Original

Evaluation of Haematological and Biochemical Parameters of Aqueous Seed Extract of *Solenostemma argel* in Male Albino Rats

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Abstract

The present study was conducted to evaluate the toxicity/safety potential of *Solenostemma argel* seeds aqueous extract at three different doses (700, 500 and 200 mg/Kg b.w) in albino rats using haematological and biomedical parameters. Male albino rats were used in this study; the rats were divided into four groups. The blood parameters measured were: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, Lym %, Gran %, Mid %, Lym #, Gran #, Mid #, PLT, MPV, PDW, PCT, P.LCR, P.LCC , RDW-CV and RDW-SD, whereas the biochemical parameters measured were : AST, ALP, ALP, Glucose , total cholesterol and triglycerides. Our study finding demonstrated that aqueous extract of *S.argel* seed at doses

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700 and 500 mg/Kg b.w caused reductions in MCV, Gran %, Gran #, PCT, PLT, P-LCC and RDW-SD, whereas at dose 200 mg/Kg b.w caused reductions in PLT, PCT and P-LCC. The extract caused increase in WBC (except at 700 mg/Kg b.w), HGB, HCT, Lym%, PDW, P-LCR, Lym # (except at dose 700 mg/Kg b.w). The rats groups at three doses showed non-significant change in liver function tests parameters (AST,ALT and ALP), whereas as it showed significant change in Glucose, triglycerides and total cholesterol (except at dose 200 and 500 mg/Kg b.w. Consumption of this plant may have tremendous impact on subjects suffering from hyper-triglyceridermia. This supports the safety use of the aqueous extract of *S.argel* in pharmacological studies.

Keywords: *Solenostemma argel* , Seeds, Aqueous extract, Haematological parameters, Biochemcal parameters, Albino rats.

Introduction

Plants have been a source of medicine for many years and medicinal plants play a crucial role in the primary health care of more than 80% of the population in developing countries. *Solenostemma argel* is a perennial herb that is commonly used in Sudan and folkloric medicine for the treatment of cold, diabetes and gastrointestinal cramps. It is also used for the treatment of respiratory disorders, rheumatism, stomach pain and febrifuge. Leaves are commonly used in traditional medicine as a purgative, antipyretic and antispasmodic remedy [1-5].

The topical anti-inflammatory activity of *Solenostemma argel* leaves was evaluated. Several compounds of argelosides and stemmosides have been shown to reduce cell proliferation in a dose-dependent manner in different human and mouse tumour models. Different extracts of the aerial parts showed hepatotoxic and nephrotoxic effects on albino rats.

From the pericarps, hairy seeds and leaves contains 14,15-secopregnane glycosides, namely argelosides A-J, and nine 15-ketopregnane glycosides, namely stemmosides C-K, have been isolated. Argelosides A-J. Stemmosides D-J. The antiproliferative activity of argelosides and stemmosides compounds against Kaposi's sarcoma cells was evaluated [5-7].

A range of 14,15-secopregnane glycosides, argelosides, were isolated from different parts of the plant. From the fruits argelosides A–B were isolated, from the seeds argelosides C–J and from the leaves argelosides K–O. From different parts also a range of 15-keto pregnane glycosides were isolated. Stemmosides A–B from the leafy stem, stemmosides C–D from the pericarp, and stemmosides E–K from the leaves. Also found in the leaves were β-sitosterol and the pentacyclic triterpenoids α-amyrin and β-amyrin [5, 8-12].

The lack of records of previous toxicological studies of the seeds extracts of *Solenostemma argel* stimulated us to initiate them through the present research work. The objectives of this research work were: (1) To assess the effect of aqueous extracts of

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Solenostenmma argel seeds at concentrations (doses) 700, 500 and 200 mg/Kg body weight in albino male rats when administered for 14 days. (2) To study the effect of *S.argel* seeds extracts on biochemical parameters (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP), Glucose, total cholesterol and triglyceride in albino male rats compared to representing control. (3) To study the effect of *S.argel* seed extracts on haematological parameters (WBCs, RBCs. HGB, HCT, MCV, MCH, MCHC, Lym, Gran, Mid, PLT, MPV, PDW, PCT, P-LCCR, P-LCC, RDW-CV and RDW-SD) in albino male rats compared to representing control.

Experimental:

Plant material

The seeds of *Solenostemma argel* (Del.) Hayne (Apocynaceae) were collected in the month of September from Abu Hasheem region, River Nile State, Northern Sudan. The plant material was authenticated by Prof. Hatil Hashim EL-Kamali. A voucher specimen of *S.argel* (Voucher no: BD/FST/OIU/ 9/2020-214) was deposited in the Departmental herbarium for future reference.

