Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Diyala

College of Veterinary Medicine

EFFECT OF SENEGALIA GREGGII EXTRACT ON IMMUNOLOGICAL AND BIOCHEMICAL MARKERS IN ALBINO MALE RAT

A Thesis
Submitted to the Council of The Veterinary Medicine College at The University of Diyala in Partial Fulfillment of the Requirements for The Degree of Master of Science in Veterinary Microbiology.

By:

Rawand Burhan Abdul-AlRaheem

B. V. M. S. (2015)

Supervised by:

Assist.Prof.Walaa Najm Abood

Assist.Prof.Ehssan N.Jasim Al-Obaidy

October/2022 **IRAQ** **Rabi'al-Awwal/1444**

وَبَشِّرِ الَّذِينَ آمَنُواْ وَعَمِلُواْ الصَّالِحَاتِ أَنَّ لَهُمْ جَنَّاتٍ تَجْرِي مِن تَحْتِهَا الأَنْهَارُ كُلَّمَا رُزِقُواْ مِنْهَا مِن ثَمَرَةٍ رِّزْقًا قَالُواْ هَذَا الَّذِي رُزِقْنَا مِن قَبْلُ وَأُتُواْ بِهِ مُتَشَابِهًا وَلَهُمْ فِيهَا أَزْوَاجٌ مُّطَهَّرَةٌ وَهُمْ فِيهَا خَالِدُونَ۝

صدق الله العظيم

سورة البقرة – اية (25)

Supervisors Certification

We certify that this thesis entitled **(Effect of Senegalia greggii Extract on Immunological and Biochemical markers in Albino male Rat)** was prepared by **(Rawand Burhan Abdul-AlRaheem)**

under our supervision at the Department of Microbiology, College of Veterinary Medicine, University of Diyala, as partial fulfillment of the requirements for the Master degree of Science in Veterinary Medicine/ Vterinary Microbiology .

Supervisors:

Assist.Prof.Dr.Walaa Najm Abood

College of Veterinary Medicine

University of Diyala

/ /2020

Assist.Prof.Dr. Ehssan N.Jasim Al-Obaidy

College of Veterinary Medicine

University of Diyala

/ /2022

In view of the valuable recommendation, we forward this thesis for debate by the examining committee.

Assist. Prof.Dr

Khalid Ibrahim Abd Alkhazraji

Vice Dean of Postgraduate Studies and Science Affairs

College of Veterinary Medicine University of Diyala

/ / 2022

Examination Committee Certification

We, the examination committee , certify that the entitled thesis (**Effect of Senegalia greggii Extract on Immunological and Biochemical markers in Albino male Rat**) by (Rawand Burhan Abdul-Raheem) has been examined and read through all of its contents and related topics. The committee recommends that the student passed and awarded the degree of master of science in Veterinary medicine(veterinary microbiology).

Professor

Ahmed Jassim Mohammed

(Chairman)

/ / 2022

Asst .Prof .Dr.

Dunya Fareed Saloom

(Member)

/ /2022

Asst. Prof. Dr

Tamara Amer Taha

(Member)

/ /2022

Prof. Dr.

Amer Khazaal Salih Al-Azzawi
Head of Department of Microbiology

/ /2022

Asst. Prof. Dr.

Khalid Ibrahim Abd-Al-Khazraji
Dean of College Veterinary

Medicine Diyala University

/ /2022

Dedications

To those who cover me with boundless love … My dear father and mother.

To my love husband ,dear sister, and my beloved son……who support me every time.

To my friends ……..who help me every time.

I present my modest effort and deepest and sincere gratitude for their support.

Rawand….

Acknowledgements

By the name of Allah, the first who deserves all thanks and pleasure for granting me with well, strength and help with which this research has been accomplished.

I'd like to express my deep gratitude to my teacher and supervisor **Assistant professor Dr.Walaa Najm Abood f**or her knowledge, advice, instruction and wisdom and writing guidance.

Great thanks should go to supervisor **Assit.Prof.Dr Ehssan N.Jasim Al-Obaidy** for helping me with the lab work and advises.

