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Antimicrobial Activity, Toxicological, Hematological and Biochemical Effects of Fagonia cretica linn in Sudan

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بسم الله الرحمن الرحيم

قال تعالى:

{وَقُل رَّبِّ زِدْنِي عِلْماً}

صدق الله العظيم

سورة طه الآية (114)

Dedication

This humble research is dedicated to the souls of my beloved parents, Abbas Mohamed Jahalla and Zainab Ali Fadlalla, the thesis is also dedicated to the soul of my beloved son ,(the loss of the youth) Samawal Omer,and to my beloved wife Nawal Bakheet

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Hereby I want with all my heart to betray my hearty gratitude to my supervisor Omer Musa Ezz-Eldeen who supervised this research with all patience and courtesy putting all his experience at my hands, my gratitude is extended to Dr. Aimun A/Ghafar the head department of pharmacology in Omdurman Islamic University faculty of pharmacy, who put himself and the higher studies laboratory at my disposal all the time, my Acknowledgment is postponed to Dr. Yousif Mohamed Yousif the previous Dean faculty of higher education in Shendi University. My thanks also included Ustaz Mohamed Osman Ali the Dean faculty of medical laboratory sciences, who effectively participated in the completion of this research. My gratitude is extended to my son Dr. Fuad whose help, participated largely in the success of this research. At last all my heart gratitude is extended to all who encouraged me with an action or a sentence until I finished this work.

List of abbreviation

A.D	Aqueous decantation
A.niger	*Aspergillus niger*
AOT	Acute oral toxicity
A O T U D	Acute oral toxicity- Up-and-Down
B. subtilis	*Bacillus.subtilis*
BUN	Blood urea nitrogen
C. Albicans	*Candidas albicans*
C.F.U	Colony forming unit
E. coli	*Escherichia coli*
FC	*Fagonia cretica linn*
G O D	Glucose oxidase determination
H b	Hemoglobin
HC	Hemoglobin concentration
Hct	Red blood cells Hematocrit
HMEpC	Human mammary epithelial cells
MCF-7	Michigan Cancer Foundation-7
MCH	Mean corpuscular hemoglobin
MCHC	Mean cell hemoglobin concentration
MDA-MB-231.	M.D. Anderson - metastatic breast-231
MPV	Mean Platelet Volume
OECD	Organization for Economic Co-operation and Development
OGD	oxygen-glucose deprivation
P. aeruginosa	Pseudomonas. Aeruginosa
PBS	Phosphate Buffer Saline
Pct	platelets-Hematocrit
PCV	Packed cell volume

Ppm	Part per million
RBCs	Red blood cells
RC	*Rubia cordifolia*
(RDW-CV)	RBCs distribution width co efficient variation
RDW-SD	RBCs distribution width standard deviation
S. aureus	*Staphylococcus aureus*
SGOT	Serum glutamate oxaloacetate transferase
SGPT	Serum glutamate pyruvate transferase
TC	*Tinospora cordifolia*
TSH	Thyroid stimulating hormone
TWBCs	Total white blood cells
USA	United states of America
W/W	Weight per weight

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Abstract

The use of the plants as medicines is an ancient and reliable practice. *Fagonia Cretica* is well known herbal plant used in traditional medicine of Pakistan, India and Far East, it is reputed to obtain a profitable therapeutical properties and it has been used in treatment of fever, thirst, vomiting, dysentery, asthma, urinary discharges, and liver troubles. Externally applied as a paste on tumors and other swellings of the neck. Reported to possess potent antibacterial properties against pathogenic organisms, also the scientific studies of the plant proved the presence of hematological, neurological, anticancer, and hepato- activity. The present study targeted the extraction and fractionation of the active components of the plants and conducting phytochemical screening, study of antimicrobial, toxicity, Hematological, and biochemical properties of the extract. Different methods were adopted to achieve the objectives of this study this included Harborne methods for extraction and phytochemical screening, the antimicrobial activity was done using cup-plate method, the toxicological effects was achieved by using *OECD 425* protocol for determining *LD50*, different laboratory tests were used to determine the hematological, biochemical effects of the plant. The screening of the preliminary Phytochemical yield the presence of flavonoids, saponins, steroids, tannins, alkaloids and absence of anthroquinone, cyanogenic glycosides and cardiac glycosides. Both chloroformic and ethanolic extracts showed potential activity against the used microorganisms, the toxicological determination of (LD50*)* was found to be greater than (5000) mg/Kg. This study showed for the first time the effect of *Fagonia Cretica* on the following hematological parameters (RDW)*, (RDW-SD), (RDW-CV), (Pct), and (MPV).* The obtained results of the effect of *Fagonia Cretica* extract revealed no effect in rat's body weight, or body organs weights, different effect were observed on hematological and biochemical parameters.

Further studies targeting the identification of the active phytochemical components and their role of action are recommended, also pharmaceutical formulation of *fagonia* as herbal medicine is highly recommended.

Keywords: *Fagonia cretica linn*, extraction, toxicological, hematological, and biochemical properties.

مستخلص الدراسة

حياة الإنسان والحيوان كانت تدور حول النبات واستخدامه كطعام ولباس ومسكن ،لكنه أيضاً استخدمه للسيطرة على الأمراض وتخفيفها لهذا استخدام النبات كعلاج مقبولة ومعقولة منذ القدم.

أم شويكة كنبات طبي قد عرفت في الطب الشعبي في كل من باكستان والهند والشرق الأقصى وقد أشيع عنها أنها تمتلك ميزات علاجية فائقة وذات جدوى كبيرة مما أدى للإستخدامها في علاج كثير من الأمراض كالحمى والعطش والغي والدوسنتاريا والأزمة والإفرازات البولية ومشاكل الكبد .وتم استخدامها خارجياً كلبخة للأورام والخراجات فى الرقبة ،وقد دون عنها أيضاً إمتلاكها قدرة فائقة كمضاد حيوى ضد كثير من الباكتيريا الضارة.

الدراسات العلمية أثبتت أن للنبات أثر على كل من معدلات الدم،الجهاز العصبى،السرطان وأمراض الكبد.

استخدمت الدراسة طرقا مختلفة للوصول للنتائج حيث استخدمت الدراسة الطرق الموصوفة في هاربون لاستخلاص وتصنيف المركبات الكيماوية الموجودة بالنبات ، كما استخدمت الدراسة طريقة (Cup-plate) لدراسة الأثر المضاد للبكتيريا والفطريات والعفن ، وبروتوكول (OECD 425) لإختيار وتقييم سمية النبات تحت الدراسة، ولإختبار أثار النبات المعني على المكونات الأساسيى للدم والمواد الكيماوية الموجودة بالدم اسخدمت الدراسة عدة طرق لتقييم ذلك . النتائج المتحصل عليها من هذه الدراسة خلصت إلى إستخلاص وفصل بعض المواد الفعالة من نبات أم شويكة، و أثبت المسح الأولى وجود كل من الفلافونويدس،الصابونين ،الإسترويدات ، التانينوالألكالويدس. وغياب كل من الأنثروكينون،الساينوجينك جلايكوسايدسوالكارديك جلايكوسايدس .كلا المستخلصين بالإيثانول والكلوروفورم أثبتا فعالية واضحة ضد الكائنات الحية الدقيقة المستخدمة ولتحديد الجرعة السمية القاتلة ثبت أنها أكثر من5000 مليجرام لكل كيلوجرام وزن مما يعنى أن النبات لديه هامش سلامة غالى جداً .هذه الدراسة أثبتت ولأول مرة أثر النبات على معدلات الدم الآتية درجة توزيع كريات الدم الحمراء ،الإنحراف المعياري لتوزيع كريات الدم الحمراء ومعامل توزيع كريات الدم الحمراء ، ومتوسط حجم الصفائح الدموية ، ودرجة تكدس الصفائح الدموية ، ومتوسط حجم الصفائح الدموية .

خلصت الدراسة الى أن المستخلص بالكحول الإيثيلى لا يؤثر على MPV. PCT RDW . RDW-SD, RDW-CV أوزان الفئران و أوزان الأعضاء الداخلية كالكبد ،الرئتين،الطحال،القلب والكلى. أثر مستخلص النبات على معدلات الدم ووظائف الكلى والكبد كانت متباينة ولكنها جميعاً تؤكد عدم سمية للنبات.

نوصى بإجراء دراسة مستفيضة للمواد الفعالة في النبات وفصلها ومعرفة طريقة عملها،إضافة إلى العمل على عمل تركيبة صيدلانية من النبات .

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Chapter One

1. INTRODUCTION AND LITERATURE REVIEW

1.1. The role of medicinal plants in fighting diseases :

Life and diseases go together: Where there is life, diseases are bound to exist. Dependency and sustainability of man and animal life has been revolving around plants through uses as foods, fibers and shelter, but also plants have been used to control and ease diseases, therefore the use of the plants as medicines is an ancient and reliable practice. Indigenously different plants have been used to cure a disease or several diseases at a time, but towards the middle of the (20^{th}) century the contribution of medicinal plants to medicine was reduced by approximately ($¼^{th}$) as research and development favored the use of synthetic chemicals. Now this trend is reversing once again in favor of plants, as they have been discovered to possess natural products that are chemically balanced, effective, and least injurious with none or much reduced side effects of synthetic chemicals. Therefore, herbal cures and medicines have attraction, particularly for those who failed to get use of or disappointed with other methods of treatment. [1]

Herbal medicine is the use of plants as medicines. Herbal medicine is also known as phytotherapy (especially in Europe; from Greek phyton meaning plant), botanical medicine, medical herbalism and herbiology in (USA). More specifically, the term herbal medicine refers to the therapeutic use of relatively crude and therefore chemically complex plant extracts, or simply the herb in its dried form. In this way herbal medicines are distinct from plant-derived pharmaceutical drugs, which contain single chemical compounds extracted from plants in their pure form .[2]

A herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. On a global scale there is substantial and justifiable acceptance of the many benefits that orthodox medicine brings. Simultaneously, however, the western world, birth place of orthodox medicine, is embracing complementary medicine in an emphatic way. The World Health Organization *(WHO)* estimates that the global market for complementary therapies stands at (60) billion *US* dollars a year and is growing steadily. This represents a worldwide trend that is reflected in individual countries in significant ways. Data from the British parliament support the idea that complementary medicine use in Britain is high and is increasing. In (1999) in Britain (93) million pounds were spent on complementary medicine and (5) million British visited (50,000) complementary practitioners who operate there. By (2002) the amount that Britons spent on complementary medicines had risen to (126) million pounds. Australia is also experiencing an exponential increase in the use of complementary medicine. It is estimated that Australians spent (2.3) billion Australian dollars on complementary therapies and medicine in the year (2000), which is a (120 %) increase on what they spent in (1993). Overall in (2000) Australian people spent (4) times more money from their pockets on complementary medicine than they spent on prescribed drugs. The same report indicated that in the USA expenditure on complementary medicines in (2000) was (34) billion US dollars. The popularity of complementary medicine with the American people is widely attested. Nearly (68%) of all American adults have used at least one complementary therapy at some time in their lives and of these (50%) continue to use complementary medicine (20) years later. High retention rates such as this reflect the fact that complementary medicine and its philosophy can become a way of life for those who use it. A more recent study based in El Paso, Texas, suggests that the number

of people using complementary medicine may be still increasing. This study found that (77%) of those surveyed were using complementary medicine. In France (75%) of the population has used complementary medicine at least once and in Germany (77%) of pain clinics provide acupuncture. In Germany as well, a study of adults with hay fever, asthma, eczema and food allergies found that more than (1 in 4) of these people used complementary medicine to treat their allergic condition, of these people (78%) used the complementary treatments because they assumed there would be fewer side effects. Although the assumption that 'natural is safe' is a fallacious one, the perceived relative safety of complementary medicine is proving a significant factor in driving people to embrace it as part of their health regime. Equally, however, people clearly perceive that complementary medicine is efficacious. A study from a New York City pain clinic found that (85%) of patients had used complementary medicine to relieve their pain. Further, (60%) believed that their complementary medicines had worked. This point to the fact that the ground swell of support for complementary medicine is not arising out of some misguided search for absent nurturing or feel-good treatments. When it comes to choosing health options individuals are pragmatic enough to insist that their treatments are efficacious. Often, people will turn to complementary medicine if they perceive that orthodox medicine is not delivering the health outcomes they desire. The surging tide of pragmatism that is seeing people across the world turn to a combination of orthodox and complementary medicine is leading science to address the wisdom that lies in this complementary therapies.[3]

1.2. Literature review of the selected plant*: Fagonia cretica l.*

1.2.1. Taxonomy:

Family: Zygophyllaceae.

Vern names: (Ar) Umm Showeika, Sholib, U mmShok.

1.2.2. Botanical description:

Description:

Spine scent glabrous woody diffused annual herbs up to (50) cm long leaves opposite, compound, (1-3) foliate; leaflets linear lanceolate, (1-2x0.2) cm stipulate; stipule spine scent, inflorescences axillary, solitary. Flowers purple with petals more than twice as long as sepals. Fruits capsules, ovate, (4-5) cm long (5 -sided, with persistent style.

Is a small spiny under shrub, mostly found in dry calcareous rocks throughout Pakistan. [4] It is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well documented. [5]

Habitat:

Sandy hills (Quos), low land plains.

Distribution:

In Sudan: north Kurdufan, also widespread throughout Northern and Central Sudan, it is present abundantly in Shendy region.