Preparation of Aqueous Extract: In extraction, the *dried S.argel seeds* were powdered. Three-hundred gram of powdered plant material was taken, mixed with 1500 ml of water and kept for 2 days in an orbital shaker. The mixture was then centrifuged at 2500 × *g* and the supernatant collected was concentrated under the reduced pressure in a rotary evaporator. The concentrated extract was then lyophilized. The residue obtained was used for the study, and the remaining residue was kept at –20°C for future use.

Animals and experimental set-up:

Albino rats weighing about 150–180 g were obtained from the animal house of Faculty of Veterinary Medicine, Khartoum University, and used for the study. Rats were housed at constant temperature of 21+ 2°C, humidity (55 + 10%) with a 12-h light, 12-h dark cycle and fed on standard pellets with free access to distilled water. The study was approved by Ethical Committee constituted for the purpose of Control and Supervision of Experiments on Animals, Omdurman Islamic University.

Parameters investigated [13-17]

The animals were divided into four groups of four, five animals each. Different doses of aqueous extract of *S. argel* corresponding to 200, 500 and 700 mg/kg were given orally in an intra-gastric tube to three groups of five animals each. Another group of five rats that received 1 ml of physiological saline served as control continuously for 14 days. At the end of experimental period, all the rats from each group were sacrificed under mild chloroform anesthesia. Through cervical decapitation, 5 ml of blood was collected from each rat, out of which 1 ml mixed with Dipotassium ethylene diamine tetra acetic acid was used for hematological studies. The remaining 4 ml blood collected was allowed to clot and the serum separated was used for the evaluation of biochemical parameters.

A/ Haematology:

Twenty animals were randomly divided into four groups with each group five rats. The four groups of rats were subjected to the following oral treatments once a day for 14 days:

Group I : Served as normal control were received distilled water

Group II , Group III and Group IV rats were received 700, 500 and 200 mg/kg b.w of *S.argel* aqueous extract for 14 days , respectively.

The experiment was conducted for 14 days during the experiment, readings was taken in 14 days.

Rats were anaesthetized in chloroform-saturated chamber. Animal in test and control group were sacrificed on the 14 days. Five micro liters' blood was taken from animal eyes to determine haematology parameters. The samples were collected into two different containers. The first container was a plain bottle containing no anticoagulant with the sample used for biochemical determination while the second container containing plasma is an automatic multi-pair blood cell counter for in vitro diagnostic use in clinical laboratory. It performs speedy and accurate analysis of blood parameters and detects the abnormal samples.

Parameters that were recorded included hemoglobin (HGB), Red blood cells(

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RBCS) , White blood cell (WBC) hematocrit (HCT), mean corpuscular hemoglobin and (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), Lymphocytes(Lym): Lym % and Lym # , Gran % and Gran #, Mid % and Mid # , platelet (PLT) , MPV, PDW, P.LCR, P.LCC, RDW.CV and RDW.SD. EDTA anticoagulant was used for haematological parameters. Blood samples were analyzed using established procedures and automated Sysmex KX-21 hematology analyzer.

B/ Biochemistry

Plasma was collected after centrifugation at 3000r.p.m for ten min at 400 C and divided into aliquots to avoid freezing and thawing. Aliquots were stored at 200 C pending assay. Plasma was used for determination of liver function tests (ALT, AST and ALP) , glucose and cholesterol and triglycerides.

1. Determination of Aspartate Aminotransferase (AST)

A. Principle:

Aspartate amino transferase catalyzes the transfer of the amino group from Aspartate to 2exoglutarate forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm, by means of the malate dehydrogenase (MDH).

Serum and plasma collected by standard procedure. Aspartate aminotransferase in serum and plasma stable for 7days at 2-80C, use heparin as anticoagulant.

B. Procedure

The working reagent and the instrument was adjusted reaction temperature and was Pipette into a Cuvette. The reagent was mixed and the Cuvette was inserted into the photometer. Start the stopwatch. After 1minute the initial absorbance was record and at 1minute interval thereafter for three minutes.

The difference between consecutive, and the average absorbance difference per minute was calculated (ΔA/min).

