INTRODUCTION

Livestock are an important and integrated component of agricultural production system in developing countries . They are reared under a wide variety of production systems ranging from traditional smallholders and village production system to large – scale intensive commercial farms . Like in many developing countries , the majority of the farmers in Sudan raise their livestock and traditional production as a sideline to the main agricultural activities . However , livestock production plays a significant role in supporting farmer's income .

Most developing countries of the world lie in the tropical and subtropical region . The climatic conditions provide favourable environment for development of many diseases associated with inflammations , which considered a major limiting factor in the improvement of livestock production .

The climate of Sudan ranges from completely arid to tropical zones with a wide range of bioclimatic regions , from the almost barren deserts in the North to the tropical rain forests in the extreme South of the country . The diversity of the climate of Sudan is responsible for its very rich flora . Research on medicinal and aromatic plants began along time ago , but this was carried out in a scattered and unstructured fashion until the establishment of the Medicinal and Aromatic Plants Research Institute (MAPRI) in 1972 .

The search for a novel anti-microbial urged the scientists to dig into the folklorically used plants to exploit their potential effort which was ended by many targeted options , seventy six extracts of 31 Sudanese medicinal plants belonging to 21 families were investigated for their anti-bacterail activity against four bacteria by Farouk *et al* ., (1983) . The antihelmithic activity of 14 plants species that represented seven families of the Sudanese flora was examined using the free-living nematode (Ibrahim 1992) . AlMagbol and co- workers (1992) screened a total of 573 plant extracts belonging to 111 Sudanese plants , distributed among 46 families for their anti-bacterial activity

The present work is an attempt to carry out some scientific evaluation of three medicinal plants (*Aerva javanica , Amaranthus viridis , Lepidium sativum*) used by smallholder farmers in Sudan . For example *Aerva javanica* used in North Kordofan (Western Sudan) for treating ulcers and wounds (ElTohami , *et al* ., 1997) . In White Nile Provinces the root is used as chew stick , anti-plasmodial , and for treatment of abdominal pain . In Gash Delta , the plant used against snake bites , rheumatism and for breast cancer (Elghazali , 1986) .

Amaranthus viridis found in Wad Madani and Zalingei (Western Darfour) and used as anti-helminthic and as a fodder for grazing animals (ElTohami , *et al* ., 1997) .

Lepidium sativum used in different parts of Sudan as germicide , body pain , anorexia , digestive problems , constipation and maleness (Elghazali , *et al* ., 1994) .

For these reasons the objectives of the present study were :

1. To determine the anti-inflammatory activity of Ethanolic extracts of *Aerva javanica, Amaranthus viridis* and *Lepidium sativm* , using rat Paw edema model .
2. To assess primary pharmacological screening of the three plants ethanolic extract on isolated tissues (rabbit jejunum) .
3. To study sub-chronic toxicity of the three plants (*Aerva javanica, Amaranthus viridis* and *Lepidium sativm*) in Albino rats .

CHAPTER ONE
LITERATURE REVIEW

1.1 : Ethnoveterinary medicine (EVM) :

Was defined by McCrockle , (1995) , as ‘ The wholistic , interdisciplinary study of local knowledge and its associated skills , practices , beliefs , practitioners , and social structures pertaining to the health care and healthful husbandry of food , work , and other income – producing animals , always with an eye to practical development application within livestock production and livelihood systems , and with the ultimate goal of increasing human wellbeing via increased benefits from stock raising ‘ . According to Tabuti *et al* ., (2003) and other systematic studies on ethnoveterinary medicine , the venture can be justified for three main reasons :

1. They can generate useful information needed to develop livestock healing practices and methods that are suited to the local environment .
2. Ethnoveterinary medicine could be a key for veterinary resources and could add useful new drugs to the Pharmacopoeia .
3. Ethnoveterinary medicine can contribute to biodiversity conservation .

