

Sudan University of Science and Technology
Collage of Graduate Studies

**Anti - inflammatory Activities of *Dichrostachys cinerea* Bark
and *Capparis decidua* Stem Extracts in Rats**

الأنشطة المضادة للإلتهاب لمستخلصات لحاء الكداد وجذع الطندب في الجرذان

A Thesis submitted in fulfillment of the requirements for the
Degree of Master in Pharmacology

By:

Baraa Galaldien Altybe Srag
B.V.M. (2012) SUST

Supervisor:

Professor Amel Omer Bakhiet
College of Veterinary Medicine SUST

Co-supervisor:

Professor Abdel-wahab Hassan Mohammed
Faculty of Pharmacy-National Ribat University

November 2018

الآية

قال تعالى:

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (١) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (٢) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ

(٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (٥)﴾

صدق الله العظيم
سورة العلق، الآيات (١-٥)

Dedication

I lovely dedicate this thesis to my...

Dearly loved parent

Dearly loved husband

Precious brother and sister

Great teachers

....

For all my lovers

For all those searching for knowledge

....

Baraa

ACKNOWLEDGEMENTS

In the name of Allah the most Merciful and Beneficent

I would like to thank Almighty Allah for giving me the opportunity, determination and strength to do my research.

I owe great gratitude to my supervisors; Professor Amel Omer Bakhiet and Professor Abdel wahab Abdalla Hassan for their guidance, inspiration and support making the completion of my research successful.

I am greatly indebted to Dr. Somaia Awadelkarem Ali for her intellectual guidance, invaluable support, motivation, and encouragement at all levels of my research.

I highly appreciate and acknowledge Sudan University of Science and Technology (SUST), Deanship of Scientific Research for their sponsorship and the financial support which greatly enabled me to purchase chemicals and other materials without which it would have been difficult to complete this work.

I wish to express my deepest appreciation to my family; my mother, father, sister and brothers for all of the sacrifices that they have made on my behalf.

I would also like to thanks my entire friends who supported me to strive towards my goal.

ABSTRACT

Dichrostachys cinerea (Alkadad) and *Capparis decidua* (Altondub) are used widely in folkloric medicine in Sudan due to their nutritional and medicinal values. The bark of *D. cinerea* and stem of *C. decidua* methanolic extracts were evaluated in acute and chronic inflammation models in rat. Carrageenan induced paw oedema was used to acute model to investigate the anti-inflammatory effect of *D. cinerea* bark and *C. decidua* stem at a dose of 100, 200 and 400 mg/kg. Diclofenac sodium was used as a standard drug. Sterile cotton pellets (20mg) were surgically inserted subcutaneously under anesthesia in twenty rats. *D. cinerea* bark and *C. decidua* stem extracts were tested at dose of 200 and 400 mg/kg. Diclofenac sodium was also used as a reference drug. Treatments were daily administered for 7 days. In acute models, oral administration of *D. cinerea* bark at dose of 100, 200 and 400 exhibited a significant ($p < 0.05$) dose dependent anti-inflammatory effect at 4th hours whenever, *C. decidua* stem extract at dose of 100, 200 and 400 also exhibited a significant ($p < 0.05$) dose dependent anti-inflammatory effect especially at 4th hours more than *D. cinerea* at same hours. The inhibition rates of paw oedema for *D. cinerea* were 25.41, 32.30, 38.80% respectively in bark extract and 34.4, 44.4, 65.3% respectively in stem extract of *C. decidua* and 82.0% in diclofenac sodium. The high dose of the two plant extracts (400mg/kg) was comparable to standard drug diclofenac sodium. In chronic model, the *D. cinerea* bark and *C. decidua* stem extracts significantly reduced inflammatory oedema and masked the production of granulomatous tissue induced by cotton pellet granuloma. It is calculated that the *D. cinerea* bark and *C. decidua* stem. Methanolic extracts possess potential anti-inflammatory effect in acute and chronic inflammation in rats. Further studies should be performed to explain the exact mechanism of *D. cinerea* bark and *C. decidua* stems in acute inflammation.

المستخلص

يستخدم الكداد و الطندب على نطاق واسع في مجال الفلكلور في السودان بسبب قيمهما الغذائية والطبية. تم تقييم مستخلصات المثنول للحاء الكداد وجذع الطندب في نماذج الإلتهاب الحاد والمزمن في الفئران. تم استخدام الودمة المستحثة بالكاراجينان لنموذج حاد للتحقيق في التأثير المضاد للإلتهابات للحاء الكداد وجذع الطندب بجرعة 100 و 200 و 400 ملغم / كغم. تم استخدام ديكلوفيناك الصوديوم كعقار قياسي. تم إدخال جزيئات القطن المعقمة (20 ملغ) جراحياً تحت الجلد تحت التخدير في عشرين جرّداً. تم إختبار مستخلصات لحاء الكداد و جذع الطندب بجرعة 200 و 400 ملغم / كغم. كما استخدم الصوديوم ديكلوفيناك كدواء مرجعي. كانت تداول العلاج اليومي لمدة 7 أيام. في النماذج الحادة ، أظهر إعطاء الفأر لحاء الكداد بجرعة 100 و 200 و 400 ملغم / كغم جرعة معنوية ($P < 0.05$) ذات تأثير مضاد للإلتهاب في الساعة الرابعة كلما تم ذلك ، كما أظهر مستخلص جذع الطندب بجرعة 100 و 200 و 400 ملغم / كغم ذات تأثير معنوي ($p < 0.05$) مضاد للإلتهاب بشكل خاص في الساعة الرابعة أكثر من الكداد في نفس الساعة. كانت معدلات تثبيط الودمة لمستخلص لحاء الكداد 25.41 و 32.30 و 38.80 % على التوالي و 34,4 و 44,4 و 65,3 % على التوالي في مستخلص جذع الطندب و 82,0 % في ديكلوفيناك الصوديوم. كانت الجرعة العالية من مستخلصين النباتيين (400 مغ / كغ) مماثلة للعقار القياسي دايكلوفيناك الصوديوم. في النموذج المزمن ، تقلص مستخلصات لحاء الكداد و جذع الطندب إلى حد كبير من الودمة الالتهابية وملثمين إنتاج الأنسجة الحبيبية الناجم عن الورم الحبيبي ببلية القطن. تمتلك المستخلصات الميثانولية تأثيراً مضاداً للإلتهابات في الإلتهاب الحاد والمزمن في الجرذان. يجب إجراء دراسات إضافية لشرح الآلية الدقيقة للنشاط المضاد للإلتهاب لمستخلصات لحاء الكداد و جذع الطندب في الإلتهاب الحاد.

TABLE OF CONTENT

الآية	I
Dedication.....	II
Acknowledgements.....	III
Abstract.....	IV
المستخلص.....	VI
Table of Content.....	VII
List of Table	XI
List of Figure	X
Introduction	1
Objective.....	2
Literature Review	4
1.1 Inflammation.....	4
1.1.1 Acute inflammation	4
1.1.2Chronic inflammation.....	5
1.2 Anti – inflammatory drugs.....	6
1.3 Anti – inflammatory plants.....	8
1.4 Models used to study anti – inflammatory agents.....	9
1.4.1 Acute inflammatory model.....	9
1.4.1.1 Carrageenan – induced paw edema model.....	9
1.4.2 Chronic inflammatory model.....	10
1.4.2.1 Cotton pellet granuloma.....	10
1.5 Some of anti-inflammatory plants.....	11
1.5.1 <i>Dichrostachys cinerea</i>	11
1.5.1.1 Description <i>D. cinerea</i>	11

1.5.1.2 Distribution <i>D. cinerea</i>	14
1.5.1.3 Uses of <i>D. cinerea</i>	14
2.5.1.4 Phytochemical Components of <i>D. cinerea</i>	15
1.5.1.5 Pharmacological properties of <i>D. cinerea</i>	16
1.5.2 <i>Capparis decidua</i>	16
1.5.2.1 Description of <i>C. decidua</i>	16
1.5.2.2 Distribution of <i>C. decidua</i>	18
1.5.2.3 Uses of <i>C. decidua</i>	18
1.5.2.4 Phytochemical Components of <i>C. decidua</i>	19
1.5.2.5 Pharmacological properties of <i>C. decidua</i>	20
1.6 Herbal medicine in Sudan.....	21
Materials and Methods	24
2.1 Plant materials.....	24
2.2 Preparation of plants extraction.....	24
2.3 Experimental animals.....	24
2.4 Experimental design.....	25
2.5 Experimental procedure.....	25
2.5.1 Acute Anti-inflammatory activity.....	25
2.5.2 Chronic Anti-inflammatory activity.....	25
2.6 Data analysis.....	26
Results	27
1.2 The yields of <i>D. cinera</i> bark and <i>C. decidua</i> stem.....	27
1.3 Anti-inflammatory activity of <i>D. cinera</i> bark methanolic extract on carrageenan induced paw edema.....	27
1.4 Anti-inflammatory activity of <i>D. cinera</i> bark on cotton pellet granuloma in rats.....	27

