–	–
Bile duct hyperplasia	–	–	–	–	–	–
Fibrosis	–	–	–	–	–	–
Perivascular cuff	–	–	–	–	+	+
Kidney						
Congestion	–	+	+	–	–	+
Degeneration and necrosis	–	–	–	–	–	–
Cellular infiltration	–	–	–	–	–	–
Tubular protein cast	+	+	+	+	–	+
Heart						
Congestion and hemorrhage	–	–	–	–	–	–
Myofibril degeneration and necrosis	–	–	–	–	–	–
Hyalinized vessels	–	–	–	–	–	–

– = not observed; ± = mild or absent; + = moderate; ++ = severe

dok je broj limfocita povišen kod kronične limfocitične leukemije, zaraznih bolesti poput pertusisa, virusnih bolesti poput ospica, rubeole i vodenih kozica (15). Rezultati ove studije pokazuju da su broj ukupnih leukocita i diferencijalna slika bili normalni u oba ispitivana modela i time ukazivali na nealergijsku narav pripravljenih ekstrakata. To također pokazuje kako ekstrakt nije remetio imuni status životinja.

Nedostatak željeza je glavni uzrok hipokromne mikrocitične anemije, gdje je moguć granično nizak Hb i PCV čak i kad je broj eritrocita normalan. Akutni gubitak mase krvnih stanica kakav se nalazi kod krvarenja i hemolize glavni je uzrok normokromne normocitne anemije. Kod makrocitne anemije postoji smanjenje apsolutnog broja eritrocita po jedinici volumena. U tom slučaju je pojedinačna stanica veća u volumenu i promjeru te sadrži više hemoglobina, što rezultira povišenjem MCV i MCHC. Ovaj oblik anemije nalazi se kod nedostatka folne kiseline i vitamina B_{12}. U našem ispitivanju niti akutno niti subkronično davanje ekstrakta nije značajno utjecalo na broj eritrocita (P=0,067; 0,121), PCV (P=0,343; 0,195) i Hb (P=0,730; 0,451).

like pertussis, and viral diseases like measles, rubella and chicken pox (15). The results of the present study suggested that total leukocyte and differential counts were normal in both test models, therefore being indicative of the non-allergic nature of the extracts. It was also an indication that the extract did not compromise the immune status of the animals.

Iron deficiency is the major cause of hypochromic microcytic anemia, in which case there may be a borderline low Hb and PCV even if the RBC is normal. Acute loss of blood cell mass as in hemorrhage and hemolysis is the major cause of normochromic normocytic anemia. In macrocytic anemia, there is a decrease in the absolute number of RBC *per* unit volume. In this case, the individual cell is larger in volume and diameter and contains more hemoglobin. As a result, MCV and MCHC are elevated. This form of anemia is seen in folic acid and vitamin B_{12} deficiencies. In the present study, RBC (P=0.067; 0.121), PCV (P=0.343; 0.195) and Hb (P=0.730; 0.451) were not significantly affected as a result of either acute or sub-chronic administration of the extract. In addition, other erythrocyte variables (MCV, MCH and MCHC) were not affected due to

Uz to, davanje pripravka nije utjecalo niti na druge eritrocitne parametre (MCV, MCH i MCHC). To bi moglo značiti da ovaj ekstrakt u ispitivanim koncentracijama ne izaziva nikakav oblik anemije. S obzirom na to da je terapijska doza ekstrakta daleko niža od količina koje su se rabile u ispitivanju toksičnosti, može se pretpostaviti da ekstrakt ne bi utjecao na eritropoezu, sintezu hemoglobina ili na druge čimbenike povezane s metabolizmom eritocita kad ga se uzima u terapijskoj dozi. Isto tako u ovoj studiji nije bilo značajnog utjecaja na broj trombocita (P>0,05). Slični su rezultati objavljeni za *Strychnos potatorum*, biljku koja se rabi u Indiji za liječenje većeg broja bolesti uključujući šećernu bolest (16). Međutim, Ali i Bashir (17) i Taiwo i sur. (18) izvješćuju kako su ekstrakti *Aviccenia marina* odnosno *Aloe vera* značajno utjecali na hematološke parametre kod štakora kojima su se davale akutne i subkronične doze istih.