I would like to give my appreciations to the Dean of the Veterinary Medicine **Asst. Prof. Dr. Khalid Ibrahim** Vice dean of Postgraduate Studies and Scientific Research for facilitating the difficulties and head of Microbiology depart. **Prof. Dr Ammer Ghazial**

I want to thank the department of Microbiology staff for their help and for providing me with information about the laboratory techniques and providing all facilities required for this research.

My gratitude and thanks to all M.Sc. students and my friends who help me to finish my research.

Finally , I like to thank all those who helped me in my academic career.

Declaration From

I here by declare that thesis entitted (**Effect of Senegalia greggii Extract on Immunological and Biochemical markers in Albino male Rat**) presented at the college of veterinary medicine / university of Diayala in / /2022 is my original work ,except for quotations and citations which have been duly acknowledged. I also declare that it has not been submitted previonsly or concurrently ,for any other degree at the university of Diyala or other universities.

Rawand Burhan

Date: / /2022

Abstract:

The immune system is extremely vulnerable to the action of xenobiotics for several reasons. Xenobiotics can stimulate the immune system as antigens by provoking a substantial immune response. *Senegalia greggii* one of the medicinal plants used as traditional medicine using the pods to make eyewash to treat conjunctivitis, also grounding leaves and pods into powder that prevents bleeding and soothes sore skin. As a tea it may treat diarrhea and dysentery. *Senegalia greggii*, previously known as *Acacia greggii*, is an ocean of tree in the genus Senegalia sweetened to the southwestern United States and northern Mexico, and distributed through the world. For its important as use as medicinal plant , it is important to investigate the acute toxicity and the effect on the immune system with the biological activity of *Senegalia greggii* seeds extract. Collected S.greggii in 25 September 2021 from Kalar region. In 25 November 2021 we get about extraction by rotary evaporater. Also was applied for GC-MS methods to identify active compounds, in Cac Chemistry Analysis Center in 25/1/2022 code of samples 894RB Gc Ms.

In animals house beginning 7 December 2021 in Vet.Medicine of Diyala Unversity, take four groups of 10 rats/ groups orally administration of various doses (250 , 500, 1000 , control) of *Sanegalia greggi* extracts.In 30 December 2021 Collected the liver and kidney for histopatholoigcal examination, and blood samples for clinical biochemistry examinations to identification level of CD4 T

cell, CD8 T cell, TNF- a, TGF-B level, and the level antioxidant enzyme GSH and MDA in 9 January 2022. The results revealed all the rats in the *Sanegalia greggii* groups increased in the weight of rat at dose depended manner, normal hepatic structure but minimal inflammatory cell infiltration. There was also a decrease in the numbers of CD4 and CD8 in all rat groups. Cytokine like TNF-α was also decreased while increased in TGF-ß level in a dose depended manner. Also increased in the level of antioxidant enzyme GSH but, decreased in the level of MDA in the treated rats. The *Sanegalia greggi* seeds extracts was inhibited the growth of E.coli, K. pneumoniae, and S.aureus used various doses (60, 125, 250, control) in 13 February 2022. Senegalia greggii plant's chemical analysis revealed the existence of many active chemical components. I finished my practical experience in March 2022.

The conclusion of this study is that Immunotoxicology plays a significant role in the evaluation of the safety of drugs and substances and maybe use the *Senegalia greggii* seed extract in the anti-inflammatory drug discover.

List of Contents

Table No.	Titles	Page No.
	Supervisors Certification	**I**
	Examination committee certification	**II**
	Dedication	**III**
	Acknowledgements	**IV**
	Declaration from	**V**
	Abstract	**VI**
Tables	**List of contents**	**VIII**
	List of tables	**XIII**
	List of figures	**XV**
	List of abbreviation	**XIX**
	Chapter one: Introduction	**1**

1	**Introduction**	**1**
1.1	**Aims of study**	**3**
	Chapter two: Literature Review	**4**
2.1	**Medicinal Plants**	**4**
2.2	**Senegalia greggii**	**5**
2.2.1	**Taxonomy and Systemic of Senegalia greggii**	**5**
2.3	**Ethanopharmacology**	**7**
2.4	**Senegalia Greggii as a Medical Plant**	**10**
2.5	**Acacia's Antimicrobial Action**	**12**
2.6	**Antioxidant**	**18**
2.7	**Immunotoxicity**	**23**
2.8	**The population biology of dispersal and gene flow in the desert shrub Acacia (Senegalia) greggii A. Gray in the Mojave National Preserve**	**25**
2.9	**Senegalia greggii distinctest**	**27**