Universally:

It is found in India, Pakistan, China, Bangladesh and Egypt.[6]

1.2.3. Chemical composition:

Triterpenoid, saponins, alkaloids, Coumadin's, flavonoids and tannins.[7]Water soluble protein was isolated and purified from the water extract of dried *Fagonia cretica* plant. The acid hydrolysate of the material showed the presence of phosphate, sugar, and amino acids in the molar ratio as seen in table one.[8]

Amino acid	Residue percent	Molar ratio
Lysine	8.34	1.00
Threonine	16.71	2.00
Aspartic acid	25.12	3.01
Serine	25.15	3.02
Glutamic acid	24.68	2.96

Table 1: Amino acids composition of water soluble protein of *Fagonia cretica*

Four new triterpenoid saponins were isolated and identified from the aerial parts of *Fagonia cretica*. They were characterized as 3-*O*-[β-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl] hederagenin 28-*O*-β-D-glucopyranosyl ester, 3-*O*-[β-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl] oleanolic acid 28-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] ester, 3-*O*-[β-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl] 27-hydroxy oleanolic acid 28-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] ester and 3β-*O*-[β-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl] olean-12-en-27-al-28-oic acid 28-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] ester. The structures of the saponins were assigned by spectral analyses. To the best of knowledge the genin 3β hydroxy olean-12-en-27-al-28-oic acid is new.[9]

1.2.4. The medicinal properties of the plant:-

In the last (15) years, this plant and related species have been investigated mainly for the presence of flavonol and terpenoid glycosides.

Most of the flavonol glycosides have been isolated from various Egyptian *Fagonia* species and their phylogenetic affinities have also been investigated.[10] Several saponin glycosides have been separated and characterized.[11] Other constituents, such as docosyl docosanoate from hexane extract[12] and water soluble proteins from aqueous extract of air-dried *Fagonia cretica* plants, have been isolated[13] furthermore nahagenin[14] (hederagnin, ursolic acid and pinitol from other *Fagonia* species have also been separated and characterized [15] antimicrobial activity of its flavonoid compounds has been explored previously[16] while the nutritive values of it and of other species growing wild in the Rajasthan region of India, have also been evaluated.[17]

A-Folkloric Use of *Fagonia cretica l.:*

An aqueous decoction of the plant is a popular remedy for cancer in the indigenous system of medicine[5] the maceration of the whole plant is used as antispasmodic. The powdered fruits mixed with sour milk are taken instantly as anti-purgative.[6]

Rubia cordifolia, *Fagonia cretica linn* and *Tinospora cordifolia* are tropical herbs and have been extensively used in the treatment of various types of hematological, hepatic, neurological and inflammatory conditions.[18]

The plant is bitter and used for the treatment of fever, thirst, vomiting, dysentery, asthma, urinary discharges, liver troubles, typhoid, toothache, stomach troubles, and skin diseases. Boiled residues of the plant in water are used to induce abortion. Externally applied as a paste on tumors and other swellings of the neck.

Leaves and twigs are used for snake bite. Reported to possess potent antibacterial properties against pathogenic organisms.[4]

B. The investigated biological activities:

Antimicrobial activity of *Fagonia cretica* plant extracts against selected microbes:

The methanolic extract obtained from the flowers of *Fagonia cretica* was tested for its antimicrobial activity against fungi (i) *Aspergillus niger* (ii) *Aspergillus fumigates* for antifungal activity; and bacteria (i) *Escherichia coli* (ii) *Proteus vulgaris* (iii) *Streptococcus agalactiae* and (iv) *Bacillus anthracis* for antibacterial activity. The results are shown in the tables' below.[19]

S. No.	Bacterial species	Methanolic extract from flowers of *Fagonia cretica*	Control
1.	*Escherichia coli* (+)	14	25
2.	*Proteus vulgaris* (+)	20	17
3.	*Streptococcus agalacties* (–)	24	28
4.	*Bacillus anthracis* (–)	24	28

Table 2: Antibacterial activity of methanolic extracts of *Fagonia cretica* flowers

S.No	Fungal species	Methanolic extract flowers of *Fagonia cretica*	Control
1.	Aspergillus niger	26	30
2.	Aspergillus fumigates	18	30

Table 3: Antifungal activity of methanolic extracts of *Fagonia cretica* flowers

***Fagonia cretica l.* flowers antibacterial zones of inhibition:**

Eleven compounds have been isolated from the first time from the whole plant of *Fagonia cretica* and their structures are elucidated by extensive spectroscopic studies. All the isolated compounds are tested to their antimicrobial activity:-

1-Linoleic acid.

2-β-sitosterol-3-O-β-D-(6'-hexadecanoyl)-glucopyranoside.

3-Methyl triacontanoate.

4-Taraxerol.

5-β-Amyrin acetate.

6-Oleanolic aldehyde acetate.

7-Octacosonic acid.

8-Triacontanoic acid.

9-Taraxerone.

10-2α 3α23 Trihydroxyolean-12-en-28-oic acid (arjunolic acid).

11- 3α-23-Dihydroxyurs 12-en-28-oic acid.[20]

Microorganism	Zone of inhibition diameter (mm)											
Bacteria	1	2	3	4	5	6	7	8	9	10	11	Standard drugs imipenem
Gram-positive												
Bacillus subtilis	15	20	5	25	28	20	5	0	20	22	15	33
Shigella flexneri	5	25	0	20	10	18	5	5		25	10	27
Staphylococcus aureus	10	18	5	25	28	15	12	10		20	16	33
Gram-negative												
Escherichia coli	12	25	0	30	14	10	10	10		18	22	30
Pseudomonas aeruginosa	17	20	9	20	12	10	5	0		20	15	24
Salmonella typhi	10	20		12	10	17	5	0		18	20	25
Fungi												Miconazole
Trichophyton longifusus	62	85	0	88	30	70	0	0		65	70	70
Candida albicans	68	70	10	76	65	70	20	50		75	85	110.8
Aspergillus flavus	12	68	0	90	60	55	20	20		70	75	20
Microsporum canis	45	60	0	85	65	60	0	0		85	80	98.4
Fusarium solani	50	25	22	50	50	60	45	30		55	88	73.25
Candida glabrata	72	50	20	50	50	60	20	25	68	70	80	110.8

Table 4: Antibacterial and antifungal activity of (11) compounds isolated from *Fagonia cretica linn.*

In the present study testing the active phytocomponents of *Fagonia cretica* using phytochemical analysis the study tested antimicrobial (Table 4) antibacterial and antifungal activity of (11) compounds isolated from *Fagonia cretica linn*. Activity of *Fagonia cretica* plant extracts (aqueous, methanolic, ethanolic) of different concentrations against *S. aureus, B. subtilis S. epidermidis E. coli,* and *P. aeruginosa* by disc diffusion method. *(MIC)* of plant extract was also carried out against *S. aureus, P. aeruginosa* and *B. subtilis.*

The preliminary phytochemical screening of *Fagonia cretica* plant showed the presence of bioactive compounds. Aqueous and methanolic extracts showed more activity against all the tested bacteria as compared to ethanolic extract.

The results scientifically validate the use of this plant in the traditional medicines and isolation and characterization of the active principle for further exploitation in medical microbiology.[21]

2-Biological effect:-

I- Neurological effect:

Rubia cordifolia, *Fgonia cretica linn* and *Tinospora cordifolia* exerts neuroprotection by modulating the antioxidant system in the rat hippocampal slices subjected to oxygen glucose deprivation. The major damaging factor during and after the ischemic/hypoxic insult is the generation of free radicals, which leads to apoptosis, necrosis and ultimately cell death. *Rubia cordifolia, Fagonia cretica linn* and *Tinospora cordifolia* have been reported to contain a wide variety of antioxidants and have been in use in the eastern system of medicine for various disorders. However, their mechanism of action was largely unknown. The herbs selected for this are used to test their neuroprotective ability and the associated mechanism in rat hippocampal slices subjected to oxygen-glucose deprivation.[18]

II-Effects of *Fagonia cretica l.* constituents on various hematological parameters in rabbits:

Investigating the effects of powdered *Fagonia cretica* plant and its two majors triterpenoid Saponin (Saponin-I and Saponin-II), isolated from its ethanolic extract, on red blood cells count, hemoglobin concentration, mean corpuscular hemoglobin and on total leukocyte count of normal male rabbits. Their obtained results showed that the Saponin treated 3 dose groups of animals indicated significant decrease in count during the experimental period of (16) days. This effect was more pronounced in animals treated with Saponin-II than Saponin-I. The (0.50) and (1.0) gm crude drug treated animals and (10) and (20) mg of both

the Saponin treated animals, indicated a decreasing tendency of (*HC)* up to the (4^{th}) day, which increased afterwards up to the (16) day. The (3^{rd}) dose of these materials (1.50) gm of crude drug and (30) mg of both saponins exhibited a highly significant decreasing pattern, up to the (16) days. The decreasing effect of hemoglobin concentration was more distinct in the saponin-II treated animals than saponin-I and the crude drug. The (*MCH)* followed a reverse pattern than (*RBCs)* and (*HC)*. A continued decreasing trend was found in total (*WBCs)* during the (16) days treatment. An amount of (1.50) gm of the crude drug and (30) mg of both the saponin had highly significant decreasing effects on the amount of total leukocyte count of rabbits blood.[22]

Methanolic extract from the aerial parts (leaves and twigs) of *Fagonia cretica l.* on a hemorrhage induced by venom from Naja naja snake. The hemorrhagic response of venom was dose dependent from (0.1) to (4.0) µg per (1.5) µL phosphate buffer saline (*PBS*) on vitalize veins of fertilized hens' eggs in their shells. The extract effectively eliminated and neutralized, in a dose-dependent manner, the hemorrhagic activity of snake venom. The minimum effective neutralizing dose of *Fagonia cretica* extract was found to be (15) µg per (1.5) µL (*PBS)*. The extract possesses potentials as hemorrhagic inhibitor against snake venom compared to the standard antiserum and various plants reported in the literature. This study also provides a scientific base for the use of *Fagonia cretica* in traditional medicine for the treatment of snake bite.[23]

III-Effect of *Fagonia cretica linn* on cholesterol level:-

A recent study concluded that, the aqueous extract of the whole plant of *Fagonia cretica l.* possess a significant antihyperlipidemic effect at the doses of (250) and (500) mg/kg body weights. These effects were due to their presence of active constituents which present in whole plant of *Fagonia cretica l.* Further

studies required to establish the efficacy of the whole plant of *Fagonia cretica l.* as an antihyperlipidemic drug. [24]

IV-*Fagonia cretica* and the treatment of cancer:-

When the A.D (aqueous decantation) of *Fagonia cretica* was given for a couple of days before the next course of chemotherapy to a patient suffering from Fumigating Cauliflower-Type. Stage 1V Adeno-carcinom*.* Of chemotherapy, it is observed that there was no change in the blood count (no myelo-suppression or reduction in the blood count or the Hemoglobin level) so there was no need of blood transfusion. Patient continued A.D of *Fagonia cretica* during the rest of her treatment and showed excellent improvement. Her hair started growing back, skin color became normal and there was no need of blood transfusion over and above showed impressive improvement and regression of the tumor. [25]

V- *Fagonia cretica* and the treatment of Thalassemia major:

The herbal preparation (A.D of *Fagonia cretica*) was given to a female patient suffering from Thalassemia major she started showing good improvement in a couple of days and after about (6) months. When her blood samples were evaluated she showed no signs of Thalassemia major. Their unpublished data revealed that this herbal treatment showed regression in the blood requirements in over (68 %) of the patients and almost (100 %) of the patients had an improvement in the quality of life as they were behaving like the normal members of the society. [25]

The herbal preparation (A.D of *Fagonia cretica*), was given to a child diagnosed as B. Thalassemia major at the age of (3) months. His blood picture on the first time of diagnosis, showed very low parameters of (*Hb),* (total *RBCs),*

(Hct), (MCV), (MCH), (MCHC) and Platelets count, with the presence of reticulocytes. When the blood parameters were examined within (3) month and (20) days after treatment, there was a very good progress in all hematological parameters mentioned, with the absence of Retics.

VI- Toxicity, analgesic, antipyretic, and anti-inflammatory effect:

Study of the saponin mixtures of *Fagonia cretica* and *Fagonia mollis* for their toxicity, analgesic, antipyretic and anti-inflammatory effects. Oral doses up to (5000) mg/kg did not induce any death, sluggishness being observed only at the highest doses. At the three dose levels used (250-500-1000 mg/kg), showed significant anti-inflammatory activity and considerable analgesic and antipyretic effects.[26]

VII- Endocrinological effect:

a- Two major triterpenoid compounds, saponin-I and saponin-II, were isolated from its ethanolic extract. Radio-immunological assay was used for the estimation of blood hormones of crude drug and saponin-treated animals.

Both the saponin in (30) mg doses had significant decrease in prolactin and in the serum (*TSH)* levels as compared with crude drug treatment and control groups. The thyroxin level was also significantly reduced by (*saponin-II*) in a (30) mg dose while the crude drug and (*saponin-I*) had non-significant effects on thyroxin after (16) days. A significant increase in serum cortisol occurred with the crude drug in a (1) gm dose and with both saponin in (30) mg doses. Maximum increase in the serum cortisol occurred with saponin-II after (16) days.[27]

b- The effect of alcoholic extracts of the aerial parts of *Fagonia cretica* on estrous cycle and implantation in female albino rats was studied, it induced random

omission of heat phase toxic manifestation, however it was found to have some strong androgenic anti-progestational activity, and immune stimulating property.[28]

VIII-Cytotoxic and antitumor potential of *Fagonia cretica*:

A significant cytotoxic activity against brine shrimps at ***LD50 (118.89) ppm,*** was found when analyzed at the laboratory level, the traditional knowledge that *Fagonia cretica* has medicinal potential especially against cancer and tumors by performing cytotoxic, antitumor (potato disc) and (*DNA*) damage.