1.5 Anti-inflammatory activity of <i>C. dicitua</i> stem methanolic extract on carrageenan induced paw edema.....	29
4.5 Anti-inflammatory activity of <i>C. dicitua</i> stem on cotton pellet granuloma in rats.....	29
Discussion	34
Conclusion and Recommendations	36
References	37

LIST OF TABLES

Table no.	Title	page
(1)	The yield percentage of <i>D. cinera</i> bark and <i>C. dicitdua</i> stem	28
(2)	Anti-inflammatory activity of <i>D. cinera</i> bark methanolic extract on carrageenan induced paw edema	30
(3)	Anti-inflammatory activity of <i>D. cinera</i> bark on cotton pellet granuloma in rats	31
(4)	Anti-inflammatory activity of <i>C.dicitdua</i> stem methanolic extract on carrageenan induced paw edema	32
(5)	Anti-inflammatory activity of <i>C. dicitdua</i> stem on cotton pellet granuloma in rats	33

LIST OF FIGURES

Figure no.	Title	Page
(1)	<i>Dichrostachys Cinerea</i> plant	13
(2)	<i>Capparis Decidus</i> Plant	17

INTRODUCTION

Inflammation is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury, or tumor growth leading to local accumulation of plasma fluid and blood cells (Sobota et al., 2000). Edema formation, leukocyte infiltration, and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000). Non-steroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, asthma, and cardiovascular disease (Conforti et al., 2009). However, because many NSAIDs are associated with side effects such as gastrointestinal bleeding and suppressed function of the immune system (Hougee, 2008), attention has shifted to alternative pharmacotherapies (Conforti et al., 2008, Buhrmann et al., 2011).

Recently interest in medicinal plants has increased tremendously especially on their effects on human beings (Ojezele and Agunbiade 2013). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Oyesomi and Ajao, 2011). The bark, leaf, nut, fruit apple and the extracts from these plant parts have been used medically (Ijeh et al., 2010). Yedjou et al. (2008) estimated that 80% of the population of Africa depends on medicinal plants to satisfy their health care requirements.

Dichrostachys cinerea belongs to the family Fabaceae is a deciduous thorny shrub or small rounded tree found in tropical and subtropical condition. Traditionally the plants are used as vermifuge and in leprosy, syphilis, dysentery, headache, toothache etc. All these traditional uses indicate that there must be some antibacterial and analgesic properties (Mishra et al., 2009). Flavonoids are part of the polyphenol family. They are present in plants and are mainly used to give color

to plants. Flavonoids are recognized like antitumor, antiviral, anti-allergy, anti-inflammatory (Hhadi, 2004) and vascular protective more antiseptic or antibacterial. Antioxidants are documented in several publications to mitigate the inflammatory processes and this plants activity has been ascribed to the phenolic compounds present in it, particularly flavonoids (Reine et al., 2014).

The genus *Capparis* comprises 250 species including shrubs, trees and woody climbers. *Capparis decidua* (Forsk.) Edgew commonly known as karel, karer, karil, karuetc, is a densely branching shrub or small tree of the Thar Desert. It is also found in the subtropical and tropical zones and other arid regions in southern Asia with a mass of slender, 4-5 m high, or occasionally a small tree with many green vinelike apparently leafless branches, hanging in bundles (Sushila et al., 2010). Different parts of the plant especially root bark and fruits have been used traditionally to cure various ailments. All studies indicated that the plant have significant pharmacological activities like hypercholesterolemia, anti-inflammatory and analgesic, antidiabetic, anti-microbial, anti-plaque, anti-hypertensive, anti-helminthic activities (Verma et al., 2011).

The present study was undertaken to investigate the pharmacological potential of *DichrostachysCinerea* barks extracts and cappers disiduas extract by using various animals models and thus to explore the plant for their potent anti-inflammatory effect.

Objectives:

The objectives of this study were:

1. To evaluate the anti-inflammatory effect of *Dichrostachys cinerea* bark extracts using various experimental animals' models.
2. To evaluate the anti-inflammatory effects of *Capparis dicitua* stem extracts using various experimental animals' models.
3. To study the histopathological effect of *D. cinerea* and *C. dicitus* plants using various experimental animals' models.

CHAPTER ONE

LITERATURE REVIEW

1.1 Inflammation:

Inflammation is derived from a Latin word "inflammation" means to set on fire, is an important process in the body's defense system, which acts to remove and repair damaged tissue or to neutralize harmful agents (Ferrero et al., 2006; Maslinska and Gajewski 1998). The cascade includes elevated permeability in micro vessels, attachment of circulating cells to the vessels in the vicinity of injury site, migration of several cell types, growth of new tissue and blood vessels (Geert, 2006). Inflammation may release or generate a diverse population of pro-inflammatory mediators like bradykinins, serotonin, histamines, prostaglandins and nitric oxide. These substances contribute to the classic clinical picture of heat (calor), redness (rubor), pain (dolor), swelling (tumor) and diminished function associated with inflammation and may produce hyperalgesia or allodynia (Howard, 2006).

Even though the innate cascade process of inflammation is complex, it is mainly divided into two parts i.e. acute and chronic which could either be beneficial or detrimental.

1.1.1 Acute inflammation:

Acute inflammation is characterized by rapid onset and is of short duration. It is characterized by the exudation of fluids and plasma proteins; and the migration of leukocytes, most notably neutrophils into the injured area. This acute inflammatory

response is believed to be a defense mechanism aimed at killing of bacteria, virus and parasites while still facilitating wound repairs.

1.1.2 Chronic inflammation:

Chronic inflammation is of a more prolonged duration and manifests histologically by the presence of lymphocytes and macrophages, resulting in fibrosis and tissue necrosis. The persistent chronic inflammation increases the development of the degenerative diseases such as rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer, asthma, acquired immunodeficiency disorder(AIDS), cancer, congestive heart failure (CHF), multiple sclerosis (MS), diabetes, infections (bacteria, fungi, parasites), gout, IBD-inflammatory bowel disease, aging and other neurodegenerative CNS depression, all of which are associated with immune-pathological that appears to play a key role in the onset of the condition (O'Byrne and Dalglish 2001; Dalglish and O'Byrne 2002).

Sometimes the acute process subsides but the stimulus persists sufficiently to evoke a subsequent chronic inflammation. In other cases, with a stimulus that typically induces chronic inflammation; the tissues response may be acute in type for the first day or so. Suffice it to say at this stage that the tissue response differs considerably in acute and chronic. The cellular component involves the movement of white blood cells (leukocytes) from the blood vessels into the inflamed tissue. They extra-vacate from the capillaries into tissue, and act as phagocytes, picking up bacteria and cellular debris. They may also aid by walling off an infection and preventing its spread. Influx of neutrophils is one of the earliest stages of the inflammatory response. These cells mount a rapid, nonspecific phagocytic response. Later, monocytes/macrophages and cells of other lymphocyte lineages (specific subsets of T cells and B cells) appear at the site of injury; cell types which are associated with antigen-specific and more tightly regulate immune responses

and once activated also produce protective and inflammatory molecules (Silva, 2016).

1.2 Anti – inflammatory drugs:

Inflammation diseases are currently treated with steroidal and non – steroidal anti-inflammatory drugs (NSAID) (Sivarman et al., 2010). Steroids are the chemical compounds released by the adrenal gland and have anti-inflammatory action by different mechanisms. As an example, glucocorticoids are the steroidal hormones which enhance the expression of nearly 130 genes which include the anti-inflammation, phagocytosis, anti-oxidative stress and suppress the pro-inflammatory genes (Franchimont et al., 2003; Yona and Gordon 2007; Barnes, 1998).

In addition, glucocorticoids may express non genomic pathways by restricting ATP consuming activities and these effects are much more rapid than genomic effects (Goulding, 2004). Corticosteroids, another type of steroid hormones, inhibit the activity of phospholipase A2 and diminish the production of AA upon activation of cells by pro-inflammatory molecules (Vane and Botting, 1998). PGs and LTs are thus inhibited by corticosteroids through the action of phospholipase A2 (Nguyen and Lee, 1992). However, a number of side effects are revealed as a result of glucocorticoid use in inflammatory diseases. Glucocorticoids enhance glucose levels by degrading proteins and modulating fatty acid metabolism partly. This catabolic interference by corticosteroids leads to tissue remodeling, osteoporosis, insulin resistance and diabetes (Kleiman and Tuckermann, 2007). Long term use of glucocorticoids increases the apoptosis of hypertrophic chondrocytes in growth plate which reduces the longitudinal growth of bones (De Luca, 2006).

