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Effects of aqueous and ethanol extract of dried leaves of *Pseudocalymma alliaceum* (Bignonaceae) on haematological and biochemical parameters of wistar rats

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ABSTRACT

Objective: To perform the toxicological evaluation of aqueous and ethanol extract of dried leaves of *Pseudocalymma alliaceum* (*P. alliaceum*) in male Wistar rats by oral administration for 14 days, and to determine the biochemical and haematological status of blood. **Methods:** The animals were completely randomized into four groups of three rats each. **Results:** No deaths were reported after oral administration of the extracts, no physical signs of toxicity or adverse effects were observed. Hematological indices of red blood cell count, hemoglobin concentration and mean corpuscular hemoglobin showed no significant abnormality; however, white series levels decrease presenting a leukopenia. Glucose, creatinine and albumin increased, while urea decreased; aspartate aminotransferase values decreased with the aqueous extract at 50 and 100 mg/kg and increased with dose of 200 mg/kg, in contrast ethanol extract caused an increase in this parameter to the doses used. The alanine aminotransferase decreased with aqueous extract and increased with ethanol extract. Triglycerides decreased when used aqueous extract and reduced with ethanol extract at 100 and 200 mg/kg, in contrast to 50 mg/kg decreased to be compared with control group. **Conclusion:** The daily intake of *P. alliaceum* did not produce acute toxicity to 50 mg/kg which may be interpreted as toxic signs or biological damage, but liver and renal function changes at dosages of 100 and 200 mg/kg; however, the reduction ability of white blood cells count could be used as a basis for specific studies on the treatment of patients with leukemia.

1. Introduction

Man uses plant species for various purposes worldwide. World Health Organization[1] mentions that over 80% of the world population uses traditional medicine and emphasizes that medicinal plants used as an alternative to conventional drugs in the treatment of diseases need to be studied to determine its toxic side effects on human health. The use and study of medicinal plants has increased significantly[2-4] to determine which plants are the best to the synthesis of conventional drugs[5,6]. Above all, *Pseudocalymma alliaceum* (Lam.) *Sandwith* (Bignonaceae) syn: *Adenocalymma alliaceum* Miers., *Mansoa alliacea* (Lam.), *Bignonia alliacea* Lam., *Pachyptera alliacea* (Lam.) and *Pachyptera hymenaea* (DC.) is a woody vine belongs to the Bignoniaceae and comprises nearly 800 species and 104 genera[7]. Particularly, when leaves are disturbed it gives off a strong smell like garlic. This plant

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species has been studied to determine their chemical composition and biological activity[8], also because its economic importance to serve as a substitute for garlic in food. The leaves and flowers are widely consumed and used in traditional medicine for populations in South America[9]. However, exist few literary antecedents with enough scientific evidence that indicate some degree of security for their used. The properties that are attributed are analgesic, anti-arthritic, anti-inflammatory, antipyretic, antirheumatic, depurative, laxative and vermifuge[10-12], the dried leaves are used to treat colds, pneumonia, strep throat and respiratory disorders[13], nausea and constipation[14]. Rao *et al*.[15] analyzed volatiles compound from dried leaves of *P. alliaceum* finding diallyl disulfide, diallyl trisulfide, diallyl tetrasulfide and 1-octen-3-ol as major compounds, organosulfur compounds derived from allicin; the strong garlic aroma present in this plant species is due to naphthaquinones derived from lapachol[16]. The methanol extract of the stem of *Pseudocalymma* has shown cytotoxic activity against colon cancer cells[17]. Chirunthorn *et al*.[18] reported antioxidant and antimicrobial activity with petroleum ether and ethanol extracts. Based on the above this work was aimed perform toxicological evaluation of aqueous and ethanol extract of dried leaves of *P. alliaceum* by oral administration for 14 days in male Wistar rats, to determine the blood biochemical parameters which allowed to evaluate the haematological status, liver and renal physiology and cardiovascular risk parameters.

2. Materials and methods

2.1. Collection of plant material

The fresh leaves of *P. alliaceum* were collected from its natural habitat in San Pedro Comitancillo (16º 29' 20.67" N, 95º 09' 15.70" O) from the Isthmus region of Oaxaca, Mexico; a voucher specimen was deposited in the CIIDIR-OAX Herbarium. The leaves of the plant collected were cleaned, dried under shade at room temperature for three weeks and then milled into powder.