Several books have been written on ethnoveterinary medicine (Mathias – Mundy and McCorckle , 1989 , Anonymous , 1994 , 1996 , Bizimana , 1994 ; McCorckle *et al* ., 1996 Köhler – Rollefson *et al* ., 2001 , Martin , *et al* ., 2001) and a few data bases and websites on the subject exists (Nuffic , 2001 ; Ethnovet – web , 2003 ; Prelude , 2003 ; Spiral , 2006) . However , in most of these sources , there is only brief description of the plants used and proposed conditions that they treat . Most of the research on testing of EVM preparations has so far been carried out in Asia (Akhtar , *et al* ., 2000) . Conversely , a number of puplications have been produced that relate to diseases that affect humans and livestock in Africa (Gachathi , 1993 , Kokwaro , 1993 ; Bizimana , 1994) .

Some of the medicinal and aromatic plants found in Sudan are both wild (Table 1.1) and cultivated (Table 1. 2) (Elghazali , 1986 and ElTohami *et al* ., 1997) .

1.2 : Uses of some wild medicinal and aromatic plants in Sudan :

The dried exudation from the stem and branches of *Acacia senegal* is used as a demulcent , suspending and emulsifying agent . It is also used in textiles ,

confectionery and pastes . It contains mainly magnesium , calcium and potassium salts of glycosidal acid (known as Arabic acid), and enzyme oxydase.(El Tohami ., *et al* 1997)

Gum exudates of *Acacia nilotica* are used as an antidiarrhoetic (El ghazali ., *et al* ., 1994). Pods of *Acacia seyal* contain more than 20 % proteins and are very nourishing for livestock.(El Tohami ., *et al* 1997)

The dried mucilaginous substance obtained from leaves of *Aloes sp.*, *Aloe crassipes*, found in northern and eastern Sudan, and *A. sinkatana*, found in the East, is used in small doses as a laxative.(El ghazali , 1986)

The roots of *Balanites aegyptiaca* contain steroidal sapogenins, whereas the bulb contains sugars and saponins. The leaves and fruit contain disogenin, while the kernel has a high oil and valuable protein content. The maceration of the fruit and seeds is used as a laxative and anthelmintic. It is used in the food, animal feed and pharmaceutical industry as a precursor.(El Tohami ., *et al* 1997)

The essential oil of *Ocimum basilicum* obtained by distillation is used in perfumery, production of aroma and in the food industry as a flavouring agent. It contains volatile oil containing cineol, pinene, methyl chavicol, d-camphor and ocimene (El Tohami ., *et al* 1997)

The resin of *Citrullus colocynthis* is used as a gastrointestinal stimulant and as a powerful purgative, as well as a hydrogogue cathartic and anti-rheumatic cure in traditional medicine. (El sayed , 1993)

Cymbopogon proximus contains a bitter oleo resin, a toxic volatile oil and a saponin used extensively in indigenous medicine as a diuretic, colic painkiller and antipyretic in fever.(El sayed ., 1993)

Datura stramonium, *D. metel*, and *D. innoxia* are sources of commercial hyoscyamine. They contain alkaloids, hyoscine, hyoscyamine, as well as atropine. The constituents of *Hyoscyamus muticus* (hyoscyamine, atropine and hyoscine) relieve pain caused by the excessive use of purgatives. It is also used as a cerebral and spinal sedative.(El ghazali *et al* ., 1994)

The leaves of *Eucalyptus globulus* are used as astringents in the form of cigarettes in cases of asthma. The oil is used as an antispasmodic, deodorant and anti-irritant. (El sayed , 1993)

The resin, gum, and volatile oil of *Boswellia papyrifera* are used to make incense and as an ingredient in plasters and fuming pastilles. (El ghazali , 1986)

Fruits of *Tamarindus indica* are used as a gentle laxative, refrigerant and against malaria. They contain free and combined organic acids (tartaric, malic, and citric), potassium tartarate and 25-40 % invert sugars. (El ghazali , *et al* ., 1994)

Table 1.1 : Wild medicinal and aromatic plants of the Sudan.