C. Calculation

The AST concentration is calculated using the following general formula:

$$\Delta A/min \times \frac{Vt \times 10^6}{E \times I \times VS} = U/L$$

The molar absorbance (E) of NADH at 340nmis 6300, the light path (I)is 1cm, the total reaction volume (VT) is 1.05 at

370C and 1.1 at 300C, the sample volume (Vs) is 0.05 at 370C and 0.1at 300C, and 1 U/L are 0.0166µkat/L.

2. Determination of Alanine Aminotransferase (ALT)

A. Principle:

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from Alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm. by means of the lactate dehydrogenase (LDH)

B. Procedure:

Serum and plasma collected by standard procedures, (ALT) in serum and plasma was stable for 7 days at 2-80C.Use heparin or EDTA as anticoagulant.

The working reagent and the instrument was adjusted reaction temperature. And was pipette into a Cuvette. the reagent was Mixed and the Cuvette was inserted in to photometer. After 1minute the initial absorbance was record and at 1 minute intervals thereafter for three minutes.

The difference between conscuvetite absorbance and the average absorbance difference per minute was calculated .

C. Calculation:

The ALT concentration in sample is calculated using the following general formula:

$$\Delta A \times \frac{Vt \times 10^6}{E \times I \times VS} = U/L$$

The molar absorbance (E) of NADH at 340nmis 6300, the light path (I)is 1cm, the total reaction volume (V) is 1.05 at 370C and 1.1 at 300C, the sample volume (Vs) is 0.05 at 370C and 0.1at 300C, and 1 U/L are 0.0166µkat/L.

3. Determination of plasma Alkaline Phosphates

A. Procedure:

Firstly, three test tubes were labeled as blank, sample and standard tubes. Then 0.025ml from the sample was taken into sample tube and 0.025 ml from the standard phenol was taken in the standard tube. later 0.5ml of buffer substrate was added to each tube, after that the tube were incubated at 370C for twenty minutes then 0.25 ml of enzyme inhibitor,

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was added to each tube and the tubes were mixed well and 0.25 ml of the color reagent was added to each tube. Finally, the tubes were left for five minutes at room temperature and the absorbance of the standard and sample were read against bland at 510 MN.

B. Calculation:

Enzyme activity (IU/I) =absorbance of sample /absorbance of standard

4. Determination of blood glucose level

Recently, enzymatic methods, using the highly specific enzyme glucose oxidase, were developed for the routine.

The glucose oxidase (E.C. l.l.34) is a FAD conferment containing enzyme that catalyzes the oxidation of β-D glucose to gluconolactone. It is isolated from molds, which also contain the mutarotase enzyme which enhances the conversion of β-D glucose into the ß-D glucose form. Stoichiometric amount of H2O2 is also formed in the reaction. With the use of a third enzyme, peroxydase (E.C.l.ll.l.7.), in a coupled reaction the H2O2 is transformed into H2O while the necessary hydrogen's are removed from an organic substrate molecule (e.g. Ortho-dianisidine). The oxidized form of ortho-dianisidine is a colored compound and its amount can be determined spectrophotometrically.

A. Procedure:

One ml per chloric acid was pipette into a test tube and add 0.1 ml blood sample to the tube. Mix it well and centrifuge it (5 min. at 5000 RPM) to remove the precipitated proteins. Aliquots of the supernatants was used in the assay.

B. Calculation

Blood glucose (Mm) $= \frac{sample}{standard} \times concentration\ of\ standard$

* Since the concentration of glucose standard = 0.5 mM

5. Determination of total blood cholesterol

A. Principle:

Cholesterol is a constituent of all biological membranes and thus an essential lipid. Increased cholesterol levels represent a major risk factor for arteriosclerotic, particularly coronary heart disease.

The cholesterol concentration in a blood sample was determined photo metrically by color indicator reaction.

B. Procedure

Preparation of reagent. The reagent is ready for use. The standard is ready for use. It is included in the package with the reagent. Bring reagent to room temperature, following the instructions in the package leaflet.

10 µl of the respective sample or, in case of the reagent blank value, distilled water are mixed with 1000 µl of the reagent directly in the graduated Cuvette. After incubating at room temperature for 10 minutes, the extinction is to be measured against the reagent blank value within 60 minutes.

Following enzymatic hydrolysis of the cholesterol esters, the entire cholesterol complex is oxidized by oxygen from the air. In this process, hydrogen peroxide is generated, which, in combination with phenol and 4-aminophenazon under the effect of peroxydase, produces a red dye, the extinction of which is determined at 546 nm. The intensity of its colour is directly proportional to the cholesterol concentration and is calculated with the aid of a standard.