Zbirna patologija organa štakora nakon akutnog i subkroničnog oralnog davanja vodenog ekstrakta lišća *A. chevalieri*, prikazana u tablicama 7. i 8., pokazala je normalnu arhitekturu jetre, bubrega i srca. Opažene promjene nisu bile značajne i bile su ograničene na bubreg u oba ispitivana modela. Promjene su uglavnom uključivale blagu kongestiju i proteinske cilindre, što nije bilo ovisno o dozi. Kad su nastupile blage promjene, to je zabilježeno u manje od 20% ispitivanih uzoraka. Prema literaturnim podacima, akutne i subkronične doze vodenog etanolnog ekstrakta lišća *Senna alata* (19) te vodeni ekstrat i prah sjemenaka *Strychnos potatorum* (16) nisu značajno utjecale na jetru štakora. Međutim, akutno davanje ekstrakta biljke *Aviccenia marina* koja podnosi sol (17) i vodenog ekstrakta lišća sirove biljke *Aloe vera* (18) uzrokovalo je značajne promjene histologije jetre i bubrega štakora. Pokazalo se kako jetra kao prvi organ koji se susreće sa svim materijalima apsorbiranima iz probavnog sustava na različite načine odgovara na toksikološka oštećenja, što uključuje staničnu degeneraciju i nekrozu, hiperplaziju žučovoda i fibrozu (7). Bubreg je izlučni organ koji uklanja metabolizirane i ne-metabolizirane toksične tvari iz organizma; stoga je bubreg izložen višim koncentracijama štetnih tvari koje mogu uzrokovati oštećenja.

Zaključak

U zaključku, vodeni ekstrakt lišća *A. chevalieri*, za koji je opisano da ima značajan hipoglikemijski učinak kod štakora s šećernom bolesti izazvane aloksanom (2), može se smatrati relativno sigurnim u ispitanim dozama. U ranijem radu su Saidu i sur. (8) objavili kako ovaj ekstrakt ima LD_{50} iznad 3000 mg/kg tjelesne težine i nema nikakvog značajnog učinka na biokemijske parametre jetrene i bubrežne funkcije u serumu.

the treatments either. This may be an indication that the extract does not induce any form of anemia at the tested concentrations. In view of the fact that the therapeutic dose of the extract is by far lower than the amounts used in toxicity tests, it may be appropriate to suggest that the extract may not affect erythropoiesis, hemoglobin synthesis or other factors related to RBC metabolism when used at the therapeutic dose. Neither platelets, were affected significantly (P>0.05) in the reported study. Similar results have been reported for *Strychnos potatorum*, a plant used in India for the treatment of a number of diseases including diabetes mellitus (16). However, Ali and Bashir (17) and Taiwo *et al.* (18) report that the extracts of *Aviccenia marina* and *Aloe vera*, respectively, affected significantly the hematologic variables of rats treated with acute and subchronic doses.

The organ pathology scores of the rats after acute and sub-chronic oral administration of the aqueous extract of *A. chevalieri* leaves (Tables 7 and 8, respectively) indicated that the liver, kidney and heart of the rats had normal architectures. The observed changes were not significant and were restricted to the kidney in both test models. The changes were mainly mild congestion and protein cast that were not dose dependent. Where there were mild changes, they occurred in less than 20% of the tested population. It has been reported that the liver of rats treated with both acute and sub-chronic doses of aqueous ethanolic leaf extract of *Senna alata* (19) and aqueous extract and seed powder of *Strychnos potatorum* (16) were not significantly affected. Acute treatment with the extract of a salt tolerant plant, *Avicennia marina* (17) and aqueous leaf extract of raw *Aloe vera* (18), however, caused significant changes in the histology of the liver and kidney of rats. The liver, being the first organ that encounters all absorbed materials from the gastrointestinal tract, has been shown to respond to toxicologic insults in a number of ways including cellular degeneration and necrosis, bile duct hyperplasia and fibrosis (7). The kidney is an excretory organ that removes metabolized and non-metabolized toxic materials from the body; hence, it would be exposed to high concentrations of noxious materials that could cause lesions.

Conclusion

The aqueous leaf extract of *A. chevalieri,* which has been reported to exert a significant hypoglycemic effect in alloxan diabetic rats (2), may therefore be considered relatively safe at the tested doses. In an earlier work by Saidu *et al. (*8), the extract has been reported to have an LD_{50} of greater than 3000 mg/kg body weight and to show no significant effect on serum liver and kidney function biochemical parameters.

Zahvala

Autori zahvaljuju gospodinu Basseyu iz Odjela za patohistologiju, Sveučilišna bolnica Usmanu Danfodiyo, Sokoto, Nigerija, na izradi tkivnih pripravaka, te pok. Dr. T.J. Anga s Katedre za veterinarsku patologiju, Sveučilište Usmanu Danfodiyo, Sokoto, Nigerija, na pomoći u tumačenju pripravaka. Zahvaljujemo gospodinu M. Kamba na pomoći u određivanju hematoloških varijabla.