Scientific name	Local name (s)
Acacia nilotica (Linn.) Willd.exDel	Sunt, Garad (fruit)
Subsp. *nilotica* Brenan	
Subsp. *tomentosa* (Benth.)	
Acacia senegal (Linn.) Willd var Senegal	Hashab
Acacia seyal Del.	Talh
Var. *seyal* Brenan	
Var. *fistula* (Schweinf.) Olive.	
Aloe spp.	Sabbar
Argemone mexicana L	Agresone
Ambrosia maritima L.	Damsisa
Balanites aegyptiaca Del.	Heglig, Laloub
Boswellia papyrifera (Del.) Hochst.	Targ – Targ, Gafal, Luban
Citrillus colocynthis (L.) Schard	Handal
Cymbopogon proximus (Hochst.) Staph.	Mahareb
Datura innoxia Mill	Alsakran
Datura metel Mill	
Dioscoerea spp.	Dioscera
Hapolophyllum tuberculata (Forssk.) A.Juss	Haza
Rawolfia vomitoria Afz	Rawolfia
Solanum nigrum L.	Enab el Deib , Elmugad el aswad
Tamarindus indica L.	Aradib

(Elghazali , 1986 and ElTohami *et al* ., 1997)

1.3 : Uses of some cultivated medicinal and aromatic plants in Sudan :

The steam distilled fruit oil of *Pimpinella anisum* is an ingredient of carminative and expectorant medicines for children. The greatest quantities of Anise, however, are used to flavor liqueurs and in confectionery and perfumery. It

contains 3 % essential oil (aniseed oil) with 90% anethole.(El Tohami , *et al.*, 1997)

Solanostemma arghel , which locally known as Hargel contains an acidic resin, glycoside, choline, phytosterols and amyrine. It is used in indigenous medicine as an effective remedy for coughs. The infusion of its leaves is used for gastrointestinal cramps and infections of the urinary tract. (El sayed , 1993)

The ripe seeds of Black cumin *(Nigella sativa)* have camphor like scent and bitterness and an aromatic taste. They are used in cooking and as substitute for peper and can be sprinkled on bread and cakes. (El sayed , 1993)

The dried fruits of *Ammi majus* contains ammoidin, ammidin and magudin as well as oils and protein. They are used for treatment of leucoderma and skin diseases. (El ghazali , *et al* ., 1994)

Capsicum fruits *(Capsicum minimum)* contain up to 0.14 % pungent principle capsicin, non -pungent alkaloid, fixed oil and vitamin C. The fresh fruit is used as stimulant and stomach disorders and is mainly applied externally in the form of extracts, tinctures, ointments and plasters to treat rheumatism and sciatica. (El sayed , 1993)

Ricinus communis is medicinally used as a strong purgative, for cosmetic preparations, lubricant and disinfectants. Castor beans, which contain about 50 % oil, have an extremely toxic albumin (ricin) and an alkaloid ricinine.(El ghazali , 1986)

Carawaya (*Carum carvi*) is stomachic, antispasmodic, carminative galactogogic and anthelmintic. The fruit is widely used as a flavoring agent in food products such as bread, cheese, pickles and sauce. The main constituents include essential oil (3-5 %) with carvone, limonene and tannins. (El sayed , 1993)

Dried coriander (*Coriandrum sativum*) is used extensively for both bulk and flavor in sausages, corned beef and similar meat products. Some coriander seed is distilled and the solvent extracted gives an essential oil. (El ghazali , *et al* ., 1994)

Seeds of cumin (*Cuminum cyminum*) are used as a condiment, an agreeable aromatic and in veterinary medicine. (El Tohami , *et al* 1997)

Fennel oil (*Foeniculm vulgare*) is used in European countries in the flavoring of food and liqueurs and in the perfumery industry. Pharmaceutically it is used as an agreeable aromatic and carminative. The main constituents are essential oils (up to 6 %) with anethole and fenchone.(El sayed , 1993)

Fenugreek seeds *(Trigonella foenum-graceum)* include a fixed oil (about 70%), a mucilage and sapogenins (1-2 %). Seeds are generally found in most blends of curry powder and foodstuffs and animal feed.(El sayed , 1993)

Guar gum, extracted from the dried ripe seeds of *Cyamposis tetragonoloba*, contain 25% proteins and 1.6 % fat. It is used in sizing of paper and, mixed with starch, as a valuable textile size as well as a stabilizer in emulsions thickener in food, pharmaceutical and cosmetic products. Much attention had been drawn to guar as a possible oral hypoglycemic agent.(El ghazali , *et al* ., 1987)