2.10	**Emerging novel drug delivery strategies for bioactive flavonol fisetin in biomedicine (Biomedicine & Pharmacotherapy 106, 1282-1291, 2018)**	**29**
	Chapter three : Materials and Methods	**30**
3.1	**Study design**	**30**
3.2	**Material**	**31**
3.2.1	**Tools and Istruments**	**31**
3.2.2	**Tools and equipements**	**32**
3.3	**Collection and Extraction of Senegalia greggii seeds**	**34**
3.3.1	**Acute toxicity of S.greggii**	**36**
3.3.2	**Used animals in this study**	**36**
3.4	**Preparation of blood serum for assessment of CD4, CD8, TGF-β, TGFα, Glutathion and Malondialdehyde**	**39**

3.4.1	**Blood sample preparation**	**39**
3.4.2	**ELISA Kit principle used in determination of CD4,CD8, TGF-β, TGFα ,Glutathion and Malondialdehyde**	**39**
3.4.3	**Procedure for Kit principle used in determination of CD4,CD8,TGF-β,TGFα ,Glutathion and Malondialdehyde**	**40**
3.5	**Antibacterial activity of S.greggii**	**48**
3.6	**Detection of active ingredients**	**49**
3.7	**Statistical Analysis**	**50**
	Chapter four: Results	**51**
4	**Results**	**51**
4.1	**Acute toxicity of Senegallia greggii**	**51**
4.2	**Histological evalution of kidney and liver**	**53**
4.3	**Immunotoxicity of Senegallia greggii**	**58**

4.4	**Antioxidant and antibacterial activity of Senegallia greggii**	**62**
4.5	**Active compounds of Senegallia greggii**	**66**
	Chapter five : Discussion	**69**
5	**Discussion**	**69**
5.1	**Acute toxicity of Senegallia greggii**	**69**
5.2	**Immunotoxicity of Senegalia greggii**	**70**
5.3	**Antioxidant and antibacterial activity of Senegallia greggii**	**73**
5.4	**Active compounds of Senegallia greggii**	**76**
	Chapter six: Conclusion &Recommendation	**79**
6.1	**Conclusion**	**79**
6.2	**Recommendation**	**80**
	References	**81**

List of Tables

Table No.	Table Title	Page
3.1	Equipements used in this study	31
3.2	Tools used in this study	32
3.3	The chemicals and solutions used in this study	33
3.4	Kits used	33
3.5	The diluted factor for cluster of differentiation 4(CD4) standard	41
3.6	The diluted factor for cluster of differentiation 4(CD8) standard	41
3.7	The diluted factor for Transforming growth factor-beta (TGF-β)	42
3.8	The diluted factor for Tumor necrosis factorα (TNF-α)	42
3.9	The diluted factor for Glutathione (GSH) standard	43

3.10	The diluted factor for Malondaildehyde (MDA) standard	43
4.1	Showed the effect of oral administration of *S.greggii* seed ethanol extract on the body weight of rat at different doses (250,500 & 1000) mg/mL, (10 rats /groups).	52
4.2	Showes the effect of S.greggii seed ethanol on the growth of, *S.aureus. E. coli*, and *K.pneumonie*	65
4.3	Showed the active compounds of S. greggii by GC-MS analysis	67

List of Figures

Figure No.	Figure Title	Page
2.1	A- The pods of *Senegalia greggii*, B- The seeds of *Senegalia greggi*	6
3.1	Explain the study designs and sample collection and procedures of samples	30
3.2	Weight the S.gragii seeds powder and soaked with etahnol	35
3.3	Steps for acute toxicity	38
3.4	The CD4 standard curve	45
3.5	The CD8 standard curve	45
3.6	The TGF-β Standard curve	46
3.7	The TNF-α Standard curve	46
3.8	The GSH Standard curve	47
3.9	The MDA Standard curve	47
4.1	Liver of rat in control group showing Normal histopathological appearance of the hepatocytes with normal structure. H&E stain 20 X .	54
4.2	Liver of rat fed 250 mg/Kg of *S. greggii* seed extract showing Normal histopathological appearance of the hepatocytes with normal	54