The second category of anti-inflammatory drugs is NSAIDs. Approximately, 60 million Americans use the non-steroidal anti-inflammatory drugs annually to treat

inflammation related diseases and especially rheumatologically disorders and arthritis (Cryer, 2005). NSAIDs show their effect by inhibiting the action of COX instead of phospholipase A2 and do not prevent the activity of LOX (Vane and Botting, 1998). NSAIDs block the production of PGs by inhibiting both (cyclooxygenase-1) COX-1 and (cyclooxygenase-2)COX-2. It is known that about 1% of chronic users of NSAIDs, such as patients with chronic inflammatory diseases develop gastrointestinal (GI) complications such as mucosal damage and bleeding (Singh et al., 2009).

Moreover, some researchers have found acute renal failure as a result of NSAIDs use (Griffin et al., 2000). NSAIDs inhibit the production of renal prostaglandins and negatively affect glomerular filtration rate and salt excretion (Clive and Stoff, 1984). These drugs appear to produce at least some of their beneficial effects by inhibiting COX-2 and their deleterious side effects by inhibiting COX-1. Hence, synthetic anti-inflammatory drugs are more associated with negative effects rather than positive effects. Thus, selective inhibition of the induced enzyme, without affecting the homeostatic one, might avoid the side effects of currently available NSAIDs. NSAIDs have also been shown to inhibit iNOS but the pharmacological inhibitors of (inducible nitric oxide synthase) iNOS are not yet in clinical use while selective inhibitors of COX-2 have recently been launched on the market (Simon et al., 1999; Singh et al., 2009; Esko and Selleck 2002).

Selective COX-2 inhibitors (COXibs) have same anti-inflammatory benefits as traditional NSAIDs with little effect on COX-1, but as inhibitors of the enzyme responsible for the production of most inflammatory PGs, their drug efficacy is upheld. COXibs have proven to be effective in suppressing experimental tumorigenesis. Furthermore, several recently reported randomized clinical trials have shown that COXibs significantly reduce the incidence of colorectal adenomas in humans. Dismayingly, these trials also identified an increased risk for

cardiovascular events associated with COXib use, suggesting that COXibs may not be sufficiently safe for general use as cancer chemo-preventive agents (Simon et al., 1999; Louise, 2007). In view of the gastric side effects of conventional NSAIDs and the recent market withdrawal of rofecoxib and valdecoxib due to their adverse cardiovascular side effects there is need to develop alternative anti-inflammatory agents with reduced gastric and cardiovascular problems (Reddy et al., 2010).

1.3 Anti – inflammatory plants:

The use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health-care settings. According to WHO, 80% of the rural population in developing countries depend on traditional medicines to meet their primary health care needs (Baravalia, 2010).

Unlike modern allopathic drugs which are single active components that target one specific pathway, herbal medicines work in a way that depends on an orchestral approach. A plant contains a multitude of different molecules that act synergistically on targeted elements of the complex cellular pathway (Sandeep et al., 2014).

Medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions (Arif et al., 2009). The use of herbal medicines becoming popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications (Arya V. and Arya, 2011).

Now a days in pharmaceuticals herbal medicines gained more interest. These herbal medicines comprises of active ingredients from different parts of plants such as roots, stems, rhizomes, leaves, seeds and fruits. The active constituents so obtained are crude in nature. Medicinal properties of plants are due many active compounds like alkaloids, glycosides, saponins, terpenoids, lactones, phenols and flavonoids (Meena et al., 2009).

Over the past two decades, numerous plant extracts and plant compounds have been investigated for their ability to modulate inflammation. Most of these investigations have been conducted in vitro or in vivo in animal models, while only a relatively small number of human trials have been conducted in this area. Plant compounds with anti-inflammatory activity have been reviewed and arachidonic acid metabolism, nitric oxide and nuclear factor kappa B (NFκB) identified as major targets (Bremner & Heinrich, 2002; Calixto et al., 2003). In many cases such anti-inflammatory activity appears to be the result of the ability of a compound to inhibit the action and/or biosynthesis of pro-inflammatory cytokines, chemokines or adhesion molecules involved in the inflammatory process, for example by activating transcription factors (incl. NFκB) and protein kinases (Calixto et al., 2004).

1.4 Models used to study anti – inflammatory agents:

Many workers have directly tested the inhibitory effect of different herbal extracts on the production of inflammatory mediators by using different cell culture techniques (Sur et al., 2009; An et al., 2004). Such in-vitro studies are helpful in developing an understanding of the mechanism of anti-inflammatory activity of herbal constituents; however, most of such in vitro studies are secondary to a preliminary in vivo evaluation of anti-inflammatory properties of plant extracts (Kim et al., 2003; Liu et al., 2010).

1.4.1 Acute inflammatory model:

1.4.1.1 Carrageenan – induced paw edema model:

Carrageenan – induced paw edema is the most commonly used method in experimental pharmacology. Carrageenan is a sulphated polysaccharide obtained from seaweed (Rhodophyceae), and by causing the release of histamine, 5-HT, bradykinin and prostaglandins it produces inflammation and edema.

Albino Westar rats weighing between 150-200gms were divided into 5 groups of 6 rats each; three animals being housed in a labelled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum. The test compounds and standard drugs are administered by oral or intraperitoneal route. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury column of plethysmometer for measuring the paw volume. The paw is measured immediately after the carrageenan injection and then at 2, 3, 4 and 6 hours. The peak effect of carrageenan usually occurs at 3 hours after the injection.

The increase of paw volume after 3 or 6 h is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema. The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The difference at the various time intervals gives some hints for the duration of the anti-inflammatory effect. A dose- response curve is run for active drugs and ED₅₀ values can be determined (Dhalendra et al., 2013).

1.4.2 Chronic inflammatory model:

1.4.2.1 Cotton pellet granuloma:

The foreign body granulomas were provoked in rats by subcutaneous implantation of pellets of compressed cotton. After several days, histologically giant cells and undifferentiated connective tissue can be observed besides the fluid infiltration. The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal. More intensive granuloma formation has been observed if the cotton pellets have been impregnated with carrageenin.

Male Westar rats with an average weight of 200 g are anaesthetized with ether. The back skin is shaved and disinfected with 70% ethanol. An incision is made in the lumbar region. By a blunted forceps subcutaneous tunnels are formed and a sterilized cotton pellet is placed on both sides in the scapular region. The pellets are either standardized for use in dentistry weighing 20 mg or pellets formed from raw cotton which produce a more pronounced inflammation than bleached cotton. The animals are treated for 7 days subcutaneously or orally. Then, the animals are sacrificed, the pellets prepared and dried until the weight remains constant. The net dry weight, i.e. after subtracting the weight of the cotton pellet is determined.

The weight of the transudate and the granuloma as well as the percent granuloma inhibition of the test drugs was calculated. The body weight gain was also recorded (Dhalendra et al., 2013).

1.5 Some of anti-inflammatory plants:

1.5.1 Dichrostachys Cinerea:

1.5.1.1 Description D. Cinerea:

Dichrostachys cinerea is a semi-deciduous to deciduous tree up to 7 m tall with an open crown. Bark on young branches green and hairy but dark grey-brown and longitudinally fissured on older branches and stems; smooth on spines formed from modified side shoots. Slash cream colored to light yellow. Strong alternate thorns, up to 8 cm long, almost at right angles, slightly recurved, grow out of the branches and may bear leaves at the base. Twigs grey brown violet, with prominent light lenticels.

Leaves bipinnate; rachis 4-8 cm, with 5-15 (max. 19) pairs of pinnae, which each bear (min. 9) 12-22 (max. 41) pairs of leaflets; terminal pair of pinnae shorter, dark green, underside pale. Leaflets are about (8 x 2.5) mm wide; leaflets and petioles very tomentose and ciliate. Flowers are very characteristic in bicolored cylindrical, dense, petiole, pendulous spikes (bottlebrush), 6-8 cm long and fragrant. Terminal lower flowers hermaphroditic, with 1 pistil and 10 yellow stamens each. Upper flowers of a hanging spike are sterile, reddish or pale purple, with protruding staminodes. Pods narrow is yellow or brown; generally twisted or spiraled, up to (100 x 15) mm, in dense, stalked, intertwined clusters; indehiscent. About 4 black seeds with a spot at one end per pod.

It seems possible that 2 subspecies can be recognized: *D. cinerea* ssp. *africana* and *D. cinerea* ssp. *nyassana*. The latter tends to grow larger and has larger and less hairy leaves and leaflets. The generic name 'Dichrostachys' means '2-coloured spike', and 'cinerea' refers to the greyish hairs of the typical subspecies, which is confined to India; from the Greek 'konis' and the Latin 'cineres'. In South Africa it is called the 'Kalahari Christmas tree', and because of the attractive 2-coloured

hanging flowers some people call it ‘tassels for the chief’s hat’. But most commonly it is known as the ‘sickle bush’, because the young pods are curved like sickles (Orwa et al., 2009).