2.2. Preparation of crude extracts

The powdered dried leaves of *P. alliaceum* (300 g) was macerated in water for 24 h and alcohol 70% for 72 h in a flask, the extract was decanted and then filtered through Whatman No. 1 filter paper to obtain a clear extract. The ethanol extraction was further concentrated at 50 ℃ using a rotary evaporator to obtain the crude extract which was stored in a refrigerator maintained at 4 ℃ until used. Extracts were later reconstituted in distilled water to give the required doses of 50, 100 and 200 mg/kg body weight used in the present study.

2.3. Experimental animals

Twenty four male Wistar rats weighing between 200 and 230 g were obtained from the School of Medicine, Cristobal Colon University, Veracruz, Mexico; the rats were maintained in individual stainless steel cages at 20-25 , with relative humidity of 50%-60% and 12:12 h light/dark cycles. They were fed with a standard laboratory diet in a solid presentation (croquette) of Harlan - Teklad 2014 brand (14% protein-3.5% fat). Administration of the solid diet and water was ad libitum. The study was conducted following the ethical procedures using experimental animals set out in the technical specifications for production, care and use of laboratory animals of the Mexican National Standard (NOM-062-ZOO-1999).

2.4. Extracts administration and animal grouping

The animals were completely randomized into four groups of three rats each. Group I (control) received orally distilled water for 14 days while Groups II, III and IV were treated like the control except that they received 50, 100 and 200 mg/kg body weight of the plant extract for each extraction. The extract and distilled water were administered daily between 900 – 930 h.

2.5. Blood samples and serum preparation

After this period, the rats were subjected to 18 h fast. The rats were subsequently anaesthetized with Pentobarbital and blood sample was collected by cardiac puncture into EDTA for haematological studies and biochemical determinations. The samples were centrifuged at 1 086 rpm for 10 min to obtain the plasma and stored at – 20 ℃ until ready for analysis. The serum was carefully aspirated with Pasteur pipette into sample bottles.

2.6. Haematological parameters

The haematological parameters RBC: Red blood cell count, HGB: Haemoglobin concentration, HCT: Hematocrit level, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Cell Distribution Width, PTL: Platelets count, VPM: Platelet Volume Average, WBC: White blood cell count, NEU: Neutrophils, LIN: Lymphocytes MON: Monocytes, EOS: Eosinophil, BAS: Basophil were analyzed according to the standard techniques mentioned by Cheesbrough[19].

2.7. Determination of biochemical parameters

Phenotypic parameters of blood serum were determined as

cholesterol (COL), triglycerides (TRI), glucose (GLU), albumin (ALB), uric acid (AUR), urea (URE), creatinine (CRE), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using enzymatic-colorimetric methods and automated equipment DXC Synchron.

2.8. Statistical analysis

Experimental data were tested for normality error and homogeneity of variance, then proceeded to develop an analysis of variance and comparison using the multiple range test of Fischer (P<0.05); the results are expressed as mean of three replicates with standard deviation. Minitab 16.1.0 statistical package was used.

3. Results

3.1. Haematological parameters

No deaths were reported after oral administration of the extracts. No physical signs of toxicity or adverse effects to treatment were observed. The toxicology results of the aqueous extract in the parameters of the red and white series of treated rats are shown in Table 1. No variations were found in the values of RBC, HGB and MCH for extracts evaluated. A significant decrease in the results of HCT and MCV was presented with the extracts administered. The MCHC showed a significant increase with the aqueous extract at 200 mg/kg of 34.14 g/dL, rats treated with the ethanol extract recorded 33.37 g/dL with doses at 50 mg/kg, while the control group had 28.50 g/dL (Table 1).

Table 1
Effect of aqueous extracts of dried leaves of *P. alliaceum* on haematological parameters of male Wistar rats (mg/kg).

