

Original Research Article

Acute and Sub-Acute Toxicity of Aqueous Extract of *Nauclea Latifolia* in Swiss Mice and in OFA Rats

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Received: 06 July 2012

Revised accepted: 16 December 2013

Abstract

Purpose: To determine the dose – toxicity profile of the aqueous extract of *Nauclea latifolia* stem bark (AQE).

Methods: Oncin France Souche A (OFA) rats were orally administered with AQE at doses of 1.8, 18 and 180 mg/kg body weight for 28 days. In parallel, oral acute toxicity test in Swiss mice was performed with AQE at doses of 2, 4, 8 and 18g/kg body weight. Blood, urine and other biochemical markers were assessed for the rats.

Results: No death was observed after 14 days of single oral administration, and hence the LD₅₀ was > 18g/kg body weight. For sub-acute toxicity in OFA rats, an elevation of some blood parameters (platelets and erythrocytes but also eosinophils) in contrast to the low serum concentrations of biochemical markers such as aminotransferases (ALT, AST) and creatinine were recorded in rats treated with 18 and 180 mg/kg body weight. Urine analysis showed high depletion of sodium and potassium ions coupled with high loss of water.

Conclusion: Known for its diuretic property, the AQE could be beneficial against anemia and may favor blood coagulation but unfortunately may exhibit allergenic properties and cause inflammatory reactions. This study suggests the no-observed-adverse-effect-level (NOAEL) of AQE range between 1.8 and 18 mg/kg body weight in OFA rats.

Keywords: *Nauclea latifolia*, Renal excretion, Hematological parameters, Biomarkers, Allergy, Inflammation, Diuretic, Toxicity

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Nauclea latifolia (Rubiaceae) commonly known as pin cushion tree is a straggling shrub or small tree native to tropical Africa and Asia and it grows up to an altitude of 200 m. Infusions and decoctions of parts of *Nauclea latifolia* are commonly prescribed traditionally as a remedy for diabetes mellitus and it has been reported that the aqueous extract of *Nauclea lalitifolia* had

been found to exert hypoglycaemic activity in alloxan-induced diabetic rats [1]. The plant is also used as a chewing stick [2] and in the treatment of ailments like gastrointestinal tract disorders, tuberculosis, sleeping sickness, prolongs menstrual flow, hypertension and malaria [3-9]. More recently, it had been reported the presence of biologically active principles in the extract with anti-nociceptive, anti-inflammatory and anti-pyretic activities that

justifies its use in malaria ethnopharmacy and subsequent development for clinical application [1]. Indeed, all plant parts of the *Nauclea* species are a rich source of monoterpene indol alkaloids namely indole alkaloid strictosamine [10-12] but also terpenes [13] saponines and active polyphenols [2]. Moreover, the ethanolic extract of *Nauclea latifolia* had been found to possess anti-hepatotoxic and trypanocidal activities [3]. Despite the numerous studies on pharmacology activities of aqueous or ethanolic extract or biologically active principles of *N. latifolia*, very few studies were reported on its systemic toxicity. The few data on the plant toxicity revealed that the LD₅₀ was higher than 1600 mg/kg/bw after intraperitoneal (IP) injection of mice [14] and alkaloid rich extracts from the *Nauclea latifolia* can interact *in vitro* with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage and cause single-strand breaks in liver, kidney and blood cells [15]. But, more recently, it has been reported that repeated administration for 28 days of high doses (200, 400, 600 and 800 mg/kg body weight) of aqueous extract of *Nauclea latifolia* evident stem may lead to damage in the kidney and liver [16].

In Côte d'Ivoire, the aqueous extract of stem bark of *N. latifolia* (AQE) is largely and daily used against malaria and its symptoms such as fever. In these circumstances, the toxicity of plant after oral repeated administration must be clarified. The available low dose toxicity profile of AQE is extremely limited. Short-term exposure to high doses of aqueous extract of plant induces various metabolic changes and toxic responses, however, extrapolation of these findings to the long-term and low-dose exposures to human are subject is highly problematic [16]. Since, additional experiments to investigate NOAEL are indispensables, OFA rats were orally administrated with 1.8, 18 and 180 mg/kg of AQE for 28 days. In parallel, we performed oral acute toxicity study in Swiss mice.