Figure (1): *Dichrostachys Cinerea* plant

www. Google search .com

1.5.1.2 Distribution *D. Cinerea*:

D. cinerea is originated in Africa and has spread to many tropical areas in Asia and Australia (Coates – Palgrave, 2002). *D. cinerea* penetrates clear-cut areas far into the rainforest zone. In Malaysia, it occurs in areas with strong seasonal climate, usually on poor, occasionally clayey soils, in brushwood, thickets, hedges, teak forest and grassland. Form dense hammocks on lateritic soils in Senegal and Sudan, while in India it occurs in dry deciduous forest. It can be an indicator of overgrazing in low rainfall areas. Usually not frost resistant and tolerance is less on poor soils, but definitely drought resistant. It is fire resistant and does not tolerate waterlogging. It is a weedy species. For instance in Cuba, the tree is unchecked and forms veritable forests on hill land or in areas on which cane growing has been discontinued. In some parts of central Cuba, there are reports that whole farms have been rendered useless by this foreign weed (Orwa et al., 2009).

1.5.1.3 Uses of *D. Cinerea*:

As food: Fruit and seeds from *D. cinerea* are edible. As fodder: Cattle, camels and game (giraffe, buffalo, kudu, Lichtenstein's hartebeest, nyala, impala, klipspringer, red duiker and Damaradik-dik) relish the juicy pods that drop to the ground and even eat the young twigs and leaves. Leaves are highly palatable, rich in protein (11-15% crude protein) and mineral content. Young shoots and pods are also browsed by smaller domestic animals. Pods and seeds do not contain hydrocyanic acid, minimizing the chance of poisoning animals.

In apiculture: The flowers are a valuable honey source. As fuel: The wood is dense, burns slowly with few sparks and emits a non-toxic smoke, making it excellent firewood. It often grows many small trunks, ideal in size for carrying in a headload. As fiber source: The bark yields a strong fiber used for various

applications such as twine. The debarked roots are used for strong plaiting work such as for racks and baskets.

As timber: *D. cinerea* yields a medium to heavy, durable hardwood with a density of 600-1190 kg/cubic m at 15% mc. Heartwood red or dark purple with darker streaks, sharply differentiated from the yellowish-brown sapwood; grain straight or slightly interlocked; texture rather fine and even. Due to its generally small dimensions, its utilization is limited making such items as walking sticks, handles, spears and tool handles. Fencing posts are durable and termite resistant, easily lasting up to 50 years.

In the medicine: The bark is used to treat dysentery, headaches, toothaches, elephantiasis and acts as a vermifuge. Root infusions are taken for leprosy, syphilis coughs, as an anthelmintic, purgative and strong diuretic. Pounded roots and leaves are used to treat epilepsy. The roots are chewed and placed on the sites of snakebites and scorpion stings, and the leaves, which are believed to produce a local anesthesia, are used for the same purpose and also as a remedy for sore eyes and toothache. Leaves are taken as a diuretic and laxative, and used for gonorrhoea and boils; powder from leaves is used in the massage of fractures. The plant is used as a veterinary medicine in India (Vennapoosa et al., 2013).

2.5.1.4 Phytochemical Components of *D. Cinerea*:

The plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral activities (Neondo et al., 2012). Also some phytochemical studies performed on *D. cinerea* extracts have revealed the

presence of sterols and triterpenes, of reductionist compounds, as well as of cardio tonic hetero-sides (Aworet-Samsenyet al., 2011).

1.5.1.5 Pharmacological properties of *D. Cinerea*:

Pharmacological report on *D. cinerea* has shown antibacterial effect and antiviral. Several authors have shown that the species inhibit protein farnesyl-transferase activity. Moreover, chemical studies revealed the presence of a new isomer of mesquitol (a main active principle), which shown free-radical scavenging property and α -glucosidase inhibitory activities (Aworet-Samsenyet al., 2011).

1.5.2 *Capparis decidus*:

1.5.2.1 Description of *C. decidus*:

Root is shows tap root system. Initially, a single primary root develops which gives rise to secondary branches. After 1 year, numerous secondary roots develop but primary root continues to dominate. In case of mature plants, the roots can penetrate up to 4 meters. For the stem, the plant is much branched; each branch is slender, smooth, terete and spinous. Mature branches are leafless as leaves are present only on young shoots. Small, sharp, straight, light brown spines occur in pair at each node of twig. Most twigs and branches are glossy and dark green in colour, but with age, bark develops which is whitish gray.

Leaves is caducous (with very short life span), present only on young shoots. Leaves are simple, linear-oblong, acute, pointed and very small, about 4 to 12 mm long and 1 to 3 mm broad. They are either sessile or with very short petiole. New leaves appear in NovJan. Inflorescence isCorymb with many flowers arising from old branches or from short lateral shoots, in the axils of the spines. For the flowers, the new shoots bear fewer flowers, while profuse flowering occurs on old shoots.

For the sepals, the outer sepals are pubescent with ciliate margins and sub-valvate aestivation. The lower sepals are saccate and acuminate, while the upper sepals are smaller in size, concave and ovate-oblong. The inner sepals are elliptic, acute and having floccose margin. The petals are pink, red-veined, narrow-oblong, gynophore about 12 mm long, androecium is stamens 8, and inserted at the base of gynophores, and pedicel is slender and about 12 mm in length.

Fruits are small, globular, glabrous, fleshy berry, beaked at the apex, resembling a cherry in shape and size. Fresh berries are green, which turn pink on ripening and blackish on drying. Numerous seeds are embedded in the pulp of fruit (Verma et al., 2011).



Figure (2): *Capparis decidua* Plant

www. Google search .com

1.5.2.2 Distribution of *C. decidus*:

It is of a common occurrence in dry places in Sind, Baluchistan, Western Rajputana, Deccan Peninsula, Egypt, Socotra, Arabia, Tropical Africa, Central India, Punjab, Gujarat, Tinnevely and Pakistan. On most occasions *C. decidua* is found to be growing with *Zizyphus mauritiana*. The plant usually grows in dry, exposed habitat, often on foothills, in wastelands. It is found in the deserts, especially of Rajputana, Punjab and Sind, southwards to Karnataka and Tamil Nadu, growing wild in Western Ghats, Rajasthan and Gujarat (Chishty and Bissu, 2014).

1.5.2.3 Uses of *C. decidus*:

Plant has its wider utility in traditional folk medicine and is used as ailments to relieve variety of pains or aches such as toothache, cough and asthma heal. The plant and its parts are widely used by traditional healers and tribal people for curing variety of ailments. The medicinal uses of *C. decidua* are also mentioned in ancient books (Verma et al., 2011).

Powder or infusion of root bark is used in gout, rheumatism, cough, dropsy, palsy, asthma, intestinal worms and intermittent fever. The powder is applied externally on malignant ulcer. A paste of coal obtained after burning the wood is applied to muscular injuries. The flowers yield a steam volatile sulphur compound (0.4%), which is active against several microorganisms. Various preparations of *C. decidua* are powder and infusion of rootbark (1 in 10), dose: ½ - 1 ounce, juice of plant powder of Leaves & root-50-125 mg.

The top shoots and young leaves are made into a powder and used in blister; they are also used in boils, eruptions and swellings and as an antidote to poison. They are very efficacious in relieving toothache when chewed, a decoction of ground

stems and leaves is used for pyorrhea. Infusion of plant is used externally for eruptions, boils, joint diseases and internally in cough and as an antidote in case of poisoning. Juice of fresh plant is used to kill worms in ear. It is also considered as a good substitute of senega.

Crushed bark of the plant is applied as poultice for treatment of wounds. Roots are considered to be sudorific, thermogenic, expectorant, carminative, digestive, stimulant, antibacterial, aphrodisiac, anodyne, anthelmintic and useful in arthritis, dyspepsia, constipation, lumbago, dentalgia, amenorrhea and dysmenorrhea.

The plant is used for its medicinal value in diabetes, rheumatism, hypertension and various stomach problems. Wood being very strong and durable is used to make the foundations around the wells and as fire wood. Flower buds are eaten to relieve stomach ache; root paste is applied on scorpion bite; powdered coal from stem is taken during fractured bone. The stem bark decoction (10-15ml) is administered twice a day in asthma and other respiratory disorders.

In folk medicine, mixture of equal quantity of fruit powder and sugar is prescribed in rheumatism. They are given in diarrhea in cattle and goats. No systematic information is available for *C. decidua* nutritional value. Immediate domestication is necessary to preserve the species and put it to economic use. Limited work on *C. decidua* is available for its diversification through chemical/nutritional and molecular parameters which are important in presenting its nutritional value and diversity level (Chishty and Bissu, 2014).

1.5.2.4 Phytochemical components of *C. decidua*:

Various phyto-constituents have been identified and isolated from different parts of *Capparis decidua* which includes alkaloids, glycosides, terpenoids, sterols, flavonoids, phenols and fatty acids (Rathee et al., 2010; Chahlia, 2009).