While antitumor assay showed that the extract inhibited tumor induction on potato discs. Significant antitumor activity was found against all the tumor-inducing. Agrobacterium strains tested (*At6, At10 and At77*), with maximum tumor inhibition (77.04%) against (*At10*). However, the extract did not show any lethal activity against Agrobacterium tumefaciens strains, and furthermore, no (*DNA)* damaging activity was observed. The overall results indicate a strong anti-cancerous potential of this plant.[29]

IX- In vitro cytotoxic activity of *Fagonia cretica* towards breast cancer epithelial cells:-

Study done in a April (2012) describes for the first time that an aqueous extract of *Fagonia cretica* shows potent *in vitro* cytotoxic activity towards breast cancer epithelial cell lines which was not seen towards normal mammary epithelial cells. Elucidation and characterization of the cytotoxic mechanism was undertaken by analyzing (*DNA)* damage, cell cycle status, apoptosis, metabolic state and expression of transcription factors and their targets. Finally, methods for the isolation and identification of active compound(s) were developed using various chromatographic techniques. An aqueous extract of *Fagonia cretica* was able to reduce cell viability significantly in two phenotypic ally different breast cancer cell

lines (*MCF-7* and *MDA-MB-231*). This activity was markedly reduced in normal mammary epithelial cells (*HMEpC*). Further investigation into the mode of action revealed that extract treatment induced cell cycle arrest and apoptosis in both (*MCF-7)* and (*MDA-MB-231)* cell lines. This coincided with the formation of (*DNA)* double stranded breaks and the (*DNA)* repair. [30]

Justification:

Fagonia cretica linn is widely used medicinal plant for the treatment of various diseases, although, it receives different scientific evaluations worldwide, no previous studies were done in Sudan. The study attempted to fill the gap in the aspects regarding antimicrobial activity, toxicological, hematological and biochemical effects of the plant, in vitro and in vivo.

General objective:

To screen the Antimicrobial activity, Toxicological, Hematological and Biochemical effects of *Fagonia cretica linn* in Sudan.

Specific objectives:

1- To test the antimicrobial activity in vitro.
2- To determine the toxicological property.
3- To evaluate the hematological effects of the plant.
4- To investigate the biochemical effects on rats in vivo.
5- To gain more knowledge about the importance of *Fagonia cretica linn* plant as a medicinal plant.
6- To explore the potentiality of *Fagonia cretica* plant as an agent that affect several blood parameters in human.
7- To obtain Sudanese data for this native plant.

Chapter Two

2-1Materials and Methods

2.1.1.Study design :-

A case control study was adapted to achieve the objectives of this research.

2.1.2. Ethical consideration:-

Ethical approval had been obtained from Omdurman Islamic University, Faculty of Pharmacy, and department of Pharmacology, under the number (01/0/2013)

2.1.3. Study Area: - No specified area in Sudan was chosen for completion of this work. The work was shared between Khartoum, Omdurman and Shendi Towns. Experimental procedures involving the experimental animals and their care were conducted in compliance with the procedure adopted in department of pharmacology, faculty of pharmacy, Omdurman Islamic. University .The preliminary phytochemical screening was also conducted in Omdurman Islamic University. The cup-plate agar diffusion method was adopted to assess the antimicrobial activity of the prepared extracts in the Sudanese National Centre for Research, Khartoum Sudan.

Blood samples for (*CBC)* were collected in (*EDTA)* tubes from the rat's eyes using non-heparinized capillary tubes. The assay was done at Shendi University using Mindray BC-3000Plus Auto Hematology Analyzer *(SHENZHEN MINDRAY BIO-MEDICAL ELECTRONICS CO, LTD).*

All biochemical investigations were done in many laboratories, governmental and private.

2.1.3. Study population:-

A total of (30), young adult Wistar rats of both sex, weighting (42.6 – 72.7) g of age of (8-12) weeks were obtained from the Sudan National Centre for Research,

Khartoum Sudan .These rats were divided into (4) groups, (3) of them are study groups which consist of (7) rats each and the (4^{th}) group was control group, that consist of (8) rats. The rat number (30) was sacrificed for the inspection of its internal organs to check its safety.

2.1.4. Sample size:-

(2) to (3) mls of blood were collected in each of heparinized and (*EDTA)* bottles from each of the (27) live rats at the end of the study period, using non heparinized capillary tubes, from rats' eyes after Anesthesia Induction.

2.1.5. Plant material collection, identification, extraction and fractionation:-

Plant collection and Identification:

The *Fagonia cretica* plants were collected from uncultivated and waste areas of Shendi town near the Faculty of medicine and health sciences, University of Shendi, Sudan during January-February (2011). Then the plant samples were authenticated by the Herbarium staff, Department of Botany, Sudan national centre for research, Khartoum, Sudan.

A voucher specimen was deposited in there for future reference.

I .Extraction methods:

Extraction was carried out according to the method described by Harborne (1984), (2000) g of plant sample was extracted successively with chloroform and (80 %) ethanol using shaker apparatus. For (72) hours for chloroform and (5) days for ethanol. The plant was washed with distilled water and allowed to dry completely before ethanolic extraction was carried out. Extraction was carried till the color of the solvent returned colorless. Solvents were evaporated under reduced

pressure using rotary evaporator apparatus. Finally extracts were allowed to dry completely under air. [31]

II. Methods of fractionation of the crude plant to flavonoid and saponin:

To obtain a fraction containing flavonoids, (5) grams of sample is weighed in at (250) ml volumetric flask. Then (100) ml of (80%) *methanol* was added at room temperature and shaken for (4) hrs in a shaker. The entire solution is passed through filter paper (No. 42). The process is again repeated. The filtrate is later transferred into a crucible and evaporated to dryness over a water path and weighed. [32]

To obtain a fraction containing saponin, (5) g of sample was weighed. (100) ml of (20%) C_2H_5OH was added. Then the suspension was heated over a hotplate for (4) hrs with continuous stirring at (55°C). The filtrate and the residue were re-extracted with another (100) ml of (20%) C_2H_5OH. The combined extracts were reduced to (40) ml over water bath at (80°C). The concentrate was transferred into a (250) ml separatory funnel. (20) ml of *diethyl ether* was added and shaken vigorously. The aqueous layer is recovered, while the ether layer was discarded.

The purification process was repeated with a (30) ml of *n-Butanol*. Then the combined extracts were washed twice with (10) ml of (5%) *aqueous NaCl* .The remaining solution was heated in a water bath. the sample was evaporated and dried in an oven. Finally the saponin content was calculated as percentage. [33]

2.2.2. Phytochemical screening methods:

The coming phytochemical tests were done according to the methods stated in phytochemical methods a guide to modern techniques of plant analysis.

2.2.2.1. Tests for flavonoids:-

(2) Grams of the powder were boiled with distilled water for (5) minutes in water bath and filtered through a filter paper the filtrate was used for the following tests:

A-Cyanidin reaction:

About (2) ml of the filtrate was taken, and then a small piece of Mg^{2+} metal followed by drop wise addition of conc. *HCl* was added; an orange color will be produced after (2-3) minutes if flavonoids (flavones type) are present.

NH4OH test:

To (10) ml of the extract (5) ml of NH_4OH followed by few drops of *conc.* H_2SO_4 was added. A yellow color will be produced and disappears on standing, if flavonoids are present. (1) ml of the filtrate few drops of *diluted NaOH* were added. A yellow color will be produced and then disappears on addition of diluted *HCl* if flavonoids are present. To (7) ml of extract (20) ml distilled water were added and filtered through cotton wool. The filtrate was acidified with few drops of *diluted HCl* and then tested for Flavonoid as follows: (i) A (10) ml of the aliquot of the filtrate was separately shaken with (5) ml of amyl alcohol in a small separating funnel; if the alcoholic layer (upper) is faintly yellow colored this indicates the presence of free flavonoid aglycones, while if it remains colorless it indicates the absence of free aglycones. (ii) A (10) ml of the aliquot of the filtrate was separately shaken with (5) ml of the amyl alcohol to remove any free aglycones. The aqueous layer was separated and boiled with (10) ml of *conc. HCl* for (2) minutes and treated as above with *amyl alcohol*; if the alcoholic layer is faintly yellow colored this indicates the presence of flavonoid glycosides.

2.2.2.2. Test for anthraquinones:-

(0.5) Grams of the powdered drug was boiled with (10) ml of *diluted* H_2SO_4 for (2) minutes. The extract was filtered while hot through a filter paper, cooled and extracted with (5) ml *chloroform.* The chloroformic layer was separated and (3) ml of (10%) *ammonia solution* was added to it. A rose, pink or red color in the ammonia layer indicates the presence of anthraquinones.

2.2.2.3. Test for cyanogenic glycosides:-

Little amount of the powdered *Fagonia cretica* was moistened with a little amount of water in a closed flask. A small piece of *sodium picrate* paper was fixed at the mouth of the flask. The flask was heated in a water bath to allow liberation of *HCN*. Turning of sodium picrate paper to brick-red will be taken as evidence of presence of either cyanogenic glycosides or glucosinolates.

2.2.2.4 Test for alkaloids:-

(2) Grams of the plant powder were moistened with (2) ml of (10%) *ammonia solution*, and then boiled with (10) ml *methanol* for 5 minutes, filtered through a cotton wool. The volume completed to (10) ml with *methanol* and transferred to a separatory funnel and portioned with equal volume of *chloroform.* The chloroform layer was taken and acidified with HCl in alcohol (HCl 1% + 95% alcohol). The aqueous layer was separated and divided into three test tubes, these were tested with: Mayer, Dragendorff and Haggers' reagents. Formation of precipitate in a form of turbidity indicates the presence of alkaloids.

2.2.2.5. Test for Saponin:

(1) Gram of the plant powder was boiled with (15) ml distilled water for (3) minutes and filtered while hot through a cotton wool. The filtrate was used for the following tests.

A-Frothing properties:

(1) ml of the above extract was placed in a test tube and shaken for (30) seconds. Formation of a persistent froth that lasts for at least a few hours will be taken as an evidence for the presence of Saponin .If not add few *Na* HCO_3 foam persist for more than (1) hour.

B-Hemolytic properties:

Place (5) ml of (5%) suspension of *red blood cells* in *normal saline* into each of (2) test tubes. To (1) test tube add (5) ml of normal saline solution .To the other add (5) ml of the plant extract into (0.045) *sodium chloride* which has been previously dissolved to render it isotonic with normal saline shake each of the test tubes (Positive in the test while control remains free of hemolysis).

2.2.2.6. Test for cardiac glycosides:

(2) Grams of the of the plant powder were boiled with (20) ml (70%) alcohol for (5) minutes; filtered while hot and the volume was adjusted to (25) ml by distilled water. (1) ml of strong lead acetate was added and the solution was filtered. The filtrate was used for the following tests:

A- Kedde's test (for lactone ring):

To (3) ml of the filtrate (1) of dinitrobenzoic acid in ethanol and (1) ml of 2 (M) NaOH were added; a reddish-brown or a yellow-brown colour indicates the presence of unsaturated lactone ring.

B- Bal jet's test (for lactones ring):

To (5) ml of the filtrate equal volume of *picric acid* was added, and then the solution was made and used for the following tests.

B-1: alkaline by addition of *NaOH* and allowed to stand for more than (15) minutes. Appearance of orange colour indicates the presence of lactones ring.

B-2: Test for deoxysugars (Keller Killiani's test):

(10) ml of the filtrate was extracted with equal volume of *chloroform*. The chloroformic extract was evaporated to dryness in a porcelain dish; cooled and the residue was dissolved in (3) ml of *glacial acetic acid*. The glacial acetic acid was carefully transferred on the side of a test tube (2) ml of *conc.* H_2SO_4. A ring with reddish brown color will be produced at the junction of liquids, and a gradually produced bluish green color in the upper layer indicates the presence of deoxysugars.

2.2.7. Test for steroids (Liebermann Burchard's method):

(1) mg of the dried alcoholic extract was dissolved in (3) drops of glacial acetic acid and (3) ml of a mixture of (5) parts acetic anhydride and (1) part of conc. H_2SO_4, green color or pink color indicates the presence of steroids or triterpenoid respectively.

2.2.2.8. Tests for Tannins

(1) Gram of the plant powder was decocted in (25) ml of *distilled water* was used for the following tests:

A-Ferric chloride test:

To (2) ml of the filtrate, (5%) $Fecl_3$ solution was added drop by drop. Appearance of green color indicates the presence of condensed tannins, while blue color indicates the presence of hydrolysable tannins.

B-Formaldehyde test:

To (2) ml of the filtrate, (3) drops of *formalin solution* plus 6 drops of (10%) *HCl* were added and boiled for (1) minute. Formation of a precipitate which is insoluble in *hot water*, *alcohol* and (5) *KOH* indicates the presence of condensed tannins.

C- Sodium nitrite test: To (3) ml of the extract, few crystals of $NaNO_2$ were added. Appearance of green color indicates the presence of ellagitannins, while brown color indicates the presence of other tannin.[31]

2.2.3. Biological activity screening techniques:-

***2.2.3.1. In vitro* antimicrobial activity:**

A-Preparation of bacterial suspensions:

(1) ml aliquots of a (24) hours broth culture were tested for organisms (obtained from department of microbiology, Medicinal and Aromatic Plant Research Institute, National Centre for research, Khartoum, Sudan were aseptically distributed onto nutrient agar slopes and incubated at (37ºC) for (24) hours. The bacterial growth was harvested and washed off (100) ml sterile normal saline, to produce a suspension containing about (10^{-10}) C.F.U/ ml/ the suspension was stored in the refrigerator (4º C) till used.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained. The average number of viable organisms

per ml of the stock suspension was determined by means of the surface viable counting technique. [34]

Sterile dilutions of the stock suspension were made in sterile normal saline solution and (0.02) ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for (2) hours at room temperature for the drops to dry and then incubated at (37ºC) for (24) hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by (50) and the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

B. **Preparation of fungal suspension:**

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at (25ºC) for (4) days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100) ml of sterile normal saline, and the suspension was used for in vitro testing of extracts for antimicrobial activity.

C. Testing of extracts for antibacterial activity:

The cup-plate agar diffusion method [35] was adopted to assess the antimicrobial activity of the prepared extracts. (1) ml of the standardized bacterial stock suspension (10^{-10}) C.F.U/ml were thoroughly mixed with (100) ml of molten sterile nutrient agar which was maintained at (45ºC). (20) ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars was left to set and in each of these plates (4) cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with (0.1)ml samples of each of the extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for (2) hours. The plates were then

incubated in the upright position at (37 ºC) for (18) hours. (2) Replicates were carried out for each extract against each of the test organisms. Simultaneously positive controls involving the addition of petroleum ether and methanol instead of the extracts were carried out separately. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

D. Testing for antifungal activity:

The same method as bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at (25 ºC) for (2) days for the Candida albicans and (3) days for Aspergillus niger.