EXPERIMENTAL

Animals

For acute toxicity, the animals used were male Swiss albino mice from the Pasteur Institute of Adiopodoumé (Abidjan) and aged 5 to 8 weeks with weight between 20 and 25 g. The animals were acclimatized to the laboratory for 1 week prior to the test.

For sub-acute toxicity, male and female OFA rats (5 – 8 week old), weighing between 100 and 120 g were obtained from the Pasteur Institute of

Abidjan. The rats were housed in environmentally protected transparent polypropylene cages with stainless steel wire tops for a period of 1 week before commencement of the tests. The rats had free access to water. Experimental diets were placed in special containers to minimize spillage. Environmental conditions included 23 – 25 °C, relative humidity of 45 – 55 %, and a 12-h light/dark cycle.

Plant

The plant material consists of stem bark of the plant which was dried and ground into powder before diluting in water. The aqueous extract (AQE) was obtained by maceration for 24h as traditional used in Côte d'Ivoire. The extract was filtered and lyophilized and the dried residue was diluted appropriately with water for oral administration to the experimental animals.

Acute toxicity test

The acute toxicity study was performed according to OECD 401 Guidelines for testing of chemicals [17]. The animals were placed in special cages for 7 days for acclimatization before the experiment. At the end of the seventh day, the animals were subjected to fasting for 24 hours before oral administration of different doses of AQE. The mice were randomly divided into 5 groups of 3 female mice. Group I: normal control group, and the mice received water; Group II: 2 g/kg body weight AQE; Group III: 4 g/kg body weight AQE; Group IV: 8 g/kg body weight AQE; and Group V: 18 g/kg body weight AQE. After administration of the extract, the animals were observed every 30 min for 8 h on the first day and once daily for 14 days. During this period, the liveliness, sensibility to painful stimulation, visual acuity and texture of the faeces were assessed. The LD₅₀ of AQE was investigated based on the eventual observed mortality.

Sub-acute toxicity test

The protocol of sub-acute toxicity study performed according to from OECD 407 Guidelines for testing of chemicals [18]. The protocol was approved by the committee of Bioethics of Nangui Abrogoua University, Abidjan (Côte d'Ivoire). Total of 40 rats (20 males and 20 females) was randomly divided into 4 groups (n = 10 per group with 5 females + 5 males) and housed under standard husbandry condition for one week before the experiment and allowed with standard feed in solid (balls). The animals were divided into the following 4 groups: Group A: normal control group and mice received water;

Group B: 1.8 mg/kg body weight AQE; Group C: 18 mg/kg body weight AQE; and Group D: 180 mg/kg body weight AQE. Each group received the appropriate vehicle (water) and AQE daily by oral administration for 28 days. All the rats were weighed on a Sartorius balance (ED 224S, Germany), with 0.0001 mg accuracy, prior to treatment. Urine and blood were collected from the rats at the end of each week for urine and hematological analysis. After 28 days, blood and urine were again collected, all the animals euthanized using chloroform, and the tissues examined.

Urinalysis

Urine samples collected by refrigerating metabolic cages (Tecniplast, Bugugiate, Italy) were analyzed for pH, albumin, glucose, ketone body using dipsticks (Ames Labstix Bayer Diagnostic). In addition, the urinary sodium and potassium ions were analyzed using a PFP7 flame photometer (Jenway).

Evaluation of hematological parameters

The analysis of blood samples was performed using an analyzer, Beckman Coulter T540. The hematological parameters including the number of leukocytes, erythrocytes, hemoglobin (Hb) concentration and the platelet count were determined. White blood cells or leukocytes were analyzed using pap test with May-Grünwald Giemsa (MGG) blood staining for the determination of percentage (%) of leukocytes including lymphocytes, monocytes and phagocytes, and granulocytes (neutrophils, eosinophils and basophils).

Assessment of biochemical parameters

The serum biochemistry parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and urea were evaluated spectrophotometrically using an automated HumaStar 80 (Human GmbH, Germany).