Phytochemical screening of *Capparis decidua* revealed high contents of isothiocyanateglucoside, glucocapparin, stachydrine, n-triacontane, β -carotene and β -sitosterol. Presence of ntriacontanol, n-pentacosane and phthalic acid. The flowers yield a steamvolatilesulphur compound (0.4%), which is active against several microorganisms (Gupta et al., 2008, Anonymous, 2007).

1.5.2.5 Pharmacological properties of *C. decidua*:

The extract of unripe fruits and shoots of *C. decidua* cause reduction in plasma triglycerides, total lipids and phospholipids; hence used as hyper-cholesterol emic. It appeared to operate through increased fecal excretion of cholesterol as well as bile acids.

Capparidisine a new alkaloid from *C. decidua* is reported to have dose dependent depressant effect on heart rate and coronary flow. Maximum fall in coronary flow was achieved at 1mg/ml, the contraction and heart rate increased at 2 ng dose and then a dose dependent fall was seen up to 128 and 32 ng, in force of contraction and heart rate respectively.

Ethanoic extract of aerial parts exhibited anti-inflammatory and analgesic activity. Iso-codonocarpine was found to be responsible for anti-inflammatory activity and anti-asthmatic activity.

Fruits possess antidiabetic activity. *C. decidua* powder has hypo-glycemic activity, decreases lipid peroxidation and alters free radical scavenging enzymes such as superoxide dismutase and catalase in erythrocytes, liver, kidney and heart in aged

alloxan induced diabetic rats. *C. decidua* powder is used against alloxan induced oxidative stress and diabetes in rats.

The aqueous extracts of roots of *C. decidua* are found to have purgative activity while the alcoholic extract of the fruit pulp and root bark possess anthelmintic activity.

The alcoholic extract of root bark possesses significant antibacterial and antifungal activities. The ethanolic extract from the root bark of *C. decidua* was tested for its anthelmintic and antimicrobial activities. The ethanolic extract was active against *Pseudomonasaeruginosa*, *Staphylococcus aureus* and *Escherichia coli*, but was inactive against *Candida albicans*. None of the test concentrations exhibited comparable activity with the standard ampicillin tri-hydrate.

On studying the antibacterial activity of the seeds it was found that glucocapparin had no activity but its iso-thiocyanateaglycon had good antibacterial activity. It was found to inhibit cell cultures of *Vibrio cholerae*, *V. ogava*, *V. inaba* and *V. eltor*.

Capparis decidua fruit and flower extract have potent activity in preventing plaque formation. Hypolipidaemic activity: In a study the Ethanolic extract of different parts of *C. decidua* i.e., fruit, flower, shoot and bark were found to have anti-hyperlipidaemic activity in rabbits. The serum cholesterol level was reduced by 61%, 58%, 48% and 28% in *C. decidua* fruit, flower, shoot and bark after a dose of 500 mg/kg body weight was given to rabbits.

In a study by Vyas and Purohit the Ethanolic extract of fruit was found to have anti-atherosclerotic activity in cholesterol fed rabbits.

The hypotensive activity of *C. decidua* ethanol extract at a dose of 1-30 mg/kg exerted a dose dependent fall in blood pressure and heart rate in experimental animals. Whereas in guinea pig atria the extract caused a concentration dependent up to one mg/ml decrease in the force and rate of atria contractions. However, the

extract displayed inhibition of nor-epinephrine or potassium induced contractions. Furthermore it inhibited the contraction at submaximal level with 1 mg extract produced with acetylcholine, histamine and histidine. All this clearly manifest that direct relaxation action of *C. decidua* extract on myocardium and blood vessels could be responsible for its hypotensive action (Rathee et al., 2010).

1.6 Herbal medicine in Sudan:

Sudanese folk medicine represents a unique blend of indigenous cultures with Islamic, Arabic and African traditions. In addition, Sudan encompasses different terrains and climatic zones, ranging from desert and semi-desert in the north to equatorial with a short rainy season (semi-arid and semi-humid) in the centre to equatorial with a long rainy season (arid-humid and equatorial humid) in the south. This variation contributes to the immense diversity of vegetation in the region. The flora of Sudan consists of 3137 species of flowering plants belonging to 170 families and 1280 genera. It is estimated that 15% of these plants are endemic to Sudan (Khali et al., 2012).

Medicinal plants represent an important component of traditional medicine in Sudan. These are coupled with ample inherited information in the field of medicinal plants and herbal traditional users which originally were unique blends of indigenous cultures of various nations.

Similar to other developing countries, traditional medical practices play an important role in Sudan. Herbal drugs are of major importance in Sudanese folk medicine. Several broad-based screenings of many Sudanese medicinal plants were conducted for their antibacterial, antifungal, antiviral, anti-malarial and anthelmintic properties (Musa, 2009).

Capparis decidua Family (Capparidaceae), *Cyperus rotundus* Family and *Tribulus terrestris* Family (Zygophyllaceae) are three plants used successfully in Sudanese traditional medicine for treatment of inflammatory disorders. In Sudan *C. decidua* is used as anthelmintic, analgesic, aphrodisiac, carminative, diaphoretic, emmenagogue and laxative. The bark extract is used in asthma and cough. The paste of young leaves and branches are applied as plaster on boils and swelling, anti-inflammatory, astringent, stomachic, laxative, antidote, and used for skin diseases. The decoction of fresh twigs is kept for 2 – 3 days and then taken against jaundice, the fumigation of the stems are used as anti-rheumatic. The water extract of the stems is used against jaundice. The stems are used as a poultice for swelling and joint pains & the poultices of the twigs are used against head-ache. A decoction prepared from the roots is used to relieve fever and is also used for jaundice. As fumigation, roots are used to treat fever and rheumatism. The aerial part is used for rheumatism, gout; externally the infusion is used for boils, eruption and ulcers, while internally as antidote to poisons (Mohammed et al., 2014).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Plant materials:

The *Dichrostachys cinera* barks and *Capparis decdua* stems were collected from local habitat regions. The plant identified by the botanist in the Medical and aromatic plants research institute (MAPRI), Khartoum Sudan. The plants were washed with water, chopped into pieces and air-dried for seven days at room temperature. The dried pieces were grounded mechanically to a coarse powder using a hammer mill.

2.2 Preparation of plants extraction:

A bout 1000 g of powdered bark of *Dichrostachys cinera* and 1000g of powdered stems of *Capparis decidua* were subjected to extraction with methanol as solvent for 45 hr. by soxhlet extractor. The extracts were filtered while hot and concentrated in vacuum under reduced pressure using rotary flask evaporator and dried in a desiccator. After exhaustive extraction, the methanol aextracts were dried at low temperature under reduced pressure in a rotary evaporator to obtain greenish-black colored residue used for anti-inflammatory activity studies.

2.3 Experimental animals:

Twenty five healthy Albino rats of either sex, weighing between 100-120 g were selected for this study; rats purchased from medical and aromatic plants research institute (MAPRI), Khartoum Sudan. The animals were kept under controlled environmental conditions in the laboratory animal house of Collage of Veterinary Medicine, Sudan University of Science and Technology (SUST), with food and waters, which were withdrawn 2 hours before the tests were started.

2.4 Experimental design:

The rats were divided randomly into 5 experimental groups with 5 rats each.

Group (1) animals were administrated distilled water only (10 ml / Kg).

Group (2) rats were treated orally with slandered anti-inflammatory drug (10 mg/kg of diclofenac sodium).

Groups (3) rats were given oral dose of the plant extract (100 mg / Kg).

Group (4) rats received the plant extract at dose of (200 mg / Kg).

Group (5) rats were administrated with (400 mg / Kg) of plants extract.

2.5 Experimental procedure:

2.5.1 Acute Anti-inflammatory activity:

The paw edema was induced by injecting 0.1 mL of carrageenan (1.0% w/v) diluted in a saline solution and administered in the intraplantar region of the right paw of the albino rat.

The volume of the rat paw was measured with digital verniercallipr before the intraplantar stimulus with carrageenan and at 0, 1, 2, 3, and 4 hours after the injection of carrageenan. The % of paw volume inhibition was evaluated using the following formula:

$$\% \text{ inhibition} = \frac{(v_F - v_O) \text{ control} - (v_F - v_O) \text{ treatment}}{(v_F - v_O) \text{ control}} \times 100$$

Where = v_O represent the volume before administration of carrageenan, and v_F represent the volume after administration of carrageenan.

2.5.2 Chronic Anti-inflammatory activity:

Albino rats weighing between 100-120 g were anaesthetized with ether. The back skin is shaved and disinfected with 70% ethanol. An incision is made in the lumbar region. By a blunted forceps subcutaneous tunnels were formed and a sterilized cotton pellet weights 20mg is placed on both sides in the scapular region. The

animals were treated orally for 7 days with the plant extract. Then, the animals were sacrificed; the pellets were removed and dried until the weight remains constant of the cotton pellet. The weight of the cotton pellet is determined.