2.2.4. *In vivo* toxicological studies:

Experimental Animals and their care:

Experimental procedures involving the experimental animals and their care were conducted in compliance with the Guidelines for Care and Use of Laboratory Animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (1984) and United States National Institutes of Health (1985). A total of thirty, young adult Wistar rats, weighting (42.6- 72.7) grams were obtained from the Sudanese National Centre for Research, Khartoum, Sudan. The rats were fed a standard rat food (a mixture of flour, oil, meat and some vegetables) and water ad libitum and were maintained at standard laboratory conditions (12/12 hr dark/light cycle, 23 ± 1 ºC temperature, and 55 ± 3 % humidity).

A-Acute oral toxicity studies in rats using limit dose test of Up-and-Down Procedure: -

Acute oral toxicity study was conducted using the limit dose test of Up-and-Down Procedure according to Organization for Economic Cooperation and Development (OECD) at a limit dose of (5000) mg/kg body weight per oral route.

Behavioral manifestations of acute oral toxicity such as lethargy, anxiety, sleep, hyperactivity, changes in eyes, diarrheal etc, were also noted. All observations were systematically recorded and individual records being maintained for each rat.

B-Oral doses administration of *Fagonia cretica:*

Before the experiment was beginning, rats were fasted overnight for (14 – 16) hours. Group C, which was the control group, received (10) ml/kg distilled water, orally, throughout the study period while Groups (1,2,3) were orally administered single, daily doses, (300 and 600) mg/kg of body weight, respectively of the *Fagonia cretica* ethanolic extract dissolved in distilled water (1gm/10ml) for (14) days using acute oral toxicity (425) protocol.[36]

2.2.4.1. General effects of *Fagonia cretica*

A- Body weight effects

Using a sensitive balance the weights of all rats were monitored at days (7 & 14) of the experiment, the obtained weights were statistically analyzed for comparison between test and control.

B- Relative organ weight:

The rats' relative vital organ weights of the (heart, liver, kidneys, lung and spleen) were monitored on the day (15) of the test, after sacrifying the rats and the obtained weights were statistically analyzed, and compared with the control.

C-Anesthesia induction time effects:

The effects of ethanolic extract of *Fagonia cretica* in doses of (100, 300 & 600 mg/kg/body weight) has been tested on the anesthesia induction time on rats after the study period of (14) days, compared to the control group.

2.2.4.2 Hematological effects evaluation:

Complete Hemogram (CBC) determination:

Blood samples for (*CBC)* were collected in (*EDTA)* tubes from the rats eyes using non-heparinized capillary tubes after induction of anesthesia using *diethyl ether* on a glass desiccators, were assayed using (Mindray BC-3000 Plus). [37]

The determined parameters included packed cell volume (*PCV*), platelet count, total leukocyte counts, (*Hb)* and, absolute values.

2.2.4.3 Biochemical studies methods:-

A-Blood glucose level evaluation:

At the day (15) of the experimental period, the fasted over night rats were anesthetized using diethyl ether on a glass desiccators and about (3) ml of blood were collected in heparinized tubes from rat eyes by non - heparinized capillary tubes. Samples were centrifuged to separate the plasma from the blood cells. The pure sera was transferred carefully to plain tubes and stored frozen. Blood glucose was determined by using crescent diagnostic glucose enzymatic colorimetric god-pap method. [38]

B-Serum cholesterol level evaluation:

The above mentioned frozen plasma aliquots were used to determine the Serum cholesterol level using crescent diagnostic cholesterol. Enzymatic, Liquid, colorimetric, (test-chod pap/ method).[39]

C-Renal function tests:

I- Blood urea estimation:

Blood urea level is determined by using crescent diagnostic urea enzymatic, colorimetric, (endpoint-Berthelot method). [40]

II- Serum creatinine level estimation:

Similar plasma samples were implemented to determine the Creatinine level using BioMed-Creatinine Intended for use for the quantitative determination of Creatinine in serum, plasma and urine.[41]

D-Liver function tests:-

I-Total protein level determination:

The total protein level was determined by using the plasma frozen samples and by applying crescent diagnostic. Total protein photometric colorimetric test (Biuret Method).[42]

II- Serum albumin level determination:

The serum albumin was determined with the same samples by using crescent diagnostic, serum albumin colorimetric test (BCG Method) .[43]

III- SGOT level was determined by using (N.S.BIO-TEC) colorimetric determination of SGOT (N.S.BIO-TEC).[44]

IV- SGPT level was determined by using (N.S.BIO-TEC) colorimetric determination of SGPT **(**S.BIO- N TEC). [44]

Chapter Three

3. RESULTS

3.1. Extraction yield:

(Figure.1): The coarse powdered plant yield (3.5 %) chloroformic extract and (7) ethanolic one

The plant, also gave a total amount (W/W) percentage (12.2%) flavonoid and (25.6%) saponin, respectively.

3.2. <u>Phytochemical screening of the main components:</u>

The crude plant powder showed presence of flavonoids, saponin, steroids, tannins, and absence of anthroquinone, cyanogenic glycosides and Cardiac glycosides.

Test	Test results
Flavonoids	+ve
Anthroquinone	-ve
Cynogenic glycosides	-ve
Alkaloids	+ ve
Saponins	+ve
Cardiac glycosides	-ve
Steroids	+ve
Tannins	+ve

(Table 5): The main chemical components of the plant

3.3. Biological activity screening:-

3.3.1. Antibacterial and antifungal activity:

Both chloroformic and ethanolic *fagonia cretica* extracts exhibited potential antibacterial and antifungal activity against different strains of bacteria and fungi as shown in (table 6). Moreover, the saponin and flavonoids fractions possess high activity against the tested organism see (table 6).

M.O	**Inhibition Zone** [Mean ± S.E.M] %n=4			
Extract	*Ethanol*	*Chloroform*	*Saponin*	*Flavonoids*
1- E. coli	14.5±0.5	13.0±0.0	20.0±0.0	20.0±0.0
2- P. aeruginosa	14.0±0.0	14.5±0.5	19.0±0.0	20.0±0.5
3- B. subtilis	14.5±0.5	18.5±0.5	19.0±0.0	17.0±0.0

M.O		Inhibition Zone [Mean ± S.E.M] %n=4			
Extract		*Ethanol*	*Chloroform*	*Saponin*	*Flavonoids*
4-	**S. aureus**	14.5±0.5	15.5±0.5	16.0±0.0	18.0±0.0
5-	**Candida. albicans**	14.0±0.0	15.5±0.5	16.0±0.0	17.0±0.0
6-	**Aspergillus. niger**	14.0±0.0	15.5±0.5	17.0±0.0	17.0±0.0

(Table *6)*: Effect of *Fagonia cretica* extracts and fractions against (4) different bacterial strains and (2) Fungi, data presented as zone of inhibition.

Standard antibiotic zones:

14	18 mm	weak
18	22	moderate
22	above	high

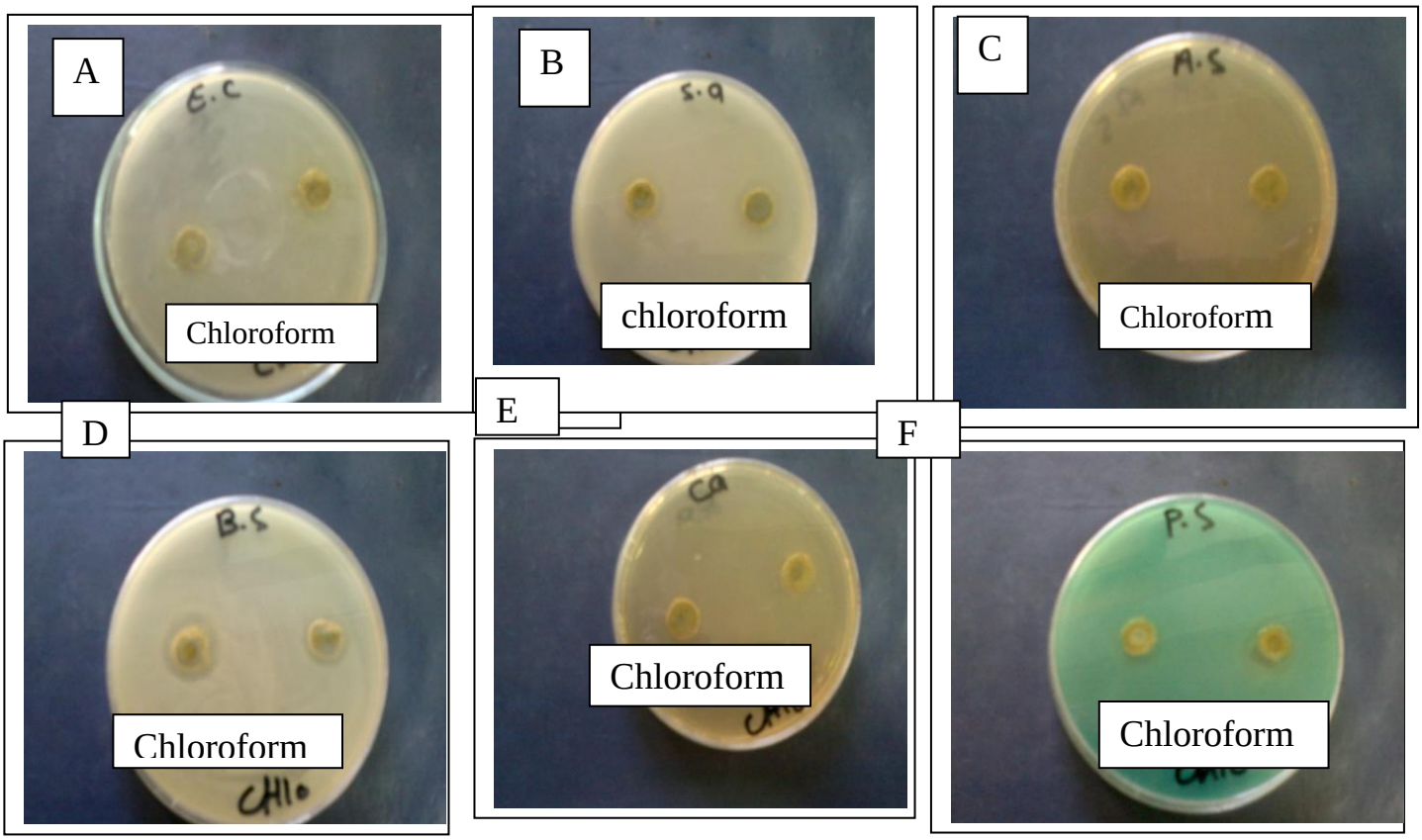
Bacteria used:

E. coli, P. aeruginosa, B. subtilis and S. aureus.

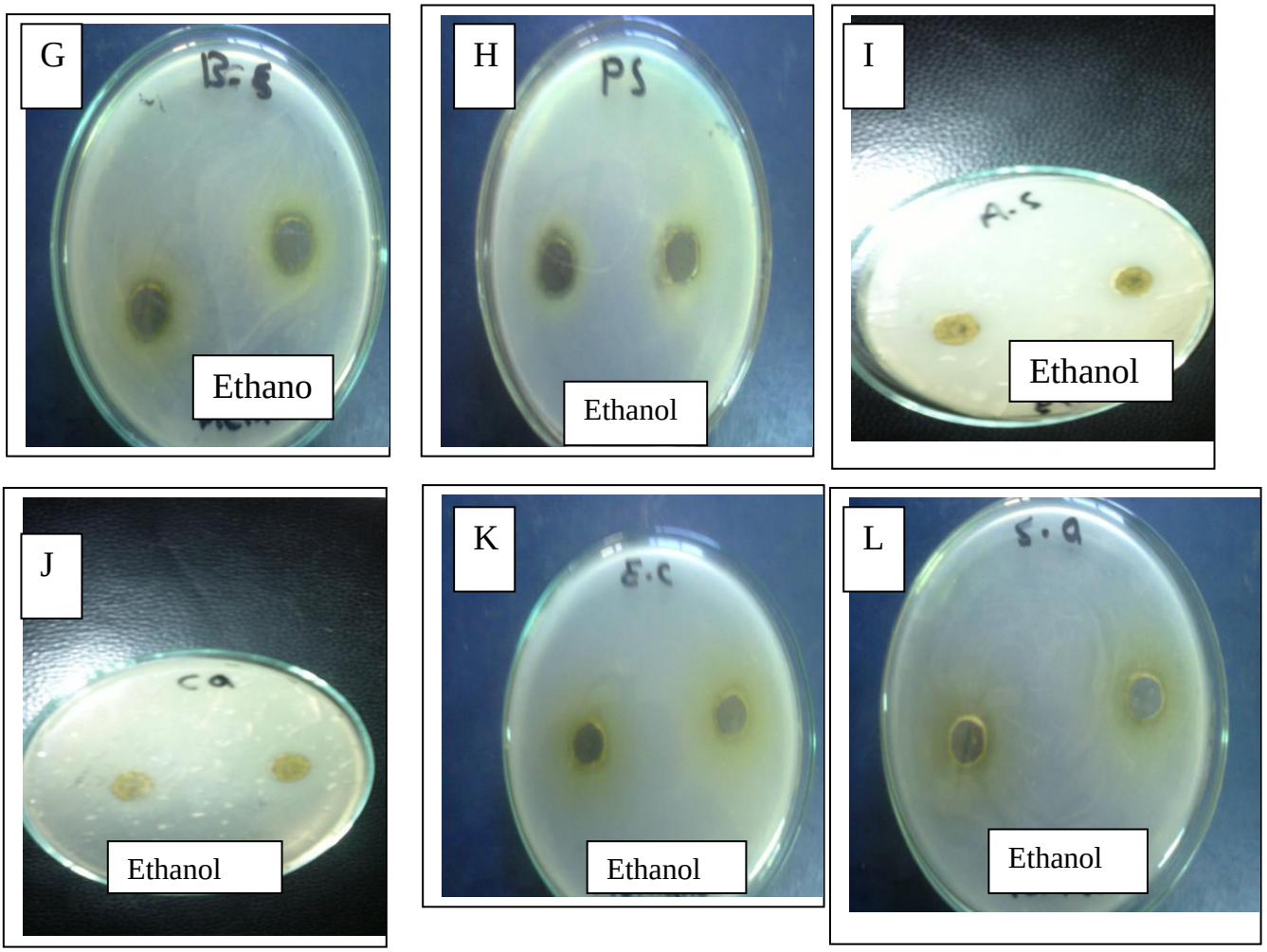
Fungi used:

Candida albicans.