Parameters	Reference Value	Control group	Aqueous extracts			Ethanol extracts		
			50	100	200	50	100	200
RBC\ERI (10^{-6}/uL)	4.00-6.00	7.06±0.20^{a*}	6.89±0.60^{a}	6.75±0.90^{a}	6.94±0.57^{a}	7.11±0.18*	7.02±0.17*	7.35±0.28*
HGB (g/dL)	11.00-7.00	14.25±0.35^{a*}	14.40±1.45^{a}	13.85±1.41^{a}	14.17±0.18^{a}	14.69±0.44*	14.15±0.79*	14.66±0.16*
HCT (%)	35.00-50.00	49.95±1.25^{a*}	43.21±3.99^{ab}	40.49±6.13^{b}	41.59±1.97^{b}	44.02±1.34$^{\#}$	42.70±2.01$^{\#}$	45.32±1.70$^{\#}$
MCV (fL)	80.00-99.90	70.75±0.25^{a*}	62.70±0.90^{b}	59.87±1.59^{b}	60.05±2.93^{b}	61.97±2.86*	60.81±2.14*	61.67±1.09*
MCH (pg)	27.00-32.00	20.50±0.20^{a*}	20.89±0.35^{a}	20.58±0.83^{a}	20.50±1.78^{a}	20.68±1.02*	20.15±0.82*	19.97±0.56*
MCHC (g/dL)	32.00-37.00	28.50±0.10^{a*}	33.31±0.32^{b}	34.39±1.90^{b}	34.14±2.07^{b}	33.37±0.23*	33.13±0.34$^{*\#}$	32.38±0.88$^{\#}$
RDW (%)	10.00-20.00	13.20±0.30^{a*}	13.14±0.29^{a}	11.79±0.39^{b}	11.61±1.29^{b}	12.36±0.38$^{\#}$	12.40±0.28$^{\#}$	12.68±0.53$^{*\#}$
PTL (10^{-3}/uL)	150.00-450.00	579.00±2.00^{ab*}	561.25±51.75^{b}	679.50±79.50^{a}	635.50±60.50^{ab}	631.50±59.50*	626.00±29.00*	613.66±24.54*
VPM (fL)	6.00-10.00	10.60±0.10^{a*}	7.00±0.49^{bc}	7.44±0.26^{b}	6.53±0.39^{c}	6.61±0.41$^{\#}$	7.22±0.49$^{\#}$	6.74±0.19$^{\#}$
WBC (10^{-3}/uL)	5.00-10.50	5.40±1.36^{a*}	2.84±0.27^{bc}	3.15±0.42^{b}	1.69±0.12^{c}	3.14±0.20$^{\#}$	2.30±0.50$^{\#}$	3.17±0.89$^{\#}$
NEU (%)	50.00-80.00	12.25±2.05^{a*}	7.20±0.30^{b}	9.85±0.05^{a}	10.85±1.85^{a}	7.05±0.25$^{\#}$	5.25±0.65$^{\#}$	7.25±0.65$^{\#}$
LIN (%)	25.00-50.00	55.55±3.95^{b*}	91.00±0.40^{a}	86.70±5.85^{a}	86.60±1.50^{a}	88.85±1.25$^{\#}$	92.86±2.57$^{\#}$	90.25±0.05$^{\#}$
MON (%)	2.00-10.00	24.85±2.65^{a*}	1.45±0.05^{b}	2.05±0.45^{b}	1.90±0.20^{b}	2.80±0.20$^{\#}$	2.05±0.75$^{\#}$	2.75±0.05$^{\#}$
EOS (%)	0.10-5.00	1.65±0.05^{a*}	0.10±0.00^{b}	0.33±0.07^{c}	0.20±0.00^{d}	0.40±0.20$^{\#\triangledown}$	0.60±0.40$^{\#}$	0.10±0.00$^{\triangledown}$
BAS (%)	0.10-2.00	1.00±0.00^{a*}	0.30±0.10^{b}	0.33±0.05^{b}	0.45±0.15^{b}	0.25±0.05$^{\#}$	0.30±0.00$^{\#}$	0.36±0.05$^{\#}$

n=3, X±SD. Values with the same letter/symbol per row are not significantly different (P> 0.05). RBC: Red blood cell count, HGB: Haemoglobin concentration, HCT: Hematocrit level, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Cell Distribution Width, PTL: Platelets count, VPM: Platelet Volume Average, WBC: White blood cell count, NEU: Neutrophils, LIN: Lymphocytes MON: Monocytes, EOS: Eosinophils, BAS: Basophils.