The weight of the granuloma as well as the percent granulomatous inhibition of the test plants was calculated.

2.6 Data analysis:

The data were analyzed using SPSS program, software version 16. The parameters analyzed using one way ANOVA test to compare between the means of different variables. The parameter with $p < 0.05$ was considered significant.

CHAPTER THREE

RESULTS

3.1 The yields of *Dichrostachys cinera* bark and *Capparis decidua* stem:

D. cinera bark and *C. decidua* stems were extracted by methanol 98% using soxhelt apparatus. The yield percentages of *D. cinera* bark and *C. decidua* stems were presented in Table (1).

3.2 Anti-inflammatory activity of *D. cinera* bark methanolic extract on carrageenan induced paw edema:

Standard drug diclofenac sodium revealed higher efficacy by significant inhibition of paw oedema. The inhibition was 62.10%.

D. cinera (100, 200 and 400 mg/kg) exhibited significant decrease in paw oedema compared to control rats which produced higher size of paw oedema induced by carragenan. The medium dose 200mg/kg found to be more effective than other doses.

3.3 Anti-inflammatory activity of *D. cinera* bark on cotton pellet granuloma in rats:

Granulomatos tissue that induced using cotton pellet method was significant inhibited in standard drug group (diclofenac sodium). Exudates and fibrous tissue were significantly reduced to percentage of 39.4% and 44.4% respectively. However, rats that received 200mg/kg exhibited high activity in exudates production and granulomatous tissue formation than other group that given 400 mg/kg.

Table 1: The yield percentage of *D. cinera* bark and *C. dicitua* stem:

Plant materials	Weight of sample	Extract	Weight of extract (mg)	Yield (%)
<i>D. cinera</i> bark	1000g	methanol	3.25 g	0.325 %
<i>C. dicitua</i> stems			25.7	2.57 %

3.4 Anti-inflammatory activity of *C. dicitua* stems methanolic extract on carrageenan induced paw edema:

Control rats that received carrageenan at a dose of 0.1ml (1.0 w/v) showed significant increase in paw edema ($p < 0.05$) compared with other groups.

There were a significant ($p < 0.05$) inhibition in paw oedema in Rats that received 200 and 400 mg/kg of *C. decdua* stem compared to control rats, especially at 4th hours. The high dose was found to be the best in decreasing paw odema.

Rat that received 200mg/kg of *C. decdua* showed significant decrease in paw oedema compared with control rats. However this group presented low anti-inflammatory activity to other test plant doses (Table 2).

4.5 Anti-inflammatory activity of *C. dicitua* stems on cotton pellet granuloma in rats:

Diclofenac sodium produced significant ($p < 0.05$) inhibition of granulomatous tissues induced by cotton pellets (44.4%).

Administration of *C. dicitua* stem methanolic extract for 7days masked significantly the production of granulomatous tissue. The result were comparable to that produced by standard drug diclofinac sodium.

High dose (400mg/kg) of *C. dicitua* stem methanolic extract inhabited potent activity compared to low dose (200mg/kg) Table 3.

Table 2: Anti-inflammatory activity of *D. cinera* bark methanolic extract on carrageenan induced paw edema:

Treatments	Increase in paw volume (mm)				Inhibition %			
	H 1	H 2	H 3	H4	H 1	H 2	H 3	H4
Controls	2.05±0.09 a	2.13±0.08 a	2.12±0.08 a	2.05±0.08 a	-	-	-	-
Standard	1.43±0.04 d	1.45±0.07 c	1.14±0.04 d	0.77±0.03 d	29.34	31.84	45.84	62.10
Dose100mg	1.80±0.02 b	1.76±0.03 b	1.66±0.04 b	1.53±0.02 b	11.74	16.64	21.30	25.41
Dose200mg	1.64±0.03 c	1.62±0.04 b	1.45±0.08 c	1.38±0.07 c	19.67	23.62	31.30	32.30
Dose400mg	1.64±0.02 c	1.61±0.04 b	1.48±0.03 c	1.52±0.05 b	19.29	24.24	29.81	38.80

Table 3: Anti-inflammatory activity of *D. cinera* bark on cotton pellet granuloma in rats:

Treatments	Cotton pellet Wt.	Increase in paw volume (mm)			
		Wet Wt.	Inhibition %	Dry Wt.	Inhibition %
Controls	20.00	197.7±4.67 ^a		46.54±2.24 ^a	
Standers		117.0±7.23 ^b	39.4	33.9±1.18 ^b	44.4
Dose 200 mg		131.7±3.87 ^{bc}	30.7	37.8±0.84 ^{bc}	28.6
Dose 400 mg		144.4±14.31 ^c	27.2	41.00±2.69 ^c	25.3

Table 4: Anti-inflammatory activity of *C. dicitua* stems methanolic extract on carrageenan induced paw edema:

Treatments	Increase in paw volume (mm) mean \pm SE				Inhibition %			
	H 1	H 2	H 3	H4	H 1	H 2	H 3	H4
Control	1.57 \pm 0.03 a	1.64 \pm 0.03 a	1.56 \pm 0.03 a	1.50 \pm 0.03a	-	-	-	-
Standard	1.46 \pm 0.10 b	1.31 \pm 0.07 c	0.700 \pm .03 d	0.27 \pm 0.09d	6.78	19.96	55.14	82.00
Dose 100 mg	1.40 \pm 0.03 b	1.39 \pm 0.04 b	1.24 \pm 0.06 b	0.978 \pm 1.1c	10.46	14.89	20.22	34.46
Dose 200 mg	1.44 \pm 0.03 b	1.45 \pm 0.05 b	1.09 \pm 0.03 c	1.44 \pm 0.03 b	8.17	11.61	29.43	44.52
Dose 400 mg	1.30 \pm 0.05 c	1.25 \pm 0.06 c	0.978 \pm 0.07 d	0.523 \pm 0.63c	17.00	23.66	48.90	65.32

Table 5: Anti-inflammatory activity of *C. dicitua* stems on cotton pellet granuloma in rat:

Treatments	Cotton pellet Wt.	Increase in paw volume (mm)			
		Wet Wt.	Inhibition %	Dry Wt.	Inhibition %
Controls	20.00	197.7±4.67 ^a		46.54±2.24 ^a	-
Standard drug		117.0±7.23 ^b	39.4	33.9±1.18 ^b	44.4
Dose 200 mg/kg		111.1±18.97 ^b	44.1	39.5±3.60 ^b	28.7
Dose 400 mg/kg		130.7±6.23 ^b	34.3	39.0±1.50 ^b	34.8

CHAPTER FOUR

DISCUSSION

Physiological or acute inflammation is a beneficial by a host response to tissue damage, but when timely resolution is delayed, it may lead to such immune-associated diseases as rheumatoid arthritis, inflammatory bowel disease (IBD), and cancer (Balkwill et al., 2005).

The present study demonstrated That anti-inflammatory activity of methanol extracts of *dichrostachys cinerea* bark and *Capparis decidua* stem in acute and chronic model of inflammation.

These plants are used in traditional system of medicine in Sudan in the treatment of various diseases including inflammation. Many medicinal plants are used in traditional medical systems to treat the relief of symptoms from pain and inflammation (Marrassini et al., 2010).

Carrageenan-induced paw edema as in-vivo model of inflammation was widely used to evaluate the anti-inflammatory activity of medicinal plant, particularly in the acute phase of inflammation (Sarika, 2012).

In carrageenan induced rat paw oedema, the initial phase of inflammation seen at the 1st hour is attributed to the release of histamine, prostaglandins and serotonin, The second phase is associated with the production of bradykinin, protease, prostaglandin, and lysosome (Saha et al, 2009)., (Brooks and Day, 1991).

Oral administration of *Dichrostacus cenara* bark methanolic extract at dose 100,200 and 400 mg/kg was significantly ($p < 0.05$) reduced the size of paw oedema especially at 4th hour (38.8%) Compere to control rats. Diclofinac sodium used as standard drug produced more effect activity in reduction of acute inflammation. The inhibition present was 62%.

Rats given *Capparis decidua* stems methanolic extract exhibited potent activity in reduce of acute inflammation induced by carrageenan. The anti-inflammatory activity of stem extract was clearly seen at higher dose (400mg/kg) especially at 4th hours.

The results were comparable to that observed by diclofinac sodium. The inhibition present was found 65.3 and 82.0% at 4th hours.

The inflammatory granuloma is a typical feature of reaction inflammation in which tissue degeneration and fibrosis is evident (Kumar, et al., 2004). The events involved in this phase of inflammation are proliferation of macrophages, neutrophils and fibroblasts. The subcutaneous implantations of sterile cotton pellets (20 mg) were performed in lumbar region to induce chronic inflammation (Lalitha and Sethuraman, 2010).