Aspergillus niger.



(Fig. 2): Inhibition zone of chloroformic extract of *Fagonia cretica* against the tested organism, (A) E. coli, (B) S. aureus (C) Aspergillus niger (D) B. subtilis. (E) Candida albicans. (F) P. aeruginosa.



(Fig.3): Inhibition zone of ethanolic extract of *Fagonia cretica* against the tested organism (G):B. subtilis. (H) P aeruginosa. (I) Aspergillus niger. (J). Candida albicans. (K) E. coli. (L) S. aureus.

3.3. 2.Toxicological studies in vivo:-

3.3.2.1. Limit test for LD50 determination:

All the (3) groups of animals survive till the experiment was over after the administration of a selected high dose (5000) mg/kg/body weight (see table5),

although some signs were observed during the limit test such as lethargy, sleeping, hyperactivity, anxiety and sometimes inactivity.

3.3.2.2. Behavior at zero time:

Group NO. (1) Dose (100) mg /kg body weight.

Rat NO. (1) & seven normal.

Rat NO. (2) & (6) inactive.

Rat NO. (3), (4) & (5) anxiety, but (3) and (4) slept after (½) an hour.

No changes during the rest of the test time.

Group NO. (II) Dose (300) mg /kg body weight.

Rat NO. (I) & (5) normal.

Rat NO. (2, 3, 4 & 7) anxiety.

Rat (4) hyperactivity.

Group NO. (III) Dose (600) mg/ kg body weight.

Rat (1, 2, 3, 4 & 7) anxiety, after (½) an hour all changed to lethargy.

Rat (5 & 6) normal but change to lethargy after (½) an hour.

Statistical Program with its default setting

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	5000	O	O
2	2	5000	O	O
3	3	5000	O	O

(X = Death, O = Survival)

The **LD50** is greater than 5000 mg/kg.

(Table 7): **Sequence and results of limit dose test of *Fagonia cretica* in rats using AOT425statpgm (Version: 1.0) (Acute Oral Toxicity (OECD Test Guideline 425)**

3.3.2.3. Main test:-

All rats were survival through the experiment by using the recommended progression doses of *Fagonia cretica* as (175, 550, 1750, 5000, 5000, & 5000) mg/kg/body weight See (table 6), (figure 5): screen shot of AOT425 statistical program of main test.

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	175	O	O
2	2	550	O	O
3	3	1750	O	O
4	4	5000	O	O
5	5	5000	O	O
6	6	5000	O	O

(X = Death, O = Survival)

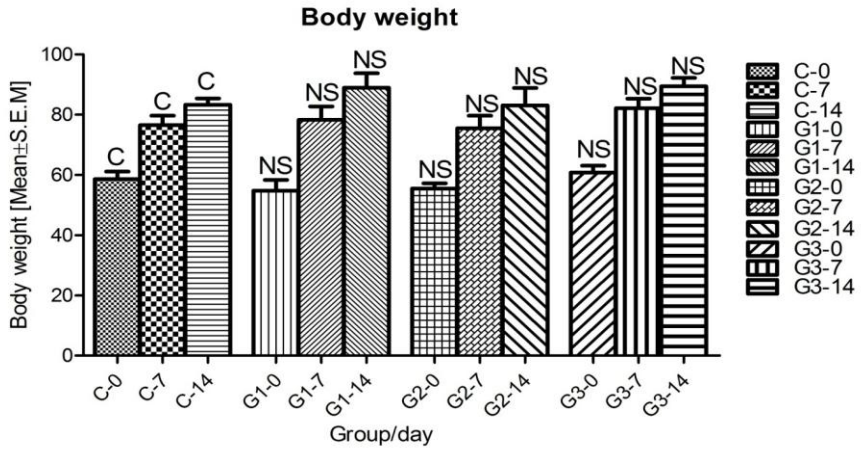
- Dose Recommendation: The main test is complete.
- Stopping criteria met: (3) at Limit Dose.
- Statistical Estimate based on long term outcomes.
- The **LD50** is greater than (5000) mg/kg.

Table 8: Sequence and results of the main dose test of *Fagonia cretica* in rats using AOT425 stat pgm (Version: 1.0) (Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program with its default setting.

3.2.4. General effects of *Fagonia cretica*:-

I- Body weight effects:

The ethanolic extract of *Fagonia cretica* in doses of (100, 300 & 600 mg/kg/body weight) has no effects on the rats body weight during the study period of (14) days compared to the control group monitored at day(0, 7 & 14) this can be shown in Fig.(4)



(Fig. 4): Rats **body weight during the study period monitored at day (0, 7 & 14)**

II- Relative organ weight:

The ethanolic extract of *Fagonia cretica* in doses of (100 & 300 mg/kg/body weight) has no effects on the rats relative vital organ weight (heart, liver, kidneys, lung and spleen) during the study period of (14) days compare to the control group whilst the dose of (600) mg/kg/body weight change the liver somewhat from the relevant control, this can be shown in (Table 9).

Relative organ weight (g/100 g):

Doses (mg/kg)	Heart	Liver	Kidneys	Lung	Spleen
Control	0.35±0.01	3.02±0.1	0.66±0.03	0.63±0.03	0.20±0.02
100	0.35±0.02	3.08±0.2	0.68±0.03	0.63±0.03	0.20±0.01
300	0.33±0.02	2.52±0.6	0.63±0.03	0.59±0.02	0.19±0.01
600	0.36±0.02	3.50±0.1*	0.66±0.02	0.60±0.02	0.20±0.01

(Table 9): **Effect of ethanol leaves extract of *Fagonia cretica* on the relative organs weight and lethality in rats**

III. <u>Anesthesia induction time effects:</u>

The ethanolic extract of *Fagonia cretica* in doses of (100 & 300 mg/kg/body weight) has no effects on the anesthesia induction time on rats after the study period of (14) days compared to the control group whilst the dose of (600) mg/kg/body retard the anesthesia induction time on rats from the relevant control, this can be shown in (Fig. 5).

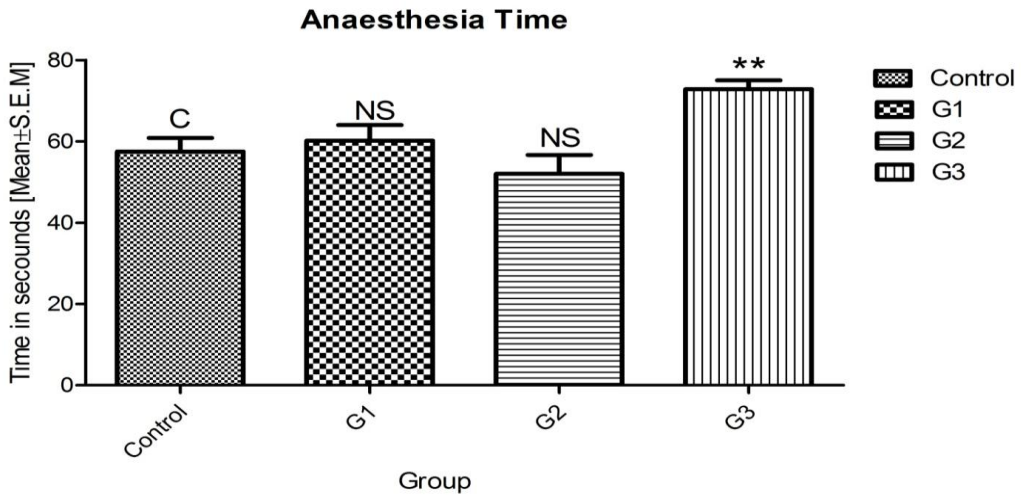


Fig. 5: **Anesthesia induction time for all groups using diethyl ether at the end of the study (day15)-sacrifying date.**

IV- <u>Hematological effects:</u>

The ethanolic extract of in doses *Fagonia cretica* of (100, 300 and 600) mg/kg/body weight) has different effects on the major blood cells in rats after the study period of (14) days compared to the control group, this can be shown in (Table 10)

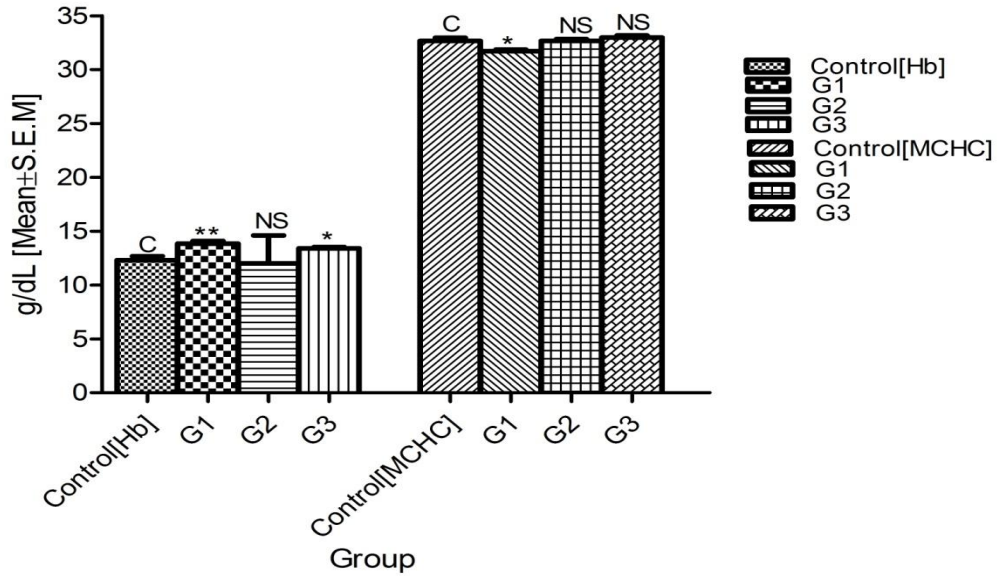
		<u>Blood cells [Mean ± S.E.M]</u>	
Doses (mg/kg)	WBCs [cmm]	RBCs[cmm]	PLTs [cmm]
Control	3.4 ± 0.3	6.8 ± 0.2	667.3± 65.2
100	5.5 ± 0.6**	7.8 ± 0.1***	926.3± 33.8**
300	6.5 ±1.0**	6.6 ±1.2	954.0± 66.6*
600	6.3 ±1.1*	7.2 ± 0.1	962.7 ± 62.3**

(Table 10): **Effect of ethanol leaves extract of *Fagonia cretica* on the major Blood cells in rats.**

***reference values (Blood cells):**

Parameter	Normal range
WBCs	6.6-12.6 x 10^3/mm
RBCs	6.76-9.75 x 10^6 /mm
Platelets	150-460 x 10^3/mm

The ethanolic extract of in doses *Fagonia cretica* of (100, 300 & 600 mg/kg/body weight) has different effects on the Hemoglobin level *Hb* and the mean cell hemoglobin concentration *MCHC* in rats blood after the study period of (14) days compared to the control group, this can be shown in
(Fig. 6).

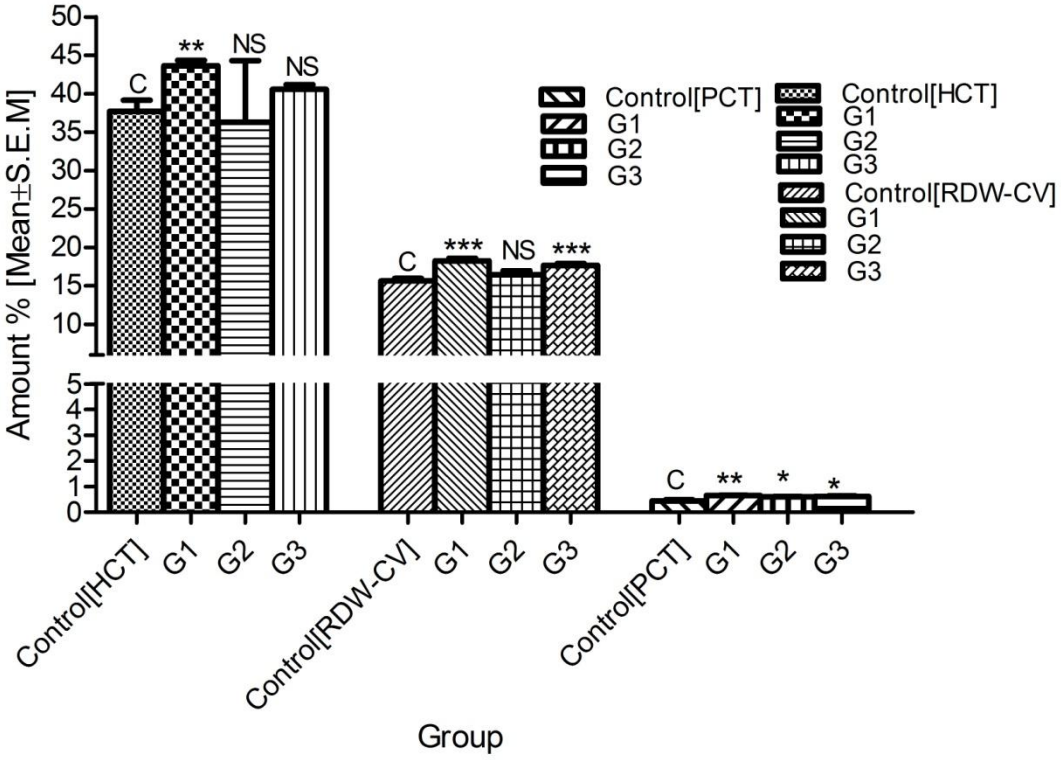


***Reference values (Hemoglobin):**

Parameter	Normal range
Hemoglobin	11.5-16.1g/dl

(Fig. 6): Effect of ethanol leaves extract of *Fagonia cretica* on *Hb* and *MCHC* of the different rats groups.