C. calculation:

$cCh\ [mgdl/\mathrm{L} = \Delta E\ Sample\ /\Delta E\ Sta. \cdot cStandard\ [mgdl/l$

* $cCh\ [mgdl/\mathrm{L}.\ 0.02586 = cCholesterol\ [mmoll/]$.

6. Triglycerides estimation :

Triacylglycerols in the sample originate, by means of coupled reactions described below, a colored complex that can be measured by spectrophotometry method.

Triglyceride + H2O $\xrightarrow{\text{Lipase}}$ glycerol + fatty acids.

Glycerol + 3 ATP $\xrightarrow{\text{Glycerol kinase}}$ Dihydroxyacetone phosphate + H2O.

2H2O + 4-aminoantipyrine + 4-chlorophenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + 4H2O.

Contents and composition of reagents:

1. Reagent (A) contains:

Buffer	45 mmol/L
Chlorophenol	6 mmol/L
Magnesium chloride	5 mmol/L
Lipase	> 100 U/ml
Glycerol kinase	> 1.5 U/ml
Glycerol 3-oxidase phosphate	> 4 U/ml
Peroxidase	> 0.8 U/ml
4-aminoantipyrine	0.75 mmol/L

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ATP	0.9 mmol/L
PH	7.0

2. Triglyceride standard: glycerol equivalent to 200 mg/dl triolein.

Procedure:

First three test tubes were labeled as blank, standard and sample tube, then 10 ml from sample was taken into sample tube. Later 10 ml from the triglyceride standard was taken in the standard tube. After that 1.0 ml of the reagent was added to each tube, they were mixed well and incubated for 15 minutes at room temperature, and the absorbance of the standard and sample was read against blank at 500 nm.

Calculation:

Triglyceride concentration = absorbance of sample /absorbance of standard x concentration of standard.

Statistical Analysis

Results are expressed as the Mean ± SE for each group. Statistical significance was evaluated by Student s t-test using SPSS version 10.0. A value of $P<0.05$ was considered to indicate a significant difference between groups.

Results and Discussion

The effect of administration of aqueous extracts of *Solenostemma argel* seeds at 700, 500 and 200 mg/Kg body weight for 14 days on haematological and some biochemical parameters in male albino rats were investigsated.

Haematological parameters:

The effect of aqueous extract of *Solenostemma argel* seeds on haematological parameters after treatment of male albino rats for 14 days:

(1) At 700 mg/Kg b.w :

The rats of GP II treated with 700 mg/Kg b.w of seed extract after 14 days were showed significant change ($P<0.05$) in PLT, MPV, PDW, PCT, P-LCR and P-LCC parameters compare to control group (GP I) , whereas showed no significant change ($P<0.05$) in WBC, RBC, HGB, HCT, MCV, MCH, MCHC, Lym %, Gran %, Mid %, Lym #, Gran #, Mid # RDW-CV and RDW-SD compare to control group (Table 1).

(2) At 500 mg/Kg b.w

The rats of GP III treated with 500 mg/Kg b.w of seed extract after 14 days were showed significant change ($P<0.05$) in PLT, MPV, PDW, PCT, P-LCR and P-LCC parameters compare to control

group (GP I) , whereas showed no significant change (P<0.05) in WBC, RBC, HGB, HCT, MCV, MCH, MCHC, Lym %, Gran %, Mid %, Lym #, Gran #, Mid # RDW-CV and RDW-SD compare to control group (Table 2).

(3) At 200 mg/Kg b.w

The rats of GP IV treated with 700 mg/Kg b.w of seed extract after 14 days were showed significant change (P<0.05) in PLT, MPV, PDW, PCT, P-LCR and P-LCC parameters compare to control group (GP I) , whereas showed no significant change (P<0.05) in WBC, RBC, HGB, HCT, MCV, MCH, MCHC, Lym %, Gran %, Mid %, Lym #, Gran #, Mid # RDW-CV and RDW-SD compare to control group (Table 3).

Biochemical parameters:

The effect of aqueous extract of *Solenostemma argel* seeds on biochemical parameters after treatment of male albino rats for 14 days:

1. At 700 mg/Kg b.w :

The rats of GP II treated with 700 mg/Kg b.w of seed extract after 14 days were showed no significant change (P<0.05) in ALP, AST and ALT parameters compare to control group (GP I) , whereas showed significant change (P<0.05) in glucose , total cholesterol and triglycerides compare to control group (Table 4).