The RDW showed statistical significance with the aqueous and ethanol extract, to administer the aqueous extract at 50, 100 and 200 mg/kg a reduction of 13.14%, 11.79% and 11.61% respectively was observed when compared with the control group that showed 13.20%. The PTL decrease with aqueous extract at 50 mg/kg to 561.25 × 10^{-3}/uL compared with the control group that recorded 579.00 × 10^{-3}/uL; platelets showed an increase using ethanol extract with three dosages employed but without statistical significance to be purchased with the control. The MPV showed a significant decrease when used the three doses and plant extracts. WBC showed statistical significance with aqueous and ethanol extract and presented a decrease in the values of 2.84, 3.15 and 1.69 ×10^{-3}/uL and 3.14, 2.30 and 3.17 ×10^{-3}/uL at 50, 100 and 200 mg/kg of ethqnol extracts respectively, while the control group reflected 5.40 × 10^{-3}/uL. WBC, neutrophils and monocytes showed significant decreases in haematological values to be compared with the control group using aqueous and ethanol extracts.

3.2. Biochemical parameters of blood

The toxicological results of aqueous and ethanol extracts on biochemical parameters are shown in Table 2. GLU showed an increase with the aqueous and ethanol extract of 189.50 and 160.66 mg/dL to 200 and 100 mg/kg respectively, the control group showed 103.33 mg/dL.

URE values increase to 47.91 mg/dL at 100 mg/kg with aqueous extract and decreased with doses at 200 mg/kg to 38.35 mg/dL, the ethanol extract was not statistically significant to be compared with the control group that showed 42.33 mg/dL. The value of CRE showed a significant increase with aqueous and ethanol extract at 100 and 200 mg/kg with values of 0.31 and 0.45 mg/dL respectively,

while control group obtained 0.26 mg/dL.

The AUR decrease to 0.96, 0.80 and 1.00 mg/dL at 50, 100 and 200 mg/kg when aqueous extracts was used, with ethanol extracts the values decreases to 0.76 and 0.83 in the first two doses, but with 200 mg/kg increase to 2.26 mg/dL, while the control group recorded 1.30 mg/dL.

A slight increase of COL values at 50 and 200 mg/kg of 106.33 and 114.00 mg/dL were observed with ethanol extract, while the aqueous extract provoked a decrease of 83.33, 89.50 and 93.50 mg/dL at 50, 100 and 200 mg/kg respectively and control group registered 97.33 mg/dL.

Triglyceride levels at 50 mg/kg decreased with ethanol extract and increased at 100 and 200 mg/kg to 172.33 and 181.33 mg/dL, these levels decreased with aqueous extract at 50, 100 and 200 mg/kg to 100.00, 115.00 and 129.00 mg/dL, while the control group showed 151.33 mg/dL (Table 2).

Table 2
Effect of aqueous extracts of dried leaves of *P. alliaceum* in biochemical values in blood of male wistar rats.

Parameters	Reference value	Control group	Aqueous extracts			Ethanol extracts		
			50	100	200	50	100	200
GLU(mg/dL)	65-110	103.33±12.01^{b}	179.33±29.28^{a}	165.50±2.50^{a}	189.50±2.50^{a}	158.33±8.32*	160.66±9.45*	160.33±11.50$^{*\#}$
URE(mg/dL)	15-43	42.33±5.68^{ab}	40.16±5.40^{b}	47.91±1.21^{a}	38.35±0.45^{b}	41.73±5.70*	38.36±1.70*	45.40±8.63*
CRE(mg/dL)	0.7-1.5	0.26±0.03^{b}	0.21±0.02^{c}	0.31±0.00^{a}	0.31±0.01^{a}	0.25±0.02*	0.30±0.05*	0.45±0.14$^{\#}$
AUR(mg/dL)	3.5-8.5	1.30±0.95^{a}	0.96±0.41^{a}	0.80±0.20^{a}	1.00±0.00^{a}	0.76±0.05$^{\#}$	0.83±0.30$^{*\#}$	2.26±1.20*
COL(mg/dL)	0-200	97.33±5.50^{a}	83.33±9.29^{b}	89.50±5.50^{ab}	93.50±1.50^{ab}	106.33±13.20*	91.33±2.08*	114.00±28.58*
TRI(mg/dL)	30-150	151.33±38.55^{a}	100.00±25.70^{b}	115.00±30.00^{a}	129.00±38.00^{a}	140.66±55.89*	172.33±27.22*	181.33±20.03*
AST(U/L)	15-46	150.00±8.00^{a}	132.66±46.50^{a}	135.50±42.50^{a}	173.00±82.00^{a}	186.00±43.86*	227.66±123.02*	447.00±184.00$^{\#}$
ALT(U/L)	13-66	70.00±21.00^{a}	70.33±12.58^{a}	59.33±5.51^{a}	63.00±9.00^{ab}	82.66±5.68$^{\#}$	121.66±70.81$^{*\#}$	182.66±63.31*
ALB(g/dL)	3.50-5.00	3.16±0.76^{b}	3.74±0.19^{b}	4.88±0.52^{a}	3.99±0.09^{ab}	4.11±0.18*	3.76±0.22$^{*\#}$	4.10±0.11*