The ethanolic extract of *Fagonia cretica* in doses of (100, 300 &600 mg/kg/body weight) has different effects on the (*RBCs- Hct)*, platelets-hematocrit *Pct* and the (*RBCs)* distribution width cell volume (*RDW-CV)* in rats blood after the study period of (14) days comparable to the control group, this can be shown in (Fig. 7).



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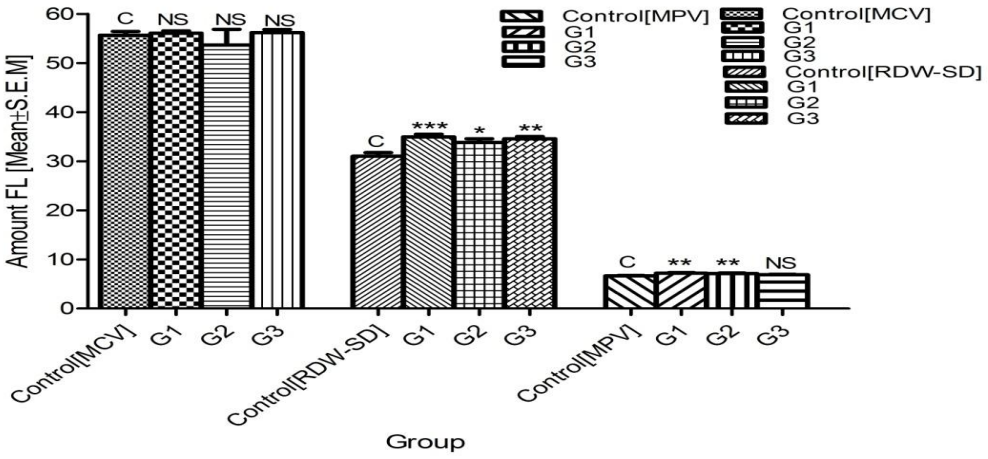
*** Reference values (Red blood cells hematocrit):**

Parameter	Normal range
Hct	37.6-50.6%)

(Fig.7): **Effect of ethanol leaves extract of *Fagonia cretica* on (*Hct), (*RDW-*CV),* and (Pct) of the different rats groups**

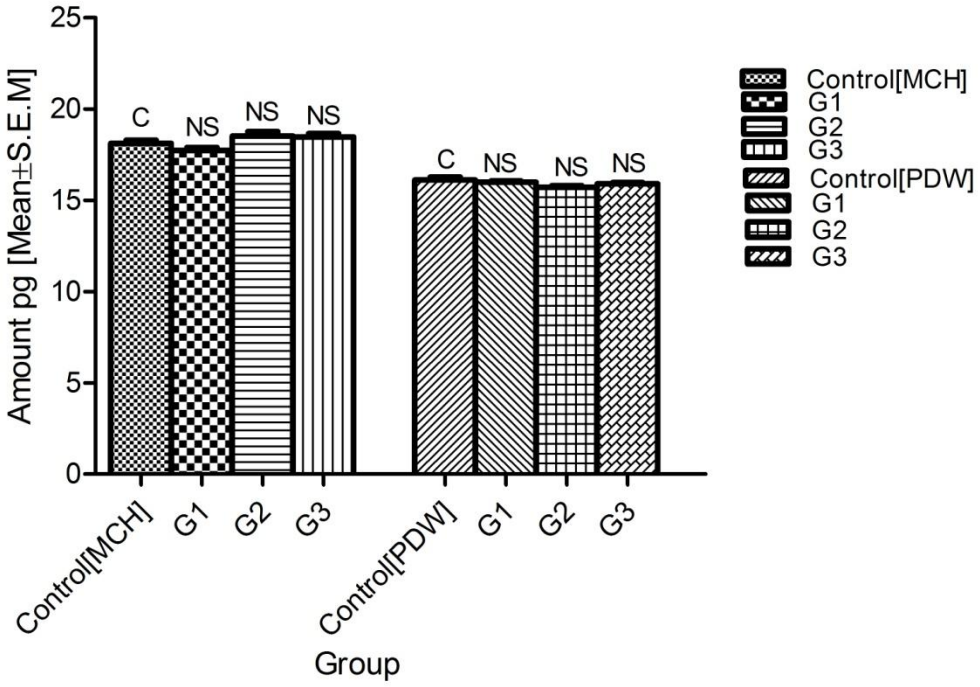
The ethanolic extract of *Fagonia cretica* in doses of (100, 300 & 600 mg/kg/body weight) has different effects on the mean cell volume (*MCV)*, mean platelets volume (*MPV)* and the (*RBCs)* diameter width standard deviation (*RDW-*

SD) in rat's blood after the study period of (14) days compared to the control group, this can be shown in (Fig**. 8).**



(Fig. 8): **Effect of ethanol leaves extract of *Fagonia cretica* on (*MCV), (RDW-SD)* and (*MPV)* of the different rats groups.**

The ethanolic extract of in doses *Fagonia cretica* of (100, 300 & 600 mg/kg/body weight) has no effects on the (*MCH)* and the platelets diameter width (*PDW)* in rat's blood after the study period of (14) days compared to the control group, this can be shown in (Fig.9**)**.

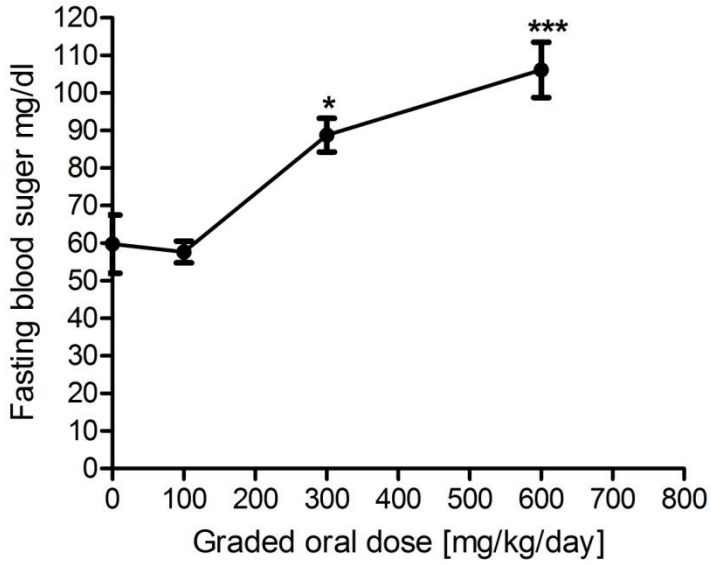


(Fig.9): **Effect of ethanol leaves extract of *Fagonia cretica* on (*MCH)* and (*PDW)* of the different rats groups.**

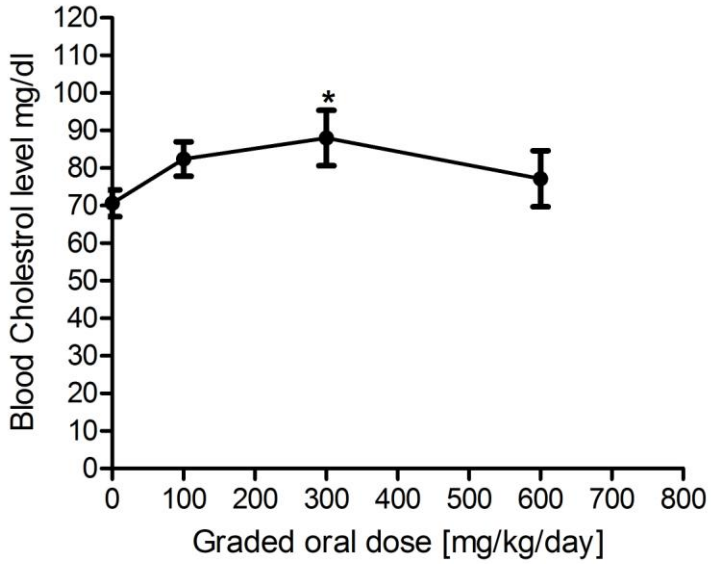
V-<u>Biochemical effects:-</u>

A-<u>Blood glucose and cholesterol:</u>

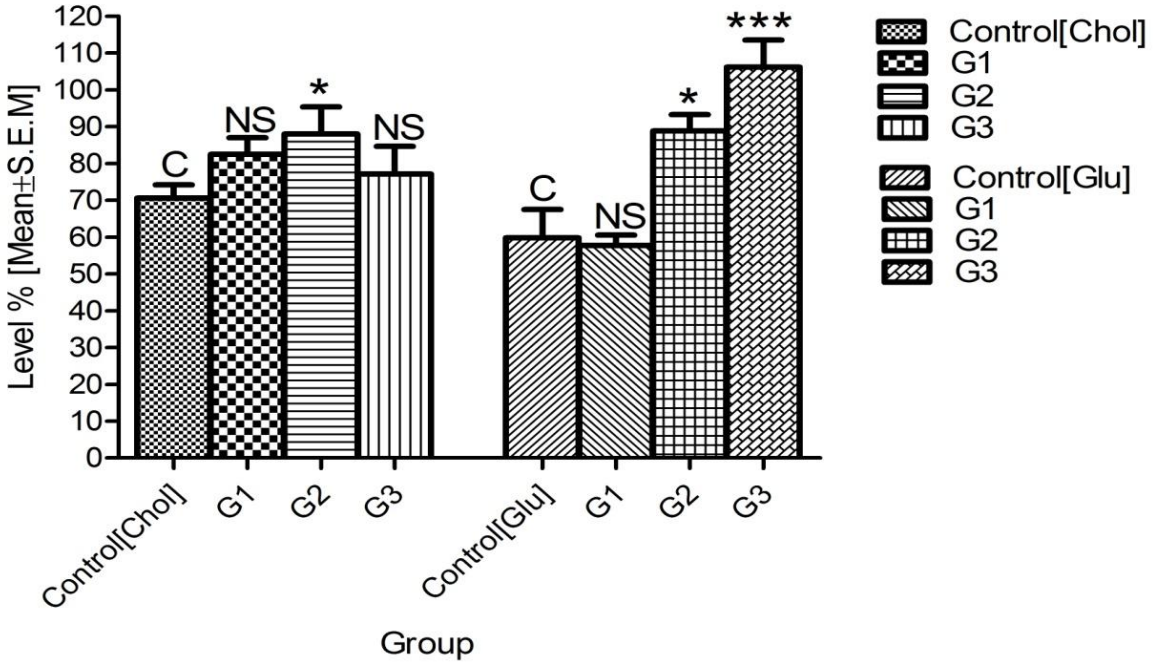
The ethanolic extract of *Fagonia cretica* in doses of (100, 300 & 600 mg/kg/body weight) has different effects on the fasting blood glucose (Fig. 10-12) and cholesterol (Fig. 11-12) in rat's blood after the study period of (14) days compared to the control group.



. (Fig. 10): **Rats glucose levels % after the study period day (15)**



(Fig.11): **Rats cholesterol levels % after the study period day (15)**



(Fig. 12): Blood glucose and Cholesterol level:

***<u>Reference values</u> (Blood glucose and Cholesterol level):**

Parameter	Normal range
Cholesterol	40-130 mg/dl
Glucose	50-135 mg/dl

<u>B-Renal function tests:</u>

The ethanolic extract of *Fagonia cretica* in doses of (100, 300 & 600 mg/kg/body weight) has no effects on the urea and creatinine as kidney function parameters, except the dose (300) mg effect on the urea level in rats blood after the study period of (14) days compared to the control group, as shown (table 11).

	Kidney function parameter[Mean ± S.E.M]	
Doses (mg/kg)	Urea [mg/dl]	Creatinine [mg/dl]
Control	31.9 ± 1.3	0.5 ± 0.1
100	32.6 ± 5.0	0.6 ± 0.1
300	40.9 ± 1.9**	0.6 ± 0.1
600	32.7 ± 5.3	0.5 ± 0.1

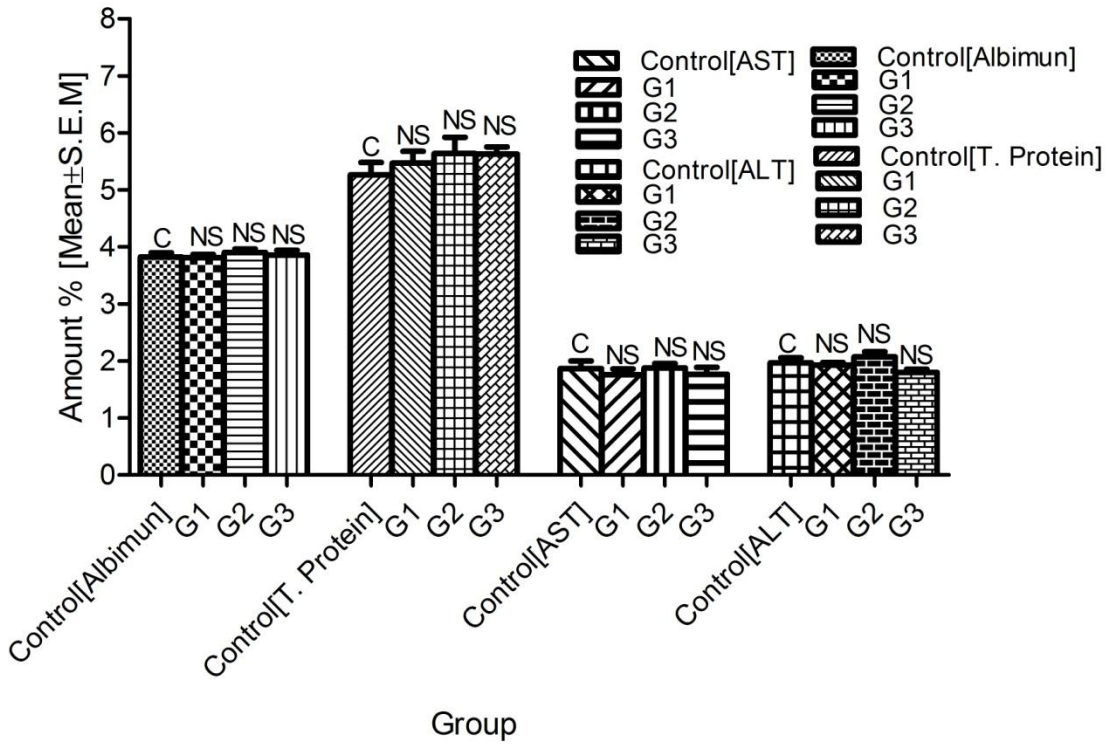
***Reference values (Kidney function tests):**

Parameter	Normal range
BUN	15-21 mg/dl
Creatinine	0.2-0.8 mg/dl

(Table 11): Effect of ethanol leaves extract of *Fagonia cretica* on the kidneys function tests (urea + creatinine) in rats.