Statistical analysis

The data generated were statistically processed using SPSS 11.19 statistical software. Analysis of variance (ANOVA), with Tukey post-hoc test to identify the variable(s) with very significant differences *vis-a-vis* the control group, was used to analyse the data from the evaluation of hematological and biochemical parameters. At 95% confidence interval, *p* values < 0.05 were considered to be statistically significant.

RESULTS

Acute toxicity

Following the administration of single dose of 2, 4, 8 and 18g/kg body weight of AQE to mice, no death was registered after 14 days post-treatment.

Sub-acute toxicity

General observation and body weight

At the end of the 4th week of the experiment (day 28), no variation of the vivacity, sensitivity to noise and to painful stimulation was noted in both experimental and control groups. In addition, the macroscopic observation of each animal's tissues was not revealed any changes but, the water was rich in the faeces of experimental animals and as compared to control, animals treated with AQE at doses 1.8 and 18mg/kg increased in body weights, in contrast to those treated with 180 mg/kg of extract (Table 1).

Biomarkers

The results of hematology and serum biochemical examinations are summarized in Tables 2 - 5. Serum creatinine, AST and ALT decreased in treated animals by AQE at doses of

Table 1: Weight of female and male rats after treatment with AQE at various doses for 4 weeks

Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28
Males					
Control	82±2.7	88±2.5	95.5±2	97.5±3	105±2.5
1.8	79±2.8	85±2.3	90.5±3	103±2.8	113±3*
18	78±2.5	88±3	91±3.2	94±3.6	100±3.2
180	80±3	87±2.4	99±2.2	101±3	93±3.2*
Females					
Control	80±2.3	86±3	90±2.9	91±2.9	102.5±3
1.8	78±2.4	84±2.6	96±3	105±3	115±2.7*
18	77±3	87±4	88±3.5	89±3.2	91±3*
180	81±2.8	88±3	89±2.7	91±3.2	86±3*

*Significantly different from control group (*p* < 0.05)

Table 2: Serum chemistry of OFA rats administered with AQE at doses of 1.8, 18 and 180 mg/kg on Day 28

Dose (mg/kg)	AST (IU/l)	ALT (IU/l)	Urea (g/l)	Créatinine (mg/dl)
Male				
Control	198.7±21	64.55±6	0.89±0.12	30.3±2
1.8	169.8±24	55.7±4.5	0.73±0.1	28.3±1.8
18	*115.2±15	*40.4±3.3	0.69±0.09	*20.5±2.2
180	*82.3±17	*32.2±7.1	0.89±0.15	*17.5±1.2
Female				
Control	163.5±10.6	65.6±8	0.62±0.08	35±3.8
1.8	161.5±14	51.5±6.3	0.65±0.06	28.33±3.2
18	*92.6±12	*40.9±3.2	0.69±0.04	*18.4±2.5
180	*62.6±9.8	*28.6±2.7	0.70±0.07	*16.4±1.5

*Significantly different from control group at $p < 0.05$ **Table 3:** Hematological data for male OFA rats treated with AQE for 4 weeks