2. At 500 mg/Kg b.w :

The rats of group (GP III) treated with 500 mg/Kg b.w of seed extract after 14 days were showed significant change (P<0.05) in glucose and triglycerides , whereas showed no significant change in ALP, AST, ALT and total cholesterol parameters compare to control group (Table 5).

3.At 200 mg/Kg b.w :

The rats of group (GP IV) treated with 200 mg/Kg b.w of seed extract after 14 days were showed significant change (P<0.05) in glucose and triglycerides , whereas showed no significant change in ALP, AST, ALT and total cholesterol parameters compare to control group (Table 6).

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Table 1. The effect of Comparison of control and *S. argel* seed aqueous extract at dose 700 mg/Kg b.w on haematological parameters in male rats for 14 days

Parameters	**Control**	**Treatment**	**t-value**	**p-value**	**S (at P < 0.05**	**Change**	**%**
WBC 10^3/µL	9.02±1.33	8.54±1.43	0.24	0.41	NS	Decrement	5.32
RBC 10^6/µL	7.00±0.89	7.58±0.32	-0.56	0.30	NS	Increment	8.28
HGB g/Dl	11.90±1.52	12.92±0.45	-0.58	0.29	NS	Increment	8.57
HCT %	49.18±6.60	51.55±2.34	-0.31	0.38	NS	Increment	4.81
MCV Fl	70.08±1.66	67.90±0.62	1.11	0.15	NS	Decrement	3.11
MCH Pg	17.04±0.12	17.05±0.14	-0.05	0.48	NS	Increment	0.05
MCHC g/Dl	24.36±0.55	25.10±0.35	-1.06	0.16	NS	Increment	3.03
Lym %	58.46±8.14	65.30±5.67	-0.65	0.27	NS	Increment	11.7
Gran %	23.06±6.58	18.12±1.10	0.66	0.27	NS	Decrement	21.42
Mid %	18.48±3.05	19.08±3.13	-0.13	0.45	NS	Increment	3.24
Lym # 10^3/µL	4.97±0.59	5.46±0.76	-0.52	0.31	NS	Increment	9.85
Gran # 10^3/µL	2.31±0.80	1.38±0.37	0.97	0.18	NS	Decrement	40.25
Mid # 10^3/µL	1.74±0.38	1.70±0.49	0.06	0.48	NS	Decrement	2.29
PLT 10^3/µL	594.20±79.49	340.50±85.95	2.16	0.03	S	Decrement	42.69
MPV Fl	7.18±0.15	7.82±0.18	-2.82	0.01	S	Increment	8.91
PDW Fl	7.66±0.26	9.80±0.59	-3.61	0.004	S	Increment	27.93
PCT %	0.42±0.054	0.26±0.059	2.02	0.04	S	Decrement	38.09
P.LCR %	11.60±0.78	15.20±1.22	-2.59	0.02	S	Increment	31.03
P.LCC 10^9/L	66.60±6.23	48.50±14.27	1.92	0.048	S	Decrement	27.17
RDW.CV %	17.38±0.27	17.48±0.25	-0.25	0.40	NS	Increment	0.57
RDW.SD Fl	49.20±1.75	47.62±0.83	0.75	0.24	NS	Decrement	3.21

Values are expressed as mean ± SE

*Significant difference at P<0.05

Table 2. The effect of Comparison of control and *S. argel* seed aqueous extract at dose 500 mg/Kg b.w on haematological parameters in male rats for 14 days