C-Liver function tests

The ethanolic extract of *Fagonia cretica* in doses of (100, 300 & 600 mg/kg/body weight) has no effects on the total protein, serum albumin, SGOT and SGPT as liver function parameters levels in rat's blood after the study period of (14) days compared to the control group, as shown in (Fig.13)



(Fig. 13): **Effect of ethanol leaves extract of *Fagonia cretica* on albumin, total protein, *SGOT* and *SGPT* of different rats groups.**

Reference value (Liver function tests):

Parameter	Normal range
Albumin	3.8-4.8 g
T protein	5.6-7.6 g/dL
SGPT	17.5-30.2 U/L
SGOT	45.7-80.8 U/L

Chapter four

4-1 Discussion

This is an *in vitro* and *in vivo* current study of the Sudanese *Fagonia cretica linn*, that attempted to examine the antimicrobial activity, toxicological, hematological and biochemical effects, in order to fill the gap due to lack of such information's in Sudan.

The extraction results revealed that the ethanolic yield was double the chloroformic one, which means that the polar components of the crude extract, was predominant over the non-polar ones, confirming the traditional methods of use that depends on the aqueous extract as preferable for public use.

The phytochemical screening of the crude powder of *Fagonia cretica* revealed the presence of flavonoid, saponin, steroids, tannins, alkaloids and absence of anthraquinones, cyanogenic glycosides and Cardiac glycosides. This agrees with (Shahina & Ghazafar, 1994), who proved the presence of the following Phytochemical components.

The biological activity of both chloroformic and ethanolic *Fagonia cretica* extracts was investigated in different aspects, regarding the antimicrobial activity, the plant exhibited potential antibacterial activity against different choosed strains and the Saponin and flavonoides fractions showed stronger activity than the crude extract, this can be taken as an evidence that flavonoides and saponin are responsible for this activity, besides the other component were with less or without activity. This agrees with (Sajid, et al, 2011) who found that *Fagonia cretica* showed antimicrobial activity using different extracts (aqueous, methanolic and ethanolic). Aqueous and methanolic extracts showed more activity, which means that the polar components of the crude extract was predominant over the non-polar ones, confirming the traditional methods of use that depends on the aqueous extract as preferable for public use. Our chloroform findings confirmed their results. My

results also agrees with (Thetwar, LK; et al.2006) who tested the antibacterial and antifungal properties of fagonia cretica flowers methanolic extract against selected microbes. The obtained results, are also matching with (Anjum, Muhammad, et al 2007) who tested eleven compounds isolated for the first time from the whole plant of *Fagonia Cretica* to their antimicrobial activity.

Nowadays, there is an increase and popular utilization of *Fagonia* for treating various health problems worldwide particularly in Pakistan, India, Far East and some other parts of the world including Sudan; hence there were limited scientific data available regarding its safety. This current case - control (14) days study evaluate the safety of *Fagonia* ethanolic extract using protocol 425 guidelines. Observations of rats' behaviors can be summarized as follows; rats were normally behaving during the adaptation period, after (½) hours of dose administration some of them from different study groups develop inactivity, lethargy and asleep associated with some rats' hyperactivity. No behavioral changes occurred during the remaining test period. Statistical estimates based on long term outcomes. The LD50 is greater than (5000) mg/kg, which indicates the high safety of the plant, which encourages its use by human. This result confirmed it's use in endogenous medicine for the treatment of breast cancer in Pakistan (*Saeed MA*. et al 1969). Also it agrees with (Lam 2012) who describes for the first time that an aqueous extract of *Fagonia cretica* shows potent *in vitro* cytotoxic activity towards breast cancer epithelial cell lines which was not seen towards normal mammary epithelial cells.

The ethanolic extract of *Fagonia* showed no effect in rat's body weight, which means absence of any substance that may cause increase in body weight.

No effects were observed on different vital organs using the tested doses, except for the large dose which has significantly increased the liver size, this hepatomegaly was not due to any pathological conditions because the macroscpical

investigations showed normal liver texture, besides our liver function tests revealed normal functionality. This was in line with the traditional use in treating liver disorders (Avinash K et. Al., 2004) also the literature survey revealed the presence of antioxidant effect that act as Hepato protective (Avanish, K et al 2004).

Only the dose of (600) mg/kg/body retarded the anesthesia induction time on rats, which means that the ethanolic extracts has a neurological effect, which agrees with (Avinash K et. Al., 2004.).

Different effects were observed on hematological parameters, For (*WBCs)* that significantly increased in dose-dependent manner which indicated the imuno-potentiatation property, that supported the antioxidant property, this result is different from the result of **(**Asif, et al., 2003) who reported the consistent (WBCs) reduction during the (16) days treatment, in contrast, it agrees with (Mitani, et al 1993) who explored the antimicrobial activity of its flavonoides compounds.

The (*RBCs)* with its hemoglobin increased in the low dose of (100) mg which means that the extract either contain erythropoietin hormone, or erythropoietin like substances that stimulate the production, of (*RBCs)* masking the (*RBCs)* fragility property of the saponin ,leading to more (*RBCs)* production these findings contradict with (Asif Saeed et al 2003) Moreover, the extract was with potential use in treating anemia, similar conclusion was reached by (Rashid. S,) who succeeded to manage Thalassemia major. These results were confirmed by the Hematocrit data.

The platelets show dose dependant increment. This can be taken as evidence that the extract inhibits the bleeding tendency which may be due to the presence of coumarins or substance possesses thrombopiotien or thrombopiotien like action. The impact of this is the use of the herbal tea of the plant as

anticancerous, where it increases the platelets number and reduces the bleeding tendency.

The ethanolic extract increased the blood glucose level in a dose dependant manner significantly starting from the (300) mg/kg body weight dose, indicating the diabetogenic features of the plant which may be due to the increase of cortisol hormone by both the crude plant and saponin I and II, this result is in line with (Asif Saeed et al. 1999), so the plant should be used with care for diabetic patients will be in need to use it, these findings can be considered as totally new, because no such informations were reported before.

The ethanolic extract showed biphasic effects on cholesterol level, indicating a hyperlipidemic property of the plant in low and moderate doses which disappeared in high doses; this result means that the plant may contain two different components, one predominant in low concentrations and the other in high concentrations. Moreover, In low concentrations the lipid biosynthesis and transportation will be enhanced, while in high concentrations the other compensatory mechanism such as negative feedback and cholesterol utilization may be stimulated. Different results were reported by (D, Senthil 2013) who reported that the aqueous extract of the whole plant of *Fagonia cretica* possess a significant antihyperlipidemic effect at the doses of (250) and (500) mg/kg body weights. The disagreement between these results is either due to the use of ethanolic extract excluding some active hypolipidemic components where the aqueous extract was used to make the components as synergetic, or because the normal range of cholesterol (40-130) mg/dl cover a wide range that masks minor changes, or because a hyperlipidemic agent was given for these rats before conducting the test and so serum cholesterol was raised to higher level then given the plant extract, this makes tracing the effect more easy.

Regarding the renal function test, the urea level is only affected significantly by ethanolic extract in moderate dose, these results were without significant clinical meaning, because the other parameters, creatinine and urea were not affected, at the same time this was confirmed by the macro pathological observation that revealed absence of any abnormal changes. Besides, these were in line with the toxicological findings which showed higher margin of safety, also the traditional plant was used as urinary antiseptic (Chopra R, et al 1982)

The ethanolic extract of *Fagonia cretica* in all doses has no effects on the investigated liver enzymes and proteins level, indicating the high safety level and agree with (Avinash K et al 2004) and supported the absence of any toxic substances, on the other hand it encourages its use by mankind.

4-2 Conclusion

Fagonia cretica linn is an ancient medicinal plant known in Asia and the Far East countries. *Fagonia cretica linn* usage is not confined to one or two diseases, but it is reputed that it has the ability to treat many disease including fever, thirst, vomiting, dysentery, asthma, urinary discharges, liver troubles, typhoid, toothache, stomach troubles etc. The plant also is reported to possess potent antibacterial properties against pathogenic organisms, and have been extensively used in the treatment of various types of hematological, hepatic, neurological and inflammatory conditions.

The screening of the preliminary Phytochemical yield the presence of flavonoides saponin, steroids, tannins, alkaloids and absence of anthraquinones, cyanogenic glycosides and Cardiac glycosides, when testing the antibacterial and antifungal activity of the plant, both chloroformic and ethanolic extracts of *Fagonia cretica* exhibited potential antibacterial and antifungal activity against different strains of bacteria and fungi, moreover, the saponin and flavonoids fractions possess high activity against the tested organism.

The toxicological determination of ***LD50*** was found to be greater than (5000) mg/Kg, estimated by the special statistical software with three stopping criteria based on long term outcomes. Which indicating wide safety margin for *Fagonia ethanolic* extract.

The evaluation of the hematological effects of the plant, different effects were observed on hematological parameters, For (*WBCs)* significantly increased in dose-dependent manner which indicated the imuno-potentiatation property. (*RBCs)* with its hemoglobin increased in the low dose.

The platelets show dose dependant increment, which can be taken as an evidence that the extract inhibit the bleeding tendency this may be due to the

presence of coumarins or substance possess thrombopiotien or thrombopiotien like action. The ethanolic extract increased the blood glucose level in dose dependant manner significantly, indicating the diabetogenic features of the plant.

Cholesterol level, renal function and liver function examination revealed different responses to the *Fagonia cretica* ethanolic extracts more knowledge about the importance of *Fagonia cretica linn* plant as a medicinal plant is gained by the advent of computerized automated blood analysis methods, revealed newer terms like (*RDW), (RDW-SD),* these terms also indicate unusual variations in the size and shape of (*RBCs)* which are highly indicative of a possible Anemic conditions which it may be due to iron, foliate or vitamin B_{12} deficiency. New hematological parameters of *fagonia cretica* were studied for the first time and used as tools for interpretation of hematological results. These parameters include (*RDW), (RDW-SD), (RDW-CV), (PCT), (MPV),* exploring the potentiality of *Fagonia cretica* plant as an agent that affect several blood parameters in rat is a achieved and a Sudanese data for *Fagonia cretica* is obtained .

The advent of computerized automated blood analysis method newer terms like (*RDW), (RDW-SD),* have come into place, these terms also indicate unusual variations in the size and shape of (*RBCs)* which are highly indicative of a possible Anemic conditions which it may be due to iron, foliate or vitamin B_{12} deficiency.

(*MPV)* Mean Platelet Volume Indicates average size of platelets; older platelets are generally smaller than younger ones and a low (*MPV)* may means a condition affecting the production of platelets by the bone marrow.

(RDW-CV RBCs) Distribution Width 'Coefficient of Variation.

Parameters including hematological blood indices, renal functions test parameters, liver functions tests parameters, blood glucose levels, and serum cholesterol levels. The outcome of this study is considered as a new Sudanese data concerning *Fagonia Cretica linn*.

4-3 Recommendations

1-Further studies targeting the identification of the active phytochemical components of *Fagonia cretica* and their role of action are recommended.

2-Pharmaceutical formulation of *Fagonia cretica* as herbal medicine is highly recommended.

3-The ***LD50*** of the plant showed a high margin of safety which encourages its use by human for the treatment of many diseases.

4-More studies on the hyperglycemic property of the plant is recommended

5-The use of the plant by diabetic patients should be with alert.

6-Further studies on the Sudanese *Fagonia cretica* as antioxidant, immune modulating agent, anticancerous, and anti-inflammatory is also recommended.

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APPENDICES

بسم الله الرحمن الرحيم

جمهورية السودان
المركز القومي للبحوث
مركز التوثيق والمعلومات

Republic of the Sudan
National Center for Research
Documentation & Information Center

النمرة: .....
التاريخ: ٢٠١٨/٤/١٥

Ref: .....
Date: .....

السيد/ _____

الموضوع:/ افادة

ارجو ان احيط سيادتكم بان
موضوع البحث/ تبات أم شويكه
Fagonia Cretica Linn
للباحث/ عمر عباس محمد جاه الله

لايوجد فى قواعد البيانات المحلية بمركز التوثيق والمعلومات – المركز القومى للبحوث.

ولكم الشكر

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المركز القومى للبحوث

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النمرة: ج أ س \ ك ص ا ق ع أ ا ل. أ. ب / بحث -2 التاريخ: ٥ \ ٥ \ 2013 م

الافاضل: .....

تصريح بإجراء تجارب معملية على الحيوانات الصغيرة

بعد نظر اللجنة فى اجتماعها رقم (..١..) بتأريخ :٤/٥/ 2013 م فى الطلب المقدم من الباحث:

عمر عباس محمد ..... ومجموعته لأجراء بحث بعنوان:

Studies on Fagonia Cretica Linn - (Acute oral toxicity using 425 protocol ..... etc.) .....

فقد منحت اللجنة الاذن بالرقم: (OIU/I.A.E.C./Exp. Tox. 2013/0.0.٦.) ويعتبر ساريا من تاريخ تحريره ولمدة .....٤..... اشهر/ سنة، (قابلة للتجديد) ..... فترة/ غير قابلة للتجديد).

وتتمنى لهم اللجنة التوفيق وترجوا تسهيل مهمتم والتعاون معهم.

ملحوظة:

- يحق للجنة سحب الترخيص وايقاع اى عقوبات تراها مناسبة فى حالة عدم التقيد بالاقرار الموقع بالطلب.
- صورة لإرشيف اللجنة.