Acknowledgments

The authors are grateful to Mr. Bassey of the Histopathology Unit, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria, for preparing the tissue slides, and the late Dr. T.J. Anga of the Veterinary Pathology Department, Usmanu Danfodiyo University, Sokoto, Nigeria, for assistance in reading the slides. Mr. M. Kamba's assistance in determination of hematologic variables is greatly appreciated.

Adresa za dopisivanje:
Yusuf Saidu
Department of Biochemistry
Usmanu Danfodiyo University
P.M.B. 2346, Sokoto
Nigeria
e-pošta: yusab@yahoo.com
tel: +234 803 613 1987
faks: +234 60 235199

Corresponding author:
Yusuf Saidu
Department of Biochemistry
Usmanu Danfodiyo University
P.M.B. 2346, Sokoto
Nigeria
e-mail: yusab@yahoo.com
phone: +234 803 613 1987
fax: +234 60 235199

Literatura/References

1. *Britannica. Encyclopaedia Britannica 2001 Deluxe Edition CD Rom.*
2. *Saidu Y, Lawal M, Isezuo SA, Shehu RA, Sahabi DM, Bilbis LS. Partial purification and elucidation of mechanism of hypoglycaemic agent of aqueous leaf extract of Albizzia chevalieri Harms (Leguminosae). J Pharmacol Toxicol 2007;2(6):513-23.*
3. *Humphrey SI, McKenna DJ. Herbs and breastfeeding. Breastfeeding Abstracts 1997;17(2):11-2.*
4. *Onoagbe TO, Ebhota AO, Udegbe HC, Omondia M, Edeni D, Ebengho SO. Assessment of some medicinal plants for hypoglycaemic activities in rats and rabbits. Bioscience Research Communications 1999;11(2):159-63.*
5. *Lohiya NK, Pathak N, Mishra PK, Manivannan B. Reversible contraception with chloroform extract of C. Papaya Lin seeds in male rabbits. Reprod Toxicol 1999;13:59-66.*
6. *Lohiya NK, Pathak N, Mishra PK, Manivannan B. Contraceptive evaluation and toxicological study of aqueous extract of the seeds of Carica papaya in male rabbits. J Ethnopharmacol 2000;70:17-27.*
7. *Akinnawo OO, Taiwo VO, Ketiku AO, Ogunbiyi JO. Weight changes and organ pathology in rats given edible larvae of Cirina forda (Westwood). Afr J Biomed Res 2005;8(1):35-9.*
8. *Saidu Y, Bilbis LS, Lawal M, Isezuo SA, Hassan SW, Abbas AY. Acute and sub-chronic toxicity studies of crude aqueous extract of Albizzia chevalieri Harms (Leguminosae). Asian J Biochem 2007;2(4):224-36.*
9. *Harborne JB. Photochemical methods; Chapman and Hall; London; 1973. pp. 1-32.*
10. *Fielding D, Matheron G. Rabbits; CTA and Macmillan; 1991. pp. 77-83.*
11. *Vijayalakshmi T, Muthulakshmi V, Sachdanandam P. Toxic studies on biochemical parameters carried out in rats with Serankottai nei, a siddha drug-milk extract of semecarpus anacardium nut. J Ethnopharmacol 2000;69:9-15.*
12. *Dancier JV, Lewis SM. Practical Haematology, 7th edition; Churchhill Livingstone; Edinburgh, London, Melbourne, New York; 1991. pp. 1-7,37-66.*
13. *QBC II Manual: Operator's manual: QBCII Centrifugal Haemat.*
14. *Kiernan JA. Histological and Histochemical Methods: Theory and Practice; Pergamon Press; Oxford, New York, Toronto Sydney, Paris, Frankfurt; 1981. pp. 1-87.*
15. *Tilkian SM, Conover MB, Tilkian AG. Clinical implications of laboratory tests; C.V. Mosby Company; St. Louis, Toronto, London; 1979. pp. 3-44; 117-32;154-9.*
16. *Sanmugapriya E, Venkataraman S. Toxicological investigations on Strychnos potatorum Linn seeds in experimental animal models. J Health Sci 2006;52(4):339-43.*
17. *Ali BH, Bashir AK. Toxicological studies on the leaves of Avicennia marina (mangrove) in rats. J Appl Toxicol 1998;18(2):111-6.*
18. *Taiwo VO, Olukunle OA, Ozor IC, Oyejobi AT. Consumption of aqueous extract of raw Aloe vera leaves: histopathological and biochemical studies in rat and tilapia. Afr J Biomed Res 2005;8:169-78.*
19. *Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX, Ngongang J. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of Senna alata (L.) Roxb (Ceasalpiniaceae). Afr J Biotechnol 2006;5(3):283-9.*