	hepatic structure. H&E stain 20 X.	
4.3	Liver of rat fed 500 mg/Kg of *S. greggii* seed extract showing mild congestion of the portal area(blue arrow). H&E stain 20X	55
4.4	Liver of rat fed 1000 mg/Kg of *S. greggii* seed extract showing dilatation and congestion of the central & portal vein(black arrow) . H&E stain 20 X.	55
4.5	Kidney of rat in control group showing tissue with a normal histopathological structure. H&E stain 20 X.	56
4.6	Kidney of rat fed 250 mg/Kg of *S. greggii* seed extract showing Normal kidney tissue with a normal histopathological structure. H&E stain 20 X.	56
4.7	Kidney of rat fed 500 mg/Kg of *S. greggii* seed extract showing some tubules with mild dilated renal tubules(black arrow). H&E stain 20 X	57
4.8	Kidney of rat fed 1000 mg/Kg of *S. greggii* seed extract showing dilated renal tubules, hyperemia in intertubular intervals(red arrow) and mononuclear cell infiltration in interstitial tissue(yellow arrow). H&E stain 20 X.	57
4.9	Shows the effect of oral administration of S.greggii seed ethanol extract on CD4 concentration of rat at different doses (250,500 & 1000) mg/kg) at 14 day after	58

	administration.	
4.10	Shows the effect of oral administration of *S.greggii* seed ethanol extract on CD8 concentration of rat at different doses (250,500 & 1000) mg/kg) at 14 day after administration**.** *Significant differences (*P* value < 0.05)	59
4.11	Show the effect of oral administration of *S.greggii* seed ethanol extract on TGF-βlevel of rat at different doses (250,500 & 1000) mg/mL) at 14 day after administration. *Significant differences (P value < 0.05)	53
4.12	Show the effect of oral administration of Sg seed ethanol extract on TNF-α level of rat at different doses (250,500 & 1000) mg/kg) at 14 day after administration. *Significant differences (*P* value < 0.05)	54
4.13	Shows the effect of oral administration of *S.greggii* seed ethanol extract on GSH level of rat at different doses (250,500 & 1000) mg/kg) at 14 day after administration.	55
4.14	Shows the effect of oral administration of *S.greggii* seed ethanol extract on MDA level of rat at different doses (250,500 & 1000) mg/kg) at 14 day after administration. *Significant differences (*P* value < 0.05).	56
4.15	Showes the inhibition zone by the effect of S.greggiii seed ethanol on the growth of , *S.aureus, E. coli,* and *K.pneumonie*	58

4.16	GC-MS analysis for *S.greggii* seed extract	59

List of abbreviations

Table N	Abbreviations	Interpreted
1	AGP	Arabinogalacton-peptide
2	CD4 Tcell	Cluster differentiation of T helper cells
3	CD8 Tcell	Cluster differentiation of T cytotoxicity cell
4	CVD	Cardiovascular disease
5	CRF	Chronic renal failure
6	CKD	Chronic kidney disease
7	CRP	C-reactive protein
8	CRF	Corticotrophin- releasing hormone
9	FDA	Flavin states
10	FAO	Food and agriculture organization of the united nations
11	GC-MS	Gas chromatography – mass spectrometry
12	GSH	Glutathione
13	GA	Gum arabs
14	GP	Glycoprotein
15	GSSG	Glutathione
16	HD patient	Hemodialysis (kidney disease)
17	HRP	Horseradish peroxidase
18	HIV	Human immunodeficiency virus
19	HCT-116	Human colon tumor
20	JECFA	The joint FAO/WHO expert committee on food additives
21	LPS	Lipopolysaccride
22	MCF7	Michigan cancer foundation-7

23	MDA	Malondialdehyde
24	NFB	National film board of canada
25	NPs	National pension system
26	NSAIDs	Non-steroidal anti-inflammatory medications
27	OS	Oxidative stress
28	OD	Optical density
29	ROS	Reactive oxygen species
30	TM	Traditional medicines
31	TGFβ	Transforming growth factor β
32	TNFα	Tumor necrosis factor α
33	TAC	Transport accident commission
34	Th	T-helper
35	U.S	United states
36	WSDF	Water-soluble dietary fibers
37	WHO	The world health organization