The sepals of *Hibiscus sabdariffa* contain flavonoids and red pigment comprising gossipten and hibiscin together with phytosterolin and organic acids malic ,citric, tartaric, ascorbic and hibiscic acids. These seeds also contain a high percentage of mucilage (62 %) and a fixed oil. The ripe calyces are used as hot and cold beverages. Medicinally it is used as antispasmodic, hypotensive, antimicrobial and for relaxation of the uterine muscle. (El Tohami , *et al* 1997)

The fruits of *Ammi visnaga* have the effect of relaxing muscles and lowering tonicity of the ureter. A decoction is used to ease the passage of kidney calculi. It is also the source of khellin.(El ghazali , *et al* ., 1994)

The dried leaves of *Lawsonia inermis* (Henna) contain lawasone, tannin mucilage and fat. They are used as a dye for hair, skin and nails; medicinally it is used as a fungicide.(El Tohami , *et al* ., 1997)

A volatile oil, distilled from the leaves of lemon grass *(Cymbopogon citratus*), is used in perfumery, cosmetics and soap.(El ghazali , *et al* ., 1987)

Peanut oil is a refined oil obtained from the seeds of one or more of the cultivated varieties of *Arachis hypogaea*. It is an edible oil which resembles olive oil, hence its use as a vehicle for liniments and as a lubricant, and in particular as a solvent for injections. It saponifies slowly but yields excellent white soap.(El ghazali , 1986)

The leaves of *Cassia acutifolia* contain sennosides, aloe-emodin and rhein. The water extract of the leaves and the fruit is taken as a laxative.(El Tohami , *et al* ., 1997) .

Table 1.2 : Cultivated medicinal and aromatic plants of the Sudan

Scientific name	Local name (s)
Azadirchta indica A. Juss	Neem
Brassica nigra (L) Koch.	Khardal aswad
Carcica papaya L	Babai
Datura stramonium L.	Sakran
Foeniculum vulgare Mill	Shamur
Grewia tenax (Forssk.) Fiori	Godeim
Hibiscus sabdariffa L.	Karkadeh
Hyoscyamus muticus L	Sakran musri
Nicotiana rustica L.	Tuback, Gamsha
Nigella sativa L.	Kamoon Aswad
Ocimum basilicum L.	Reehan
Ricinus communis L.	Khirwi
Senna alexandria Miller	Senna Maka
Solenostemma arghel (Del) Hayne	Hargel

(Elghazali , 1986 and ElTohami , *et al* ., 1997)

1.4 : Some Medicinal plants as potential source of anti-microbial agents :

Seventy six extracts of 31 Sudanese medicinal plants belonging to 21 families were investigated for their anti-bacterial activity against four bacteria by Farouk *et al* ., (1983) .Out of the 76 extracts tested , 64 exhibited inhibitory effect against at least one of the tested micro-organisms . Of these , 7 plants showed significant activity against the four tested organisms , namely *B.subtilis* , *S.aureus* , *E.coli* and *P.ueroginosa* . These plants were *Achyranthus aspera* L. (Amaranthaceae) . *Aristolochia bracteata* Lam . , (Aristolochiaceae) . *Ethulia conozoides L*. ., (Asteraceae) , *Pulicaria crispa* (Forsk) Oliv ., (Asteraceae) , *Memordica (multiflora* Hook F., (Cucurbitaceae) . *Bergia suffrulicosa* (Del.) Fenzl , (Elatinaceae) and *Withania obtusifolia Pauq*. Solanaceae) .

AlMagbol and co-workers (1992) screened a total of 573 plant extracts belonging to 111 Sudanese plants , distributed among 46 families for their anti-bacterial activity . Among the six plants belonging to the family Combretaceae ,

Anogeisuss leiocarpus (DC) Guill and Perr. was the most active plant , and its highest activity was observed with the leaf methanolic extract against the four tested organisms . The stem and root- bark chloroformic extracts of *Anogeisuss leiocarpus* showed activity against the four organisms tested , and the leaf extract exerted activity against one Gram positive bacteria and one Gram negative organism . The leaf chloroformic extract of *Plicosepalus acacia* (Zucc.) Weins and Pol. had a high activity against *E.coli* , low activity against the two Gram positive organisms and no activity against *P.aerogenosa* . The chloroformic extract of the whole plant of *Mymphaea lotus* (L.) was highly active against *S.aureus* and *E.coli* and had a low activity towards the other two organisms . The chloroformic extracts of the root-bark and stem-bark of *Anogeisuss leiocarpus* (DC) Guill and Perr. Exerted a fungicidal activity against *candida albicans* and a fungistatic effect against *A.niger* . However , the equivalent methanol and aquous extracts of its different parts tested were inactive against the two test fungi . (Tharib *et al* ., 1986) , screen four compounds isolated from the stems of the desert shrub *S.arghel* , they found that only one compound showed reasonable anti-bacterial properties against both Gram positive and Gram negative bacteria .