n=3, X±SD. Values with the same letter/symbol per row are not significantly different (P> 0.05). GLU: glucose, URE: Urea, CRE: Creatinine, AUR: uric acid, COL: Cholesterol, TRI: Triglycerides, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALB: albumin.

The AST decrease to administered the aqueous extract at 50 and 100 mg/kg to 132.66 and 135.50 U/L and increased to 173.00 U/L at 200 mg/kg; records with the ethanol extract increased to 186.00, 227.66 and 447.00 U/L at 50, 100 and 200 mg/kg respectively, while the control obtained 150.00 U/L.

The values of ALT increased to 82.66, 121.66 and 182.66 U/L with ethanol extract at 50, 100 and 200 mg/kg respectively, while aqueous extract exhibited a decrease of 63.00 U/L at 200 mg/kg; the control group had 70.00 U/L. The ALB levels increased with aqueous and ethanol extract at 100 and 50 mg/kg to 4.88 and 4.11 g/dL compared to the control that registered 3.16 g/dL.

4. Discussion

Pseudocalymma is a plant species that contains various bioactive principles with pharmacological potential, which can cause beneficial and/or harmful effects on human health. Tomlinson and Akerele[20] mention that security should be one of the main criteria for selection of useful medicinal species in public health. Saad *et al*.[21] report that the concern of users for lack of scientific evidence has favored to conduct studies regarding the toxicity and harmful effects of plant species that are used by people as natural drugs.

In this study, it was found that the aqueous and ethanol extract of *P. alliaceum* administered orally for 14 days did not cause mortality in the treated rats. The haematological indices, RBC, HGB and MCH after administration of leaf extracts showed no significant abnormality, however decrease in levels of HCT, MCV, RDW, MPV, WBC, NEU, MON, EOS, and BAS and increase in MCHC, PTL and LIN parameters was observed. The biochemical parameters that increased were GLU, CRE and ALB, while URE decreased; AST values decrease with aqueous extract at 50 and 100 mg/kg and increased when increasing dose to 200 mg/kg, in contrast with ethanol extracts all doses increased the levels of this parameter. The ALT values decreased with aqueous extract and increased with the ethanol extract.

The fact that the RBC not show statistical significance using plant extracts, indicate that there is a balance between production and destruction of red blood cells; data recorded on HGB values show that the extract does not affect the red series, which allows a normal oxygen availability for lung and tissue function, and consequently cell function. Mitruka and Rawnsley[22] mention that the increase in the number of platelets stimulus is an indicative of the clotting factor biosynthesis and this may be useful in the treatment of bleeding. In the present platelet count increased by using aqueous extracts at 100 and 200 mg/kg and ethanol extracts at 50, 100 and 200 mg/kg. The decline in platelet count when used aqueous extract at 50 mg/kg (391.00 U/L) compared with the control group (579.00 U/L), indicates that the extract had an effect on platelet production causing an induced thrombocytopenia at this dose, suggesting that *P. alliaceum* has anti-hematopoietic activity as asserts Mdhluli[23]. Dahlback[24] mentioned platelets are in charge of the procedure to reduce blood loss and vascular injury repair known as hemostasis.