Parameters	**Control**	**Treatment**	**t-value**	**p-value**	**S (at P < 0.05**	**Change**	**%**
WBC 10^3/µL	9.02±1.33	13.99±2.34	-1.85	0.051	NS	Increment	55.09
RBC 10^6/µL	7.00±0.89	8.64±0.22	-1.78	0.056	NS	Increment	23.42
HGB g/Dl	11.90±1.52	14.82±0.34	-1.66	0.071	NS	Increment	24.5
HCT %	49.18±6.60	57.60±1.97	-1.22	0.129	NS	Increment	17.12
MCV Fl	70.08±1.66	66.76±1.30	1.58	0.077	NS	Decrement	4.73
MCH Pg	17.04±0.12	17.12±0.12	-0.47	0.326	NS	Increment	0.46
MCHC g/Dl	24.36±0.55	25.68±0.53	-1.73	0.061	NS	Increment	5.41
Lym %	58.46±8.14	56.44±5.99	0.199	0.423	NS	Increment	49.64
Gran %	23.06±6.58	28.12±5.46	-0.59	0.285	NS	Increment	21.94
Mid %	18.48±3.05	15.44±2.13	0.817	0.219	NS	Decrement	16.45
Lym # 10^3/µL	4.97±0.59	7.79±1.26	-2.03	0.039	NS	Increment	56.74
Gran # 10^3/µL	2.31±0.80	4.14±1.27	-1.22	0.129	NS	Decrement	79.22
Mid # 10^3/µL	1.74±0.38	2.06±0.31	-0.67	0.262	NS	Decrement	18.39
PLT 10^3/µL	594.20±79.49	439.60±72.42	1.438	0.094	S	Decrement	26.61
MPV Fl	7.18±0.15	7.62±0.11	-2.39	0.218	S	Increment	6.12
PDW Fl	7.66±0.26	9.16±0.29	-3.86	0.002	S	Increment	19.58
PCT %	0.42±0.054	0.34±0.06	1.135	0.144	S	Decrement	19.04
P.LCR %	11.60±0.78	14.30±0.70	-2.57	0.017	S	Increment	23.27
P.LCC 10^9/L	66.60±6.23	63.20±10.78	0.273	0.396	S	Decrement	5.10
RDW.CV %	17.38±0.27	17.78±0.32	-0.95	0.184	NS	Increment	2.30

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RDW.SD Fl	49.20±1.75	47.48±8.13	0.78	0.228	NS	Decrement	3.49

Values are expressed as mean ± SE

*Significant difference at P<0.05

Table 3. The effect of Comparison of control and *S. argel* seed aqueous extract at dose 200 mg/Kg b.w on haematological parameters in male rats for 14 days

Parameters	**Control**	**Treatment**	**t-value**	**p-value**	**S (at P < 0.05**	**Change**	**%**
WBC 10^3/µL	9.02±1.33	12.46±2.94	-1.15	0.144	NS	Increment	38.13
RBC 10^6/µL	7.00±0.89	8.46±0.17	-1.42	0.099	NS	Increment	20.8
HGB g/dL	11.90±1.52	14.70±0.08	-1.62	0.074	NS	Increment	23.5
HCT %	49.18±6.60	59.25±01	-1.30	0.115	NS	Increment	20.4
MCV fL	70.08±1.66	70.07±1.31	0.002	0.499	NS	Decrement	0.01
MCH Pg	17.04±0.12	17.40±0.32	-1.13	0.117	NS	Increment	2.11
MCHC g/dL	24.36±0.55	24.88±0.78	-0.55	0.298	NS	Increment	2.13
Lym %	58.46±8.14	56.40±2.43	0.217	0.417	NS	Decrement	3.52
Gran %	23.06±6.58	26.88±4.73	-0.45	0.334	NS	Increment	16.56
Mid %	18.48±3.05	16.72±3.37	0.39	0.355	NS	Decrement	9.52
Lym # 10^3/µL	4.97±0.59	6.81±1.30	-1.39	0.103	NS	Increment	37.02
Gran # 10^3/µL	2.31±0.80	3.62±1.50	0.823	0.218	NS	Increment	56.70
Mid # 10^3/µL	1.74±0.38	2.02±0.58	-0.42	0.343	NS	Increment	16.09
PLT 10^3/µL	594.20±79.49	428.50±84.93	1.416	0.099	S	Decrement	27.88
MPV fL	7.18±0.15	7.48±0.19	-1.23	0.129	S	Increment	4.17
PDW fL	7.66±0.26	8.45±0.52	-1.46	0.093	S	Increment	10.31
PCT %	0.42±0.054	0.32±0.06	1.395	0.103	S	Decrement	23.80
P.LCR %	11.60±0.78	13.70±1.11	-1.59	0.077	S	Increment	18.10
P.LCC 10^9/L	66.60±6.23	56.00±6.89	1.139	0.146	S	Decrement	15.91

RDW.CV %	17.38±0.27	17.60±0.20	-0.62	0.277	NS	Increment	1.26
RDW.SD fL	49.20±1.75	49.53±1.31	-0.14	0.445	NS	Increment	0.67

Values are expressed as mean ± SE

*Significant difference at P<0.05

Table 4. Comparison of the control / treated of *S. argel* seed aqueous extract at dose 700 mg/Kg b.w on biochemical parameters in male rats for 14 days