1- INTRODUCTION

In recent ethanopharmacology study, the research assessment of bioactive phytoconstituents for medicinal plants to detect phytochemicals that play an important role in the activity of medicinal plants which used in the treated many diseases. For this reason, research concentrated is now directly for earning of phytoconstituents (Jeong *et al.*, 2013). Diagnosis the phytochemicals was benefit on many novel formation compounds as, nanoparticales, polymeric, solid lipid nanoparticales to progress the bioviability (Mehta *et al.*, 2018). Secondary metabolites are organic compounds which have an important role in the plants defense against pathogens so that these compounds have biological activity potential. *Senegalia greggii* one of these medicinal plants used as traditional medicine and investigated for antimicrobial and isolated biomolecule responsible for anti-HIV properties (Palacios & Ynalvez, 2015).

Senegalia greggii, known as Acacia was used America, Africa and Asia as food material for humans and cattle as well as in medicinal applications (Drabo *et al.*, 2022).

Medicinal plants are widely used in traditional medicine, and in the recent years have the important interested by researchers to discover the biological activity and the chemical contents. Many previous studies were investigated the biological activities of medicinal plants as antimicrobial activity. Scientists are under pressure to look into natural items for new antimicrobial agents as antibiotic resistance continues to develop. It is believed that people have been using plants as a kind of

medicine since the early Pleistocene epoch. Secondary metabolites from these therapeutic plants, such phenols, may have antibacterial characteristics. Studies have shown that plant species from the Senegalia and Vachellia genera have antibacterial properties. The 1940s–1960s were the peak of antibiotic research and development, but that time has long since passed. Antibiotic resistance has reached an unprecedented level, and the number of microorganisms resistant to several drugs is growing. Previously treatable illnesses with contemporary medicines are increasingly more difficult to treat as a result of rising antibiotic resistance (Gelband *et al*., 2015).

Senegalia greggii (*S.greggii*) used in traditional using the pods to make eyewash to treat conjunctivitis, also grounding leaves and pods into powder that prevents bleeding and soothes sore skin. As a tea it may treat diarrhea and dysentery, with the addition of its flowers it may also treat nausea and vomiting. The *S.greggiii* has many of biological compounds as Fisetin, is a flavone found in various plants such as *S. greggii* (Al-douri, 2000; Al-Majed *et al.*, 2003). Researchers have discovered that fisetin possesses anti-aging properties in yeast or fruit flies(Al-Majed, *et al*., 2003; Ali *et al*., 2009) (Al-Majed, *et al*., 2003; B. H. Ali *et al.*, 2009), anti-inflammatory effects in (LPS) lipopolysaccharide induced respiratory distress inflammation, and generally pro effects in (HCT-116) colon cancer cells. The protein kinase or lipid kinase routes are modulated by fisetin, which is also a strong antioxidant. *S. greggii* leaves could have the potential to be an affordable addition to roughage-based diets for sheep. (Ramirez-Lozano *et al.*,

201□) Seed extract used as a treatment for eye conjunctivitis in pigeons in comparison with other common drugs (Hussain *et al.*). Although the important of S. greggii there is no study about toxicity and biological activity of S. greggii with the less study about the compounds component. There for in this study investigate the immunotoxicity and compounds component.

1.1 Aims of Study:

This seeds is distributed, abundance and many animals eat it for that may be effect on the animal health.

The aim of this study is to identify toxic dose for *Senegalia greggii* seeds and the effect on the immune system .

The following objectives were studied to achieve the aims:

1. □nvestigate the acute toxicity of *Senegalia greggii* seed extract on the rat.

2. □valuate the effect of *Senegalia greggii* seed extract on the antioxidant enzymes M□A and Glutathione.

3. □nvestigate the □mmunotoxicity effect of *Senegalia greggii* seed extract by estimation Senegalia greggii effect on the C□4 T cell, C□□T cell, and the level of T□F-α and TGF-β.

4. □dentify the active ingredients of Senegalia greggii seeds extract using Gc-Ms techni□ue.