Inflammatory eodema and Granuloma formation were inhibited significantly after administration of methanolic extract of *Dichrostachys cinerea* bark for 7 consecutive days as compared to control group. this clearly seen by inhibition of wet and dry weight of cotton pellet. Low dose was more effect at dose in reduces oedma and fibrous tissue than high dose.

The maximum anti-inflammatory activity was seen with intermediate 200 mg/kg of CD which was comparable to that of diclofenac sodium.

In rats administrated 200 and 400mg /kg of *Capparis decidua* methanolic extract oedema and granulomatous tissue were also masked significantly ($p < 0.05$) especially at high dose ,in compered to low dose the inhibition rates were 28.7 and 34.8 in stem extract. Dichlofinac sodium used as standard drug exhibited higher activity (44.4).

CONCLUSION AND RECOMMENDATIONS

Conclusion:

Methanolic extract of *Dichrostachys cinerea* bark and *Capparis decedua* stem possess significant anti-inflammatory activities in acute and chronic inflammatory models because it reduce the volume of paw oedema and cotton pellet granuloma also restricted.

Recommendations:

Further studies should be done to explain the exact phyto-constituents responsible for antioxidant activity of *Dichrostachys* bark and *Capparis decedua* stem.

REFERENCES

- **An H. J., Jeong H. J., Lee E. H., Kim Y. K., Hwang W. J., Yoo S. J., Hong S. H. and Kim H. M. (2004).** XanthiiFructus Inhibits Inflammatory Responses in LPS Stimulated Mouse Peritoneal Macrophages, *Inflammation*, 28(5), 263-270.
- **Anonymous (2007).** The Wealth of India: A Dictionary of Indian Raw Materials & Industrial products, Raw materials, National Institute of Science Communication and Information Resources, CSIR, 2007, Vol. 3, p. 210-211.
- **Arif T., Bhosale J. D., Kumar N., Mandal T. K., Bendre R. S., Lavekar G. S. and Dabur R. (2009).** Natural Products-antifungal agents derived from plants. *Journal of Asian Natural Products Research*. 2009; 7:621-638. 13.
- **Arya V. and Arya M. L. (2011).** A Review on Anti-Inflammatory Plant Barks. *Int. J. PharmTech Res*. 2011, 3 (2).
- **Aworet-Samseny R. R, Souza A., Kpahé F., Konaté K., Datté J. Y. (2011).** *Dichrostachys cinerea* (L.) Wight et Arn (Mimosaceae) hydro-alcoholic extract action on the contractility of tracheal smooth muscle isolated from guinea-pig. *BMC Complement Altern Med*. 2011 Mar 17; 11:23.
- **Balkwill F, Charles KA, Mantovani A (2005)** Smoldering inflammation in the initiation and promotion of malignant disease. *Cancer Cell*. 2005; 7: 211–217
- **Baravalia Y. K. (2010).** Evaluation of Anti-Inflammatory and Hepatoprotective Potency of a Selected Medicinal Plant, thesis PhD, Saurashtra University.

- **Barnes P. J. (1998).** Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *ClinSci (Lond)*. 1998 Jun; 94 (6): 557-72.
- **Bremner P. and Heinrich M. (2002).** Natural products as targeted modulators of the nuclear factor-kappa B pathway. *Journal of Pharmacy and Pharmacology* 54: 453-472.
- **Brooks, P.M. and Day, R.O (1991).** Non-steroidal anti-inflammatory Drugs difference and similarities. *J N Engl Med*:324(24) 1716-25
- **Buhrmann C., Mobasheri A., Busch F., Aldeinger C., Stahlmann R., Montaseri A., et al. (2011).** Curcumin modulates nuclear factor kappa β (NF- $\kappa\beta$)-mediated inflammation in human tenocytes in vitro; Role of the phosphatidylinositol 3-kinase/Akt pathway. *J Biol Chem*. 2011; 286 (32):28556–66.
- **Calixto J. B., Campos M. M., Otuki M. F., and Santos A. R. (2004).** Anti-inflammatory compounds of plant origin. Part II. modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Medica* 70: 93-103.
- **Calixto J. B., Otuki M. F., and Santos A. R. (2003).** Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor kappa B (NFkappaB). *PlantaMedica* 69: 973-983.
- **Chahlia N. (2009).** Effect of *Capparis decidua* on hypolipidaemic activity in rats. *J. Med. Plants. Res.* 2009; 3 (6): 481-484.
- **Chishty S., and Bissu M. (2014).** Medicinal and Nutritional Importance of *C. decidua* (Forssk.) Edgew. (Capparaceae): A Review. *IJSR*. Volume 5 Issue 2, pp. 141-147.
- **Clive D. M. and Stoff J. S. (1984).** Renal syndromes associated with non steroidal anti - inflammatory drugs. *N Engl J Med*. 1984 Mar 1; 310 (9): 563–572.

- **Coates - Palgrave K. (2002).** Trees of Southern Africa, 3rd edition. Struik, South Africa.
- **Conforti F., Sosa S., Marrelli M., Menichini M., Statti G. A., Uzunov D., et al. (2008).** In vivo anti-inflammatory and in vitro antioxidant activities of Mediterranean dietary plants. *J Ethnopharmacol.* 2008; 116:144–51.
- **Conforti F., Sosa S., Marrelli M. et al. (2009).** The protective ability of Mediterranean dietary plants against the oxidative damage: the role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents, *Food Chemistry*, vol. 112, no. 3, pp. 587–594, 2009.
- **Cryer B. (2005).** NSAID-associated deaths: the rise and fall of NSAID-associated GI mortality. *Am J Gastroenterol* 100: 1694–1695.
- **Dalgleish A. G., O’Byrne K. J. (2002).** Chronic immune activation and inflammation in the pathogenesis of AIDS and cancer. *Adv. Cancer Res.* 84: 231-276.
- **De Luca F. (2006).** Impaired growth plate chondrogenesis in children with chronic illnesses. *Pediatr Res.* 2006 May; 59 (5): 625-9.
- **Dhalendra G., Satapathy T., Roy A. (2013).** Animal Models for Inflammation: A Review. *Asian J. Pharm. Res.* 2013; Vol. 3: Issue 4, Pg 207-212.
- **Esko J. D. and Selleck S. B. (2002).** Order out of chaos: assembly of ligand binding sites in heparan sulfate. *Annu Rev Biochem.* 2002;71:435-471.
- **Ferrero - Milian, L., Nielsen O. H., Andersen P. S., and Girardin S. E. (2006).** Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. *Clinical and Experimental Immunology* 147: 227–235.

- **Franchimont D, Kino T., Galon J., Meduri G. U., Chrousos G. (2002 – 2003).** Glucocorticoids and inflammation revisited: the state of the art. NIH clinical staff conference. Neuro-immunomodulation. 2002-2003; 10(5):247-60.
- **Geert W. (2006).** Analysis of inflammation, Annual Review of Biomedical Engineering, Vol. 8: 93-151.
- **Goulding N. J. (2004).** The molecular complexity of glucocorticoid actions in inflammation - a four-ring circus. Curr Opin Pharmacol. 4 (6): 629-36.
- **Griffin R. M., Yared A., and Ray A. W. (2000).** Nonsteroidal Anti-inflammatory Drugs and Acute Renal Failure in Elderly Persons. Am J Epidemiol Vol. 151, No. 5,
- **Gupta A. K., Neeraj T., and Madhu S. (2008).** Quality Standards of Indian Medicinal Plants, Medicinal Plants Unit: Published by Indian Council of Medical Research, New Delhi, 2008, Vol. 3, p. 99-105.
- **Hadi M. (2004).** La quercétine et ses dérivés: molécules à caractère peroxydant ou thérapeutiques. Thèse de doctorat 2004; Université Louis Pasteur Strasbourg I. p 155.
- **Hougee S. (2008).** Plant-derived modulators of inflammation and cartilage metabolism. In: PhD Thesis. The Netherlands: Utrecht University; 2008.
- **Howard O. Z. (2006).** Auto antigen signalling through chemokine receptors, Current opinion in rheumatology, 2006; 18(6): 642-646.
- **Ijeh I. I., Igwe K. K., Ejike C. E. C. C. (2010).** Effect of leaf aqueous extracts of *Vernonia amygdalina* Del. on contraction of mammary gland and uterus of guinea pig dams. J. Herbs Spices Med. Plants 16: in press.