Males (1)	Day 7				Day 14			
	AQE dose (mg/kg/bw)							
	0	1.8	18	180	0	1.8	18	180
Leukocytes	5.1±0.9	4.8±1.2	4.6±1	4.6±0.7	4.6±1.1	4.7±1.4	4.7±0.5	4.7±0.9
Neutrophils (%)	20	22	21	21	24	21	21	27
(10 ⁹ /l)	0.8	0.88	0.84	0.84	0.94	0.84	0.84	1.08
Eosinophils (%)	0	0	0	*2	0	0	0	*2
(10 ⁹ /l)	0	0	0	*0.08	0	0	0	*0.08
Basophils (%)	0	0	0	0	0	0	0	0
Monocytes (%)	0	0	0	0	0	0	0	0
Lymphocytes (%)	86	79	76	*74	74	78	78	71
(10 ⁹ /l)	4.3	4	3.8	3.7	3.7	3.9	3.9	3.6
Erythrocytes	3.1±0.3	3.6±0.4	2.9±0.2	3.1±0.4	3.7±0.2	3.7±0.3	4.1±0.5	4.2±0.4
Hemoglobin	6.4±0.4	8.2±0.9	5.7±0.4	5.5±0.6	7.3±0.5	7.3±0.3	8.1±0.6	5.5±0.4
Platelets	366±12	480±16	420±21	391±19	434±11	392±16	424±14	434±10
Males (2)	Day 21				Day 28			
	AQE dose (mg/kg/bw)							
	0	1.8	18	180	0	1.8	18	180
Leukocytes	4.8±1.1	4.9±1	4.9±0.9	4.9±1.3	4.8±0.9	4.8±0.7	4.7±1.2	4.7±0.9
Neutrophils (%)	23	25	*13	*16	14	16	*24	21
(10 ⁹ /l)	0.92	1	*0.52	*0.64	0.56	0.6	*0.94	0.84
Eosinophils (%)	1	1	0	2	0	0	0	*2
(10 ⁹ /l)	0.04	0.04	0	0.08	0	0	0	*0.08
Basophils (%)	0	0	0	0	0	0	0	0
Monocytes (%)	0	0	0	0	0	0	0	0
Lymphocytes (%)	76	77	*87	*84	85	84	*76	*76
(10 ⁹ /l)	3.8	3.9	4.4	4.2	4.3	4.2	3.8	3.8
Erythrocytes	4.3±0.5	4.5±0.4	4.7±0.6	4±0.3	4.8±0.2	4.7±0.4	4.8±0.3	*5.7±0.3
Hemoglobin	8.4±0.6	8.3±0.3	9.5±0.9	7.9±0.4	9.3±0.8	8.3±0.5	10.5±1	*11.5±1
Platelets	511±20	528±19	*764±9	*723±8	502±19	479±11	*574±9	*577±12

Bw = body weight; Leukocytes (10⁹/mm³); erythrocytes (10⁹/mm³); hemoglobin (g/dl); platelets (10⁹/mm³); *significantly different from control group at $p < 0.05$

18 and 180 mg/kg but the change was more pronounced at the latter dose. Erythrocytes, hemoglobin and platelets decreased at day 14 only in female rats treated at a dose of 18 mg/kg of AQE. On day 21, however, platelets increased in male rats at doses i.e. 18 and 180mg/kg body weight. Moreover, the rates of erythrocytes and hemoglobin were elevated at day 28 only for males at dose of 180mg/kg body weight. The number of lymphocytes increased at 21 day but decreased at days 7 and 28 in treated groups (18 and 180mg/kg body weight). Eosinophils were elevated in animals treated at a dose of 180

mg/kg over the period of the test. The number of neutrophils decreased on day 21 for animals that received high doses of AQE but increased at day 28 only with the highest dose in male rats, i.e., Group V (Tables 2 - 4).

The results of urinalysis showed no significant ketone, glucose and albumin in the urine of all the treated animals throughout the duration of the experiment. Urinary pH ranged from 8.5 to 9 while potassium and sodium ions (Tables 5 and 6) showed significant variation from control. High depletion of sodium ion was observed in all

Table 4: Hematological data for female OFA rats administered with AQE for 4 weeks

Females (1)	Day 7				Day 14			
	AQE dose (mg/kg)				AQE dose (mg/kg)			
	0	1.8	18	180	0	1.8	18	180
Leukocytes	4.4±0.6	4.8±0.5	4.2±0.3	*5±0.3	4.7±0.5	4.9±0.3	4.8±0.4	4.7±0.6
Neutrophils (%)	22	21	23	23	27	22	25	24
(10 ⁹ /l)	0.88	0.84	0.92	0.92	1.08	0.88	1	0.94
Eosinophils (%)	0	0	0	*5	0	0	0	*5
(10 ⁹ /l)	0	0	0	*0.2	0	0	0	*0.2
Basophils (%)	0	0	0	0	0	0	0	0
Monocytes (%)	0	0	0	0	0	0	0	0
Lymphocytes (%)	70	78	65	80	73	80	75	71
(10 ⁹ /l)	3.5	3.9	3.3	4	3.7	4	3.8	3.6
Erythrocytes	3±0.1	3.2±0.1	3±0.2	3.1±0.1	4.2±0.3	4.1±0.1	*2.8±0.2	3±0.1
Hemoglobin	5.4±0.3	6.6±0.2	5.3±0.1	5.5±0.1	7.9±0.4	8.2±0.5	*5.5±0.3	6.3±0.1
Platelets	424±19	471±16	452±15	456±12	449±20	378±16	*241±18	440±13