مقرر اللجنة: .....

رئيس اللجنة: .....

(RDW-SD) stand for the standard deviation of the red cell distribution width these are hematological parameters an underling anemic conditions

(RDW-CV) (RBCs Distribution Width Coefficient of Variation which indicates uniformity in size of RBCs, The RDW-CV is calculated from the formula:

RDW-CV = (1SD/MCV). 100

Normal ranges **RDW-SD** 39-46 fl **RDW-CV** 11.6-14.6% in adults

The Clinical interpretation of RDW and MCV is summarized in the table

Microcytic-Isocytic		Microcytic-Anisocytic		Normocytic-Isocytic		Normocytic-Anisocytic		Macrocytic-Isocytic		Macrocytic-Anisocytic	
MCV	RDW	MCV	RDW	MCV	RDW	MCV	RDW	MCV	RDW	MCV	RDW
de-creased	Normal	de-creased	in-creased	normal	Normal	Normal	in-creased	in-creased	normal	in-creased	in-creased
β-Thalassae-mia minor		Iron defi-ciency Anaemia		Anaemia of chronic Diseases		Osteomyelo-fibrosis		Aplastic anaemia		Pernicious Anaemia	

MPV :Mean Platelet Volume Indicates average size of platelets; older platelets are generally smaller than younger ones and a low *(MPV*) may means a condition affecting the production of platelets by the bone marrow

Natural view for Sudanese Fagonia cretica grown on a quaz (Shendi University)

Fagonia cretica image from the internet

Fagonia cretica image from the internet

Natural view for Sudanese Fagonia cretica grown on a quaz (Shendi University)

RDW: - RBCs Distribution Width which indicates uniformity in size of (*RBCs)* which means that very low and very high doses causes variations (*RBC's) size.*

(PCT) platelets Hematocrit.

(RDW-SD) stand for the standard deviation of the red cell distribution width These are hematological parameters an underling anemic conditions, the normal (*RBCs)* are spherical and biconcave with a diameter of (6-8) micrometer., when blood samples were analyzed manually under microscope the terms aniso and poikilocytosis were used, when such variations were observed, but with the advent of computerized automated blood analysis method newer terms like

(RDW), (RDW-SD), have come into place, these terms also indicate unusual variations in the size and shape of RBC which highly indicative of a possible anemic conditions which it may be due to iron, foliate or vitamin B12 deficiency.

(MPV) Mean Platelet Volume Indicates average size of platelets; older platelets are generally smaller than younger ones and a low *(MPV)* may means a condition affecting the production of platelets by the bone marrow
(RDW-CV) RBCs Distribution Width 'Coefficient of Variation which indicates uniformity in size of **RBCs**

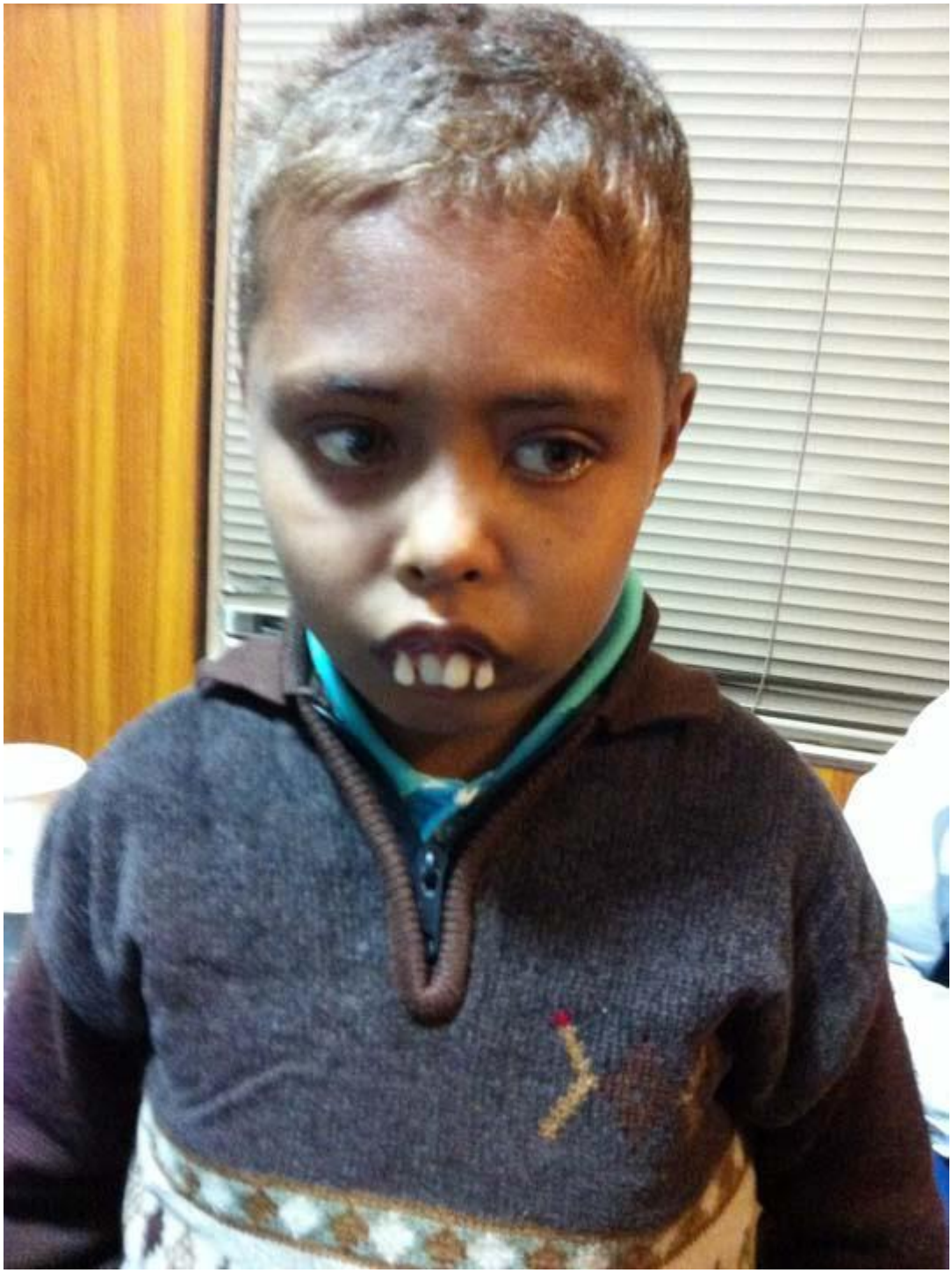
RBC	Red Blood Cell Count	Known as anemia Acute or chronic bleeding RBC destruction (e.g., hemolytic anemia, etc.) Nutritional deficiency (e.g., iron deficiency, vitamin B12 or folate deficiency) Bone marrow disorders or damage Chronic inflammatory disease Kidney failure	Known as Polycythemia Dehydration Lung (pulmonary) disease Kidney or other tumor that produces excess erythropoietin Smoking Genetic causes (altered oxygen sensing, abnormality in hemoglobin oxygen release) Polycythemia Vera—a rare disease
Hb	Hemoglobin	Usually mirrors RBC results, provides added information	Usually mirrors RBC results
Hct	Hematocrit	Usually mirrors RBC results	Usually mirrors RBC results; most common cause is dehydration
RBC indices			
MCV	Mean Corpuscular Volume	Indicates RBCs are smaller than normal (macrocytic); caused by iron deficiency anemia or Thalassemia, for example.	Indicates RBCs are larger than normal (macrocytic), for example in anemia caused by vitamin B12 or folate deficiency
MCH	Mean Corpuscular Hemoglobin	Mirrors MCV results; small red cells would have a lower value.	Mirrors MCV results; macrocytic RBCs are large so tend to have a higher MCH.

MCHC	Mean Corpuscular Hemoglobin Concentration	May be low when MCV is low; decreased MCHC values (hypochromia) are seen in conditions such as iron deficiency anemia and Thalassemia.	Increased MCHC values (hypochromia) are seen in conditions where the hemoglobin is more concentrated inside the red cells, such as autoimmune hemolytic anemia, in burn patients, and hereditary spherocytosis, a rare congenital disorder.
RDW (Not always reported)	RBC Distribution Width	Low value indicates uniformity in size of RBCs	Indicates mixed population of small and large RBCs; immature RBCs tend to be larger. For example, in iron deficiency anemia or pernicious anemia, there is high variation (anisocytosis) in RBC size (along with variation in shape – poikilocytosis), causing an increase in the RDW.
Reticulocyte Count (Not always done)	Reticulocyte (absolute count or %)	In the setting of anemia, a low reticulocyte count indicates a condition is affecting the production of red blood cells, such as bone marrow disorder or damage, or a nutritional deficiency (iron, B12 or folate)	In the setting of anemia, a high reticulocyte count generally indicates peripheral cause, such as bleeding or hemolysis, or response to treatment (e.g., iron supplementation for iron deficiency anemia)

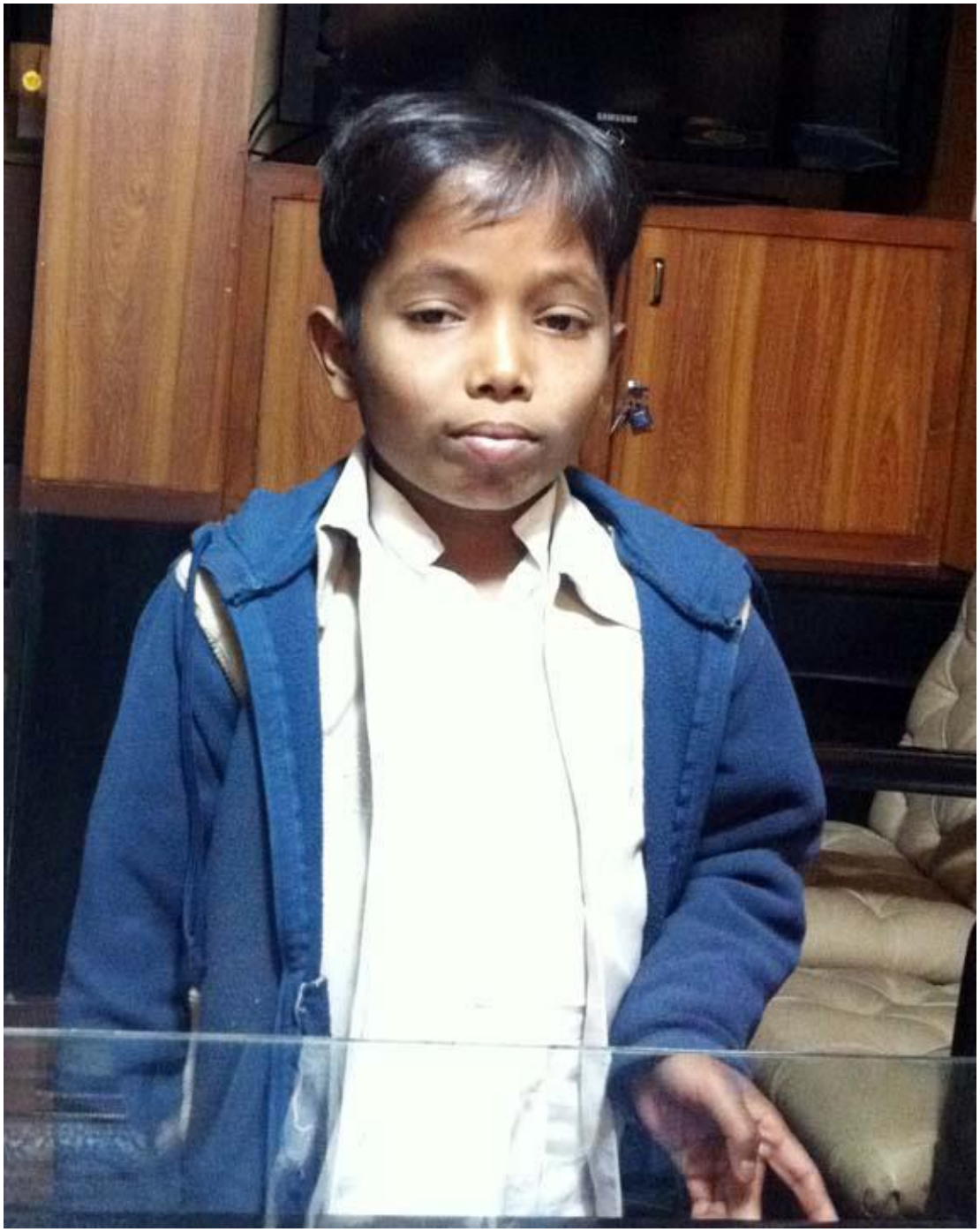
Expand Table Platelet Evaluation

Test	Full Name	examples of causes of low result	examples of causes of high result
Plt	Platelet Count	Known as thrombocytopenia: Viral infection (mononucleosis, measles, hepatitis) Rocky mountain spotted fever Platelet autoantibody Drugs (acetaminophen, quinidine, sulfa drugs) Cirrhosis Autoimmune disorders Sepsis Leukemia, lymphoma Myelodysplasia Chemo or radiation therapy	Know as thrombocytosis: Cancer (lung, gastrointestinal, breast, ovarian, lymphoma) Rheumatoid arthritis, inflammatory bowel disease, lupus Iron deficiency anemia Hemolytic anemia Myeloproliferative disorder (e.g., essential thrombocythemia)
MPV (Not always reported)	Mean Platelet Volume	Indicates average size of platelets is small; older platelets are generally smaller than younger ones and a low MPV may mean that a condition is affecting the production of platelets by the bone marrow.	Indicates a high number of larger, younger platelets in the blood; this may be due to the bone marrow producing and releasing platelets rapidly into circulation.
PDW (Not always	Platelet Distributi on Width	Indicates uniformity in size of platelets	Indicates increased variation in the size of the platelets, which may mean

reported)			that a condition is present that is affecting platelets



Thalassemia major before treatment with Fagonia cretica



Thalassemia major after treatment with Fagonia cretica

Group of rats from the experiment

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