The plant species have chemical compounds with various active ingredients which make them useful[25], according with Harnafi and Amrani[26] reported that organic extracts of *P. alliaceum* contain

alkanes, alkanols, triterpenes, flavonoids and derivatives of lapachol and mentioned that plants containing flavonoids showed platelets action in the prevention of thrombosis. das Graças *et al*.[27] and Cao *et at*.[28] mentioned that flavonoids and polyphenols have antioxidant property and filed ability to lower blood lipid levels.

Gourley and Herfindal[29] mention that VCM is a parameter which serves as an indicator to determine microcytic and macrocytic anemia, because it is an index that measures the average volume of white blood cell[30].

Therefore, the VCM decreased with the aqueous extract which recorded 62.70, 59.87 and 60.05 fL and with the ethanol extract to 61.97, 60.81 and 61.67 fL at 50, 100 and 200 mg/kg respectively; indicates no hemolytic anemia, according to the reference range for laboratory parameter (80.00 - 99.90 fL), while the control recorded 70.75 fL.

Adebayo *et al*.[31] mention that a normal reaction of rats by administering substances foreign to the body is the increase in the white blood cell count as a defense mechanism of the body that alter normal physiological processes, however in the study leukopenia was observed with decreasing values of leukocytes at all concentrations and extracts used. Sacher and McPherson[32] report that the lymphocytes are mediators of specific immune response to pathogens, while neutrophils are responsible for phagocytosis; these are mechanisms of defense against invading microorganisms and removal of dead tissue.

In this study, the CRE significantly increased at doses of 100 and 200 mg/kg with extractions employed, this parameter being an indicator of renal impairment[33]. The CRE level decreased at 50 mg/kg when aqueous extract was used, which indicates that the extract was not toxic to the kidney at that concentration. The AST increased suddenly when ethanol extract to the three dosages were used, however with the aqueous extraction at 50 and 100 mg/kg decreased and an increase was recorded with 200 mg/kg. The AST is present in almost all organs, but when it is present in blood at very high levels means there has been cell destruction, as in the case of the results obtained using ethanol extracts in this study.

The ALT did not change when aqueous extracts was administered, but suffered an increase with the doses of ethanol extraction. The ALT is located in the liver and its function is the production of glucose. Transaminases are enzymes that are indicators of liver function and as biomarkers of toxicity and the increase of these enzymes in the blood indicating the existence of cell injury in the liver, heart, kidney or muscle[34].

Therefore, the results reported in this study indicate that P. alliaceum alters hepatic and renal function of rats treated with ethanol extraction and with the aqueous extract at 200 mg/kg.

The AST decreased at 50 and 100 mg/kg and ALT at 100 and 200 mg/kg with aqueous extracts possibly due to the presence of flavonoids in the extracts used, as mention Singab *et al*.[35] and Wu *et al*.[36] in studies with *Vicia calcarata* Desf. and *Laggera alata* (D. Don) which succeeded in reducing the levels of AST and provide protection to the liver. The increased level of URE at 100 and 200 mg/kg, when aqueous and ethanol extract were administered indicate azotemia respectively. The TRI at 100 and 200 mg/kg decreased to 100.00, 140.66, 122.00 and 106.33 mg/dL with the aqueous extract and ethanol respectively; COL values decreases with the aqueous extract at three concentrations evaluated denoting a possible cardioprotective action, similar results are reported by Verma *et al*.[37] where mention that the leaf extract *Pachyptera hymenaea* (DC.) (syn: *P. alliaceum*) evaluated in rats submitted hypolipidemic activity at low doses and antihyperlipidemic at high doses in normal rats and with hypercholesterolemia induced by 28 days. Srinivasan and Srinivasan[38] by incorporating dried flowers of *P. alliaceum* in the diet of experimental rats for six weeks observed a decrease in blood cholesterol.

In conclusion, the daily intake for 14 days of *P. alliaceum* did not produce acute toxicity to 50 mg/kg which may be interpreted as toxic signs or biological damage, but changes in liver and renal function at dosages of 100 and 200 mg/kg; however, the reduction ability of white blood cells count could be used as a basis for specific studies on the treatment of patients with leukemia.

Conflict of interest statement

We declare that we have no conflict of interest.

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