- **Khalid H., Abdalla E. W., Abdelgadir H., Opatz T., and Efferth T. (2012).**Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. *Natural Products and Bioprospecting*, June 2012, Volume 2, Issue 3, pp 92–103
- **Kim D. W., Son K. H., Chang H. W. , Bae K. H., Kang S. S., and Kim H. P. (2003).** Anti-Inflammatory Activity of *Elsholtzia splendens*, *Arch. Pharm. Res.*, 26(3), 232236(2003).
- **Kleiman A. and Tuckermann J. P. (2007).** Glucocorticoid receptor action in beneficial and side effects of steroid therapy: lessons from conditional knockout mice. *Mol. Cell. Endocrinol.* 2007; 275: 98–108.
- **Kumar V, Abbas AK, Fausto N (2004)** Acute and Chronic Inflammation. In: *Pathologic Basis of Disease*, 7th Edition: Philadelphia. Saunders; 47-86.
- **LalithaKG, Sethuraman MG (2010).** Anti-inflammatory activity of roots of *Ecbolium viride* (Forsk) Merrill. *Journal of Ethnopharmacology*, 128: 248-250
- **Liu X., Hu Z., Shi Q., Zeng H., Shen Y., Jin H. and Zhang W. (2010).** Anti-inflammatory and Anti-nociceptive Activities of Compounds from *Tinospora sagittata* (Oliv.) Gagnep, *Arch. Pharm. Res.*, 33(7), 981-987.
- **Louise R H. (2007).** Inflammation and breast cancer. Cyclooxygenase / prostaglandin signaling and breast cancer. *Breast Cancer Res.* 2007; 9 (4): 210.
- **Marrassini C, Acevedo C, Miño J, Ferraro G, Gorzalczany S. (2010)** Evaluation of Antinociceptive, Anti-inflammatory Activities and Phytochemical Analysis of Aerial Parts of *Urticaurens* L. *Phytother Res.* 2010; 24: 1807–1812.

- **Maslinska D. and Gajewski M. (1998).** Some Aspects of the Inflammatory Process, *Folia Neuropathologica*, Vol. 36, No. 4, 1998, pp. 199-204.
- **Meena A. K., Bansal P. and Kumar S. (2009).** Plants-herbal wealth as a potential source of ayurvedic drugs. *Asian J Trad Med* 2009; 4:152-70.
- **Mishra U. S, Behera S. R., Murthy P. N., Manish K. and Kumar D. (2009).** Antibacterial and analgesic effects of the leaves of *Dichrostachys cinerea*. *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 1, Issue 2, Oct-Dec. 2009
- **Mitchell R. N. and Cotran R. S. (2000).** In *Robinsons Basic Pathology*, pp. 33–42, Harcourt Pvt., New Delhi, India, 7th edition.
- **Mohammed S. M., Khalid S. H., Osman J. A. W and Muddathir A. K. (2014).** A Review on Phytochemical Profile and Biological Activites of Three Anti-Inflammatory Plants used in Sudanese Folkloric Medicine. *Am. J. PharmTech Res.* 2014; 4(4).
- **Musa A. A. H. (2009).** Quality of medicinal plants traditionally used in Sudan as affected by ionizing radiation treatments. Doctor thesis of Philosophy, in Botany and Agricultural Biotechnology, University of Khartoum.
- **Neondo O. J., Mbithe M. C., Njenga K. P., and Muthuri W. C. (2012).** Phytochemical characterization, antibacterial screening and toxicity evaluation of *Dichrostachys cinerea*. *Int. J. Med.Plants.Res.*, Vol. 1 (4), pp. 032-037.
- **Nguyen K. D. and Lee D. A. (1992).** Effect of steroids and non steroidal antiinflammatory agents on human ocular fibroblast. *Invest Ophthalmol Vis Sci.* 1992 Aug; 33 (9):2693-701.

- **O’Byrne K. J., and Dalglish A. G. (2001).** Chronic immune activation and inflammation as the cause of malignancy. *Br. J. Cancer* 85: 473-483
- **Ojezele, M. O. and Agunbiade S. (2013).** Phytochemical Constituents and Medicinal Properties of Different Extracts of *Anacardium Occidentale* and *Psidium Guajava*. *Asian Journal of Biomedical and Pharmaceutical Sciences* 3(16) 2013, 20-23.
- **Orwa C., Mutua A., Kindt R., Jamnadass R., Anthony S. (2009),** Agroforestry Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.
- **Oyesomi T. O. and Ajao M. S. (2011).** Histological effect of aqueous extracts of *Anacardium occidentale* (cashew) stem bark on adult Wistar rat testis. *Med Pract Rev*, 2 (7):73-77.
- **Rathee S., Mogla O. P., Rathee P., and Rathee D. (2010).** Quantification of β Sitosterol using HPTLC from *Capparis decidua*. *Der PharmaChemica*.2 (4): 86-92.
- **Rathee S., Rathee P., Rathee D., Rathee D., Kumar V. (2010).** Review: Phytochemical and pharmacological Potential of Kair (*Capparis Decidua*). *International Journal of Phytomedicine* 2 (2010) 10-17.
- **Reddy T. S. (2010).** Paraoxonases in Inflammation, Infection, and Toxicology. Humana Press; 2010 edition, New York, USA.
- **Reine R. R. A. S., Konaté K., Aboughe A. S., Noreen K. M., Jacques Y. D. (2014).** *Dichrostachys Cinerea*, (L.) Wight Et Arn (Mimosaceae), Potential on Anti-Inflammatory Activity and Protection, in Anaphylactic in Conscious Guinea Pig. *International Journal of Pharmacognosy and Phytochemistry*, ISSN: 2051-7858, Vol.29, Issue.1.

- **Saha A, Ahmed M. (2009).** The analgesic and anti-inflammatory activities of the extract of albizialebeck in animal model. Pak. J. Pharm. Sci. 2009; 22(1): 74-77.
- **Sandeep K., Singh B. B. and Narinder K. (2014).** Physico-Chemical and Phytochemical Investigation of Plant Sesbaniasesban. RJPBCS, 5 (1), Page No. 110.
- **Sarika, A (2012).** Anti-inflammatory activity of lactobacillus on carrageenan -induced paw oedema in male wistar rats. International journal of inflammation (2012-01-10), 2012(1), 752015.
- **Silva L. (2016).** A Literature Review of Inflammation and Its Relationship with the Oral Cavity. Glob J Infect Dis Clin Res 2(1): 001-007.
- **Simon L. S., Weaver A. L., Graham D. Y., Kivitz A. J., Lipsky P. E., Hubbard R. C., Isakson P. C., Verburg K. M., Yu S. S., Zhao W. W., and Geis G. S. (1999).** Anti-inflammatory and upper gastrointestinal effects of celecoxib in rheumatoid arthritis: a randomized controlled trial. JAMA. Nov 24; 282 (20):1921-8.
- **Singh M., Shakya S., Soni V. K., Dangi A., Kumar N., and Bhattacharya S. M. (2009).** The n-hexane and chloroform fractions of *Piper betle* L. trigger different arms of immune responses in BALB/c mice and exhibit antifilarial activity against human lymphatic filarid *Brugiamalayi*. Int Immunopharmacol. 2009; 9:716–728.
- **Sivaraman D., Muralidaran P., Kumar S. S. (2010).** Evaluation of Anti-microbial and anti- inflammatory activity of methanol leaf extract of *Ipomoea aquatic* Forsk. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 1: 258-264.
- **Sobota R., Szwed M., Kasza A., Bugno M., and Kordula T. (2000).**Parthenolide inhibits activation of signal transducers and

activators of transcription (STATs) induced by cytokines of the IL-6 family, *Biochemical and Biophysical Research Communications*, vol. 267, no. 1, pp. 329–333.

- **Sur R., Martin K., Liebel F., Lyte P., Shapiro S. and Southall M. (2009).** Anti-Inflammatory Activity of Parthenolide Depleted Feverfew (*Tanacetum parthenium*), *Inflammopharmacology*, 17, 42–49.
- **Sushila R., Permender R., Dharmender R., Deepti R., and Vikash K. (2010).** Phytochemical and pharmacological Potential of Kair (*Capparis Decidua*). *International Journal of Phytomedicine*, 2, 10-17.
- **Vane J. R. and Botting R. M. (1998).** Anti-inflammatory drugs and their mechanism of action. *Inflamm Res.* 1998 Oct; 47 Suppl 2: S78-87.
- **Vennapoosa S., Sandeep Devareddy, K. Sumathi N., Kumar S. (2013).** phytochemical and antimicrobial evaluations of *Dichrostachys cinerea*. *IRJP*, Volume 4, Issue 1, Jan 2013, pp. 106-111.
- **Verma P. D., Dangar R. D., Shah K. N., Gandhi D. M. and Suhagia B. N. (2011).** Pharmacognostical Potential of *Capparis decidua* Edgew. *Journal of Applied Pharmaceutical Science* 01 (10); 2011: 06-11.
- **Yedjou C. G., Rogers C., Brown E., Tchounwou P. B. (2008).** Differential effect of ascorbic acid and N-acetyl 1-cysteine on arsenic trioxide mediated oxidative stress in human leukemia (HL-60) cells. *J. Biochem. Mol. Toxicol.*, 22: 85-92.
- **Yona S. and Gordon S. (2007).** Inflammation: Glucocorticoids turn the monocyte switch. *Immunol Cell Biol.* 85 (2):81-82.