Females (2)	Day 21				Day 28			
	AQE dose (mg/kg)				AQE dose (mg/kg)			
	0	1.8	18	180	0	1.8	18	180
Leukocytes	4.7±0.8	4.8±0.5	5.1±0.9	4.9±0.8	4.8±0.6	4.8±0.9	4.7±0.7	4.7±0.6
Neutrophils (%)	26	20	*14	20	23	19	20	23
(10 ⁹ /l)	1.04	0.8	*0.78	0.8	0.92	0.76	0.8	0.92
Eosinophils (%)	1	2	0	*4	0	0	1	*7
(10 ⁹ /l)	0.04	0.08	0	*0.16	0	0	0.04	*0.28
Basophils (%)	0	0	0	0	0	0	0	0
Monocytes (%)	0	0	0	0	0	0	0	0
Lymphocytes (%)	72	78	*86	78	77	81	78	70
(10 ⁹ /l)	3.6	3.9	*4.3	3.9	3.9	4.1	3.9	3.5
Erythrocytes	5±0.2	3.8±0.2	4.2±0.5	4.6±0.3	5.6±0.3	5.6±0.2	5.4±0.2	5.5±0.4
Hemoglobin	9.3±0.5	7.1±0.3	7.7±0.3	9.1±0.5	12.1±1	11.9±0.8	11.3±1	11±0.9
Platelets	502±17	449±12	549±15	*739±9	573±13	607±17	590±18	554±16

Leukocytes (10³/mm³); erythrocytes (10⁶/mm³); hemoglobin (g/dl); platelets (10³/mm³); *significantly different from control group at p <0.05

Table 5: Urinary concentrations of sodium ((Na⁺) and potassium (K⁺) ions for OFA male rats administered with AQE

Males Dose (mg/kg)	Day 7		Day 14		Day 21		Day 28	
	Na ⁺ (mEq/l)	K ⁺ (mEq/l)						
Control	68.7±4	113.6±8	58.3±3.7	119±14	72.1±10	167.5±24	56±7	158±16
1.8	66±5	116.5±7.3	64.7±5	141.7±16	62.17±7.5	139.8±21	51±6.5	147±20
18	66.8±4.6	103.1±7	76.2±4.3	146.8±14	60.74±6.2	147±42	63.7±9	132.2±19
180	*50.2±3.4	*74.2±6	*82.7±4.7	*90±4.3	*34.3±5.6	*59.3±9.7	*91.8±8.6	143.4±22

*Significantly different from control group at p <0.05

Table 6: Urinary concentrations of sodium ((Na⁺) and potassium (K⁺) ions for OFA female rats administered with AQE

Females Dose (mg/kg)	Day 7		Day 14		Day 21		Day 28	
	Na ⁺ (mEq/l)	K ⁺ (mEq/l)						
Control	40.9±7.9	144.2±21	80.7±9	128±9.2	108±18	131.2±8.2	53.7±4	126.7±9.2
1.8	*81.1±9.6	131±23	72.6±6.5	120.9±7.5	104±13	135.7±14	47±4	129±10
18	*80.4±12	125.7±17	71.4±8	135.1±9	94±17	150.3±22	79.3±4	119.96±9
180	*107.7±13	*211.2±29	*58.6±4.5	*101±5.5	*34.6±11	*89.5±7.6	*91.8±4	144.3±12

*Significantly different from control group at p <0.05

female rats treated by AQE at day 7 and this high depletion was also observed on day 28 only in the female rats that received 180 mg/kg but decreased on days 14 and 21, compared to control. At the same dose, in male rats, sodium ion levels were elevated on days 14 and 28 but

diminished on days 7 and 21. For potassium ion, at dose of 180mg/kg body weight, the values of urinary concentration decreased only on days 7, 14 and 21 in males and on days 14 and 21 in female rats, but showed an elevation on day 7.