Parameters	**Control**	**Treatment**	**t-value**	**p-value**	**S (at P < 0.05**	**Change**	**%**
ALP	16.80	17.00	-0.094	0.464	NS	Increment	1.19
AST	9.38	10.80	-1.058	0.163	NS	Increment	15.14
ALT	7.36	7.60	-0.287	0.391	NS	Increment	3.26
Glucose	77.40	84.50	-2.134	0.035	S	Increment	9.17
Cholesterol	64.60	69.75	-1.903	0.049	S	Increment	7.97
Triglycerides	83.20	93.00	-2.046	0.939	S	Increment	11.78

Values are expressed as mean ± SE

*Significant difference at P<0.05

Table 5. Comparison of the control / treated of *S. argel* seed aqueous extract at dose 500 mg/Kg b.w on biochemical parameters in male rats for 14 days

Parameters	**Control**	**Treatment**	**t-value**	**p-value**	**S (at P < 0.05**	**Change**	**%**
ALP	16.80	19.40	-0.1.37	0.104	NS	Increment	15.48
AST	9.38	9.12	0.215	0.417	NS	Decrement	2.77
ALT	7.36	6.10	1.60	0.073	NS	Decrement	17.12
Glucose	77.40	83.00	-1.87	0.05	S	Increment	7.24

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Cholesterol	64.60	70.80	-1.68	0.066	NS	Increment	9.60
Triglycerides	38.20	90.80	-2.143	0.032	S	Increment	137.69

Values are expressed as mean ± SE

*Significant difference at P<0.05

Table 6. Comparison of the control / treated of *S. argel* seed aqueous extract at dose 200 mg/Kg b.w on biochemical parameters in male rats for 14 days

Parameters	**Control**	**Treatment**	**t-value**	**p-value**	**S (at P < 0.05**	**Change**	**%**
ALP	16.80	15.00	1.111	0.152	NS	Decrement	10.71
AST	9.38	9.78	-0.354	0.367	NS	Increment	4.26
ALT	7.36	7.28	0.088	0.466	NS	Decrement	1.08
Glucose	77.40	70.25	1.974	0.044	S	Decrement	9.24
Cholesterol	64.60	70.25	-1.495	0.089	NS	Increment	8.75
Triglycerides	38.20	94.00	-2.679	0.016	S	Increment	146.07

Values are expressed as mean ± SE

*Significant difference at P<0.05

Discussion

The need to evaluate the toxicity profile of *Solenostemma argel* extracts was necessitated by their extensive therapeutic uses by natives of North Central Sudan.

Various haematological parameters investigated in this study (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, Lym %, Gran %, Mid %, Lym #, Gran #, Mid #, PLT, MPV, PDW, PCT, P.LCR, P.LCC , RDW-CV and RDW-SD, serve as useful indices in the evaluation of toxicity of plant extract in animals. Evaluation of haematological parameters is not only used to determine the extent of harmful effects of extracts on the blood of an animal, it is also used to elucidate blood relating functions of a plant extract or its products [18]. Analysis of blood

parameters is very important in risk assessment because changes in the haematological parameters predicts human toxicity when the data are translated from animal studies. Haematological parameters are equally very important for assay because the hematopoetic system is a major target for toxic substances. These parameters are also used to monitor the pathological and physiological status of animals and humans [19].

Increase in the WBC count observed in rats administered *S.argel* (at doses 200 and 500 mg/Kg b.w) suggest that *S.argel* extract contains agents that could stimulate the production of leucocytes and could serve as immune booster. WBC an indication of pathological condition which may imply challenge on the immune system by the plant extract. The significant increase in WBC following the treatment of the plant extract indicates a boost in the immune system (as in the experimental groups GPIII and GP IV). Such effects may also be due to increase in vascular permeability. Increase in haemoglobin content can be correlated with no induction of anemia in experimental animals after treatment with plant extract.

The non-significant effect of the plant extract doses on the RBCs and indices relating to it (Hb, PCV,MCV,MCH and MCHC) through the experimental period is an indication that there was no destruction of matured RBCs and no change in the rate of production of RBCs (erythropoiesis).

The non-significant effect on the RBCs and Hb implies that there was no change in the oxygen – carrying capacity of the blood and amount of oxygen delivered to the tissues following the plant extract treatment since RBCs and Hb are very important in transferring respiratory gases. Increase in the RBC and its indices following administration of the extracts is an indication of normal erythropoiesis.

Administration of *S.argel* (200, 500 and 700 mg/Kg b.w) led to a decrease in platelet counts in rats, also observed was a dose dependent and statistically significant reduction in PLT of rats.