DISCUSSION

The maximum acute dose of AQE administered (18 g/kg) is 7 times greater than 2.5 g/kg recommended for screening of plant toxicity [19]. For comparison, it was found that the LD₅₀ of mebendazole was 640 mg/kg body weight in mouse [20]. The results of this study are supported by previous findings obtained from intraperitoneal and oral administration to mouse of both aqueous and ethanol extracts of the root and stem bark with the highest dose of the plant at 16 g/kg [14,21]. Therefore, the results of acute toxicity in the present study confirm the low toxicity of a single consumption of the stem bark extract of *Nauclea latifolia* as previously reported [14,21].

Although acute toxicity helps to determine substances that are non-toxic, it is important to assess sub-acute toxicity profile because it helps to evaluate morphological and physiological changes in organs. Toxicological evaluation by repeated doses have been generally accepted as a fundamental test for safety assessment. Although there are previous reports on the toxicological profile of AQE [16], however, available toxicological profiles of low doses of the plant including hematological parameters and NOAEL are limited. Furthermore, there is controversy over the sub-acute toxicity of AQE in rats [16,22]. It has previously been reported that the aqueous extract of *N. latifolia* did not significantly affect the liver, kidney, heart and hematological system of rats but could cause reduction in feeding and water consumption as well as body weight at the highest dose tested [22]. However, more recently, evident liver and kidney toxicities have been revealed [16].

In the present study, the mean gain in weight was high in groups which received AQE which may be due to the beneficial effect of the extract. After the consumption of AQE, animals showed a slight stimulation of appetite for food and water. It is known that in addition to their therapeutic properties, medicinal plants can affect positively the nutritional status of an animal [23]. However, the highest dose of AQE caused serious weight loss in the rats which could be explained by the high urinary excretion of water to the diuretic properties of *N. latifolia*.

With the exception of serum urea, all serum parameters analyzed, namely, creatinine, ALT and AST decreased in a time- and concentration-dependent manner. Since, these serum parameters were analyzed only at the end of the experiment, i.e., day 28, the high excretion of these parameters coincided with the very high

urinary excretion of blood sodium and potassium ions. The kidney plays a very important role in the removal of metabolic wastes from the blood stream. Its functionality, therefore, can be assessed among many others by determining the serum concentration of excretory constituents [24]. The significant decrease in serum creatinine, sodium ion and potassium ion was probably linked to metabolic alkalosis as revealed by the values of urine pH of 8 - 9 for the 4 weeks of study.

AST and ALT levels in the groups which received high doses of the plant extract (18 and 180 mg/kg) for 28 days may be suggestive of an alteration in the secretory and excretory functions of the kidney [24,25] caused by the extract of *N. latifolia* stem. Our findings are partially supported by a previous report [16] which found a decrease in serum creatinine and urea but increase in serum AST and ALT in rats treated with the aqueous extract of *N. latifolia*. The authors concluded that the extract of the plant may not only adversely interfere with amino acid metabolism of the liver but also cause a destruction of liver tissue in a time- and concentration-dependent manner [16]. That could explain the elevation of serum ALT and AST in contrast to the decrease in these serum parameters in the present study. The doses of AQE used in the present study (1.8, 18 and 180 mg/kg) when compared to those previously used (200, 400, 600 and 800 mg/kg) [16] are not high enough to cause damage to liver tissue based on serum AST and ALT elevation.

The effect of AQE on erythrocytes, hemoglobin and platelets was slightly beneficial at 18 and 180 mg/kg doses on days 21 and 28. AQE probably exerts activity against anemia and may also enhance the blood coagulation. The effect of AQE on lymphocytes count was beneficial on day 21 of the experiment but on day 28, caused a reduction in lymphocytes indicating possible lymphocyte cell death. Moreover, AQE induced eosinophilia which occurs in some allergic diseases and infestations by large parasites. More likely, however, AQE may contain biologically active principles with allergenic properties. AQE modulated (diminished and increased) time-dependently neutrophil count; elevation of neutrophils could indicate inflammatory diseases induced by AQE or could have been a killer of neutrophils.

CONCLUSION

Oral exposure of acute doses of AQE did not cause mortality. Low doses, however, led to weight gain. Although AQE may possess

beneficial effect against anemia and favor blood coagulation (platelets elevation); unfortunately, it seems that it may contain principles that exert that have allergenic and inflammatory properties.

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