Administration of *S.argel* extract dose at 700 mg/Kg b.w caused reductions in WBC, MCV, Gran %, Gran #, P-LCC and

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RDW-SD, whereas an increase in HGB, HCT, MCHC, Lym%, Mid %, PDW and P-LCR. Administration of *S.argel* extract at dose 500 mg/Kg b.w caused reductions in MCV, Lym%, Mid %, Lym #, PCT, P-LCC and RDW-SD. Administration of *S.argel* extract at dose 200 mg/Kg b.w caused reductions in Lym %, Mid %, PCT and P-LCC .

Red blood cells are highly susceptible to lipid peroxidation because they have unsaturated membrane lipids. Increase in HCT, heamoglobin, white blood cells (at doses 200 and 500 mg/Kg b.w) , red blood cells, mean corposcular heamoglobin concentration and lymphocytes (at 700 mg/Kg b.w) in the experimental animals suggests that *S.argel* seed aqueous extracts may have haematopoietic properties which will promote erythropoiesis in animals and are therefore nontoxic. This justifies the use of studied plants in traditional medicine. The calculated blood indices MCV, MCH and MCHC have a particular importance in anemia diagnosis in most animals. Increased levels of MCH and MCHC indicates that phytoconstituents in the extract stimulated the secretion of erythropoietin which stimulates the stem cells in the bone marrow to produce RBC [20].

HCT, HGB, RBC and MCHC are particularly important for the diagnosis of anaemia in humans and most animals. The values obtained in this current study for HCT, HGB, RBC and MCHC seen to be within the normal range as reported by Mitruka and Rawnsley, [21], suggesting the animals were not anaemic.

Platelet size correlates with platelet activity and can be assessed by platelet indices. The platelet indices (P-LCR, P-LCC) are universally available with routine blood counts and are an attractive index to study in clinical scenarios. There are extremely useful in assessing the etiology of thrombocytosis , in assessing cardiovascular risk of thrombiosis.

Serum biochemical parameters evaluated include glucose, triglycerides, total cholesterol, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP).

In the present study, liver function parameters were evaluated for groups administered with *S.argel* aqueous extract. At all studied doses (700, 500 and 200 mg/Kg b.w were administered for 14 days , it showed that non-significant

change in liver parameters (AST,ALT and ALP) compared to control.

Generally, enzymes such as ALT, AST and ALP are marker enzymes for function and integrity of organs such as liver. The enzymes found within organs and tissues are released into the blood stream following cellular necrosis and cell membrane permeability and are used as diagnostic measure of liver damage. Furthermore, serum ALT/AST has been used as an index to monitor liver pathology. The ratios higher than unity are indicative of adverse pathological effects on the liver . Calculated ALT/AST ratios were far below unity in treated rats and values were not significantly different.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) have been identified as indicators of hepatocellular injury, while Alkaline phosphatase (ALP) is a marker of cholestasis [20]. Results obtained show that aqueous extracts of the seeds of *S.argel* did not significantly elevate the activities of liver function enzymes AST, ALT and ALP in the serum of test animals. This suggests that these extracts did not induce hepatocellular injury in animals. Decrease in the level of these enzymes in the serum of test animals may be due to non- leakage of hepatocytes which normally occurs from peroxidative damage of their membranes leading to increased membrane permeability. This results indicate that the seed aqueous extract of studied plant was not harmful to the liver.

Alkaline phosphatase (ALP) is a marker ecto-enzyme for the plasma membrane and often used to assess the integrity of the plasma membrane (*Akanji et al.*, 1993). Liver damages/infections are characterized by elevated ALP concentration in serum as in groups 500 and 700 mg/Kg b.w .

Glucose and triglycerides levels were also evaluated in this study. Significant change were found in the three doses of *S.argel* aqueous extract (700, 500 and 200 mg/Kg b.w) during 14 days. Total cholesterol levels were also evaluated in this study. Non-significant changes were found at doses 500 and 200 mg/Kg b.w of *S.argel* (except at dose 700 mg/Kg b.w , significant increase was shown).

Conclusion:

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The medicinal uses of the seed extract of *S. argel* has not been reported in many scientific literatures. There is paucity in scientific reports on the medicinal properties of the seed extract. The present study showed that *Solenostemma argel* aqueous extract did not induce a various haematological changes in the male albino rats for 14 days (except PLT, P-LCR, P-LCC, RDW-SD), while the effect of extract in rats revealed also no changes in liver enzymes (AST, ALT and ALP) at $P<0.05$. Consumption of this plant may have tremendous impact on subjects suffering from hyper-triglyceridermia. This supports the safety use of the aqueous extract of *S.argel* in pharmacological studies.

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