Ross and co- workers (1980) reported that *Solenostemma argel* has a marked anti-fungal activity . Tannin from *Nymphaea tetragona* Georgi (Nymphaeaceae) was isolated and assayed for activity against two species of bacteria pathogenic to fish and inhibited the growth of both *Aromonas salmonicide* and *Pseudomonas fluorescens* (Kurihara *et al* ,1993) .

1.5 : Inflammation and anti-inflammatories :

1.5.1 : Inflammation :

It is part of the complex biological response of vascular tissues to harmful stimuli , such as pathogens , damaged cells , or irritants . (Ferrero *et al* . , 2007) . The classical signs of acute inflammation are pain , heat , redness , swelling and loss of function , and therefore it is considered as a mechanism of innate immunity , as compared to adaptive immunity , which specific for each pathogen . (Abbas and Lichtman , 2009) .

Inflammation can be classified as either acute or chronic . Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes especially granulocytes , from the blood into the injured tissues . A cascade of biochemical events propagates

and matures the inflammatory response , involving the local vascular system , the immune system , and various cells within the injured tissue . Prolonged inflammation , known as chronic inflammation , leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process . (Parakrama *et al* . , 2005) . The process of acute inflammation is initiated by cells already present in all tissues , mainly resident macrophages , dendritic cells , histiocytes , kupffer cells and mastocytes . These cells present on their surfaces certain receptors named pattern recognition receptors (PRRs) , which recognize molecules that are broadly shared by pathogens but distinguishable from host molecules , collectively referes to as pathogen – associated molecular patterns (PAMPs) . At the onset of an infection , burn , or other injuries , these cells undergo activation (one of their PRRs recognize a PAMP) and release inflammatory mediators responsible for the clinical signs of inflammation . Vasodilatation and its resulting increased blood flow causes the redness and increased heat . Increase permeability of the blood vessels results in an exudation or leakage of plasma proteins and fluids into the tissues resulting in edema , which manifests itself as swelling . Some of the released mediators such as bradykinin increase the sensitivity to pain i.e. hyperalgesia . The mediator molecules also alter the blood vessels to permit the migration of leukocytes , mainly neutrophils , outside of the blood vessels i.e. extravasation into the tissues . The neutrophils migrate along a chemotactic gradient created by the local cells to reach the site of injury . (Cotran *et al* . , 1998) . The loss of function is probably the result of a neurological reflex in response to pain .

In addition to cell - derived mediators , several acellular biochemical cascade systems consisting of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response . These include the complement system activated by bacteria and the coagulation and fibrinolysis systems activated by necrosis . (Cotran *et al* . , 1998) . The exudative component involves the movement of plasma fluid , containing important proteins such as fibrin and immunoglobulins (antibodies), into inflammed tissue . The complement system (C3 and C5a) when activated , creates a cascade of chemical reactions that promotes opsonization ,chemotaxis , and agglutination (factor XII) and produces the Membrane Attack Complex (MAC) i.e. complex of the complement proteins C5b ,C6 ,C7 , C8 and C9 . The kinin system (bradykinin generates proteins capable of sustaining vasodilatation and other physical inflammatory effect . The coagulation system or clotting cascade which forms a

protective protein mesh over sites of injury . The fibrinolysis system , which acts in opposition to the coagulation system , to counterbalance clotting and generates several other inflammatory mediators , such as production of chemokines and nitric oxide . (Eming *et al* . , 2007) .

The cellular components involves leukocytes , which normally reside in blood and must move into the inflamed tissue via extravasation to aid in inflammation . Some act as phagocytes , ingesting bacteria , viruses and cellular debris . Others release enzymatic granules (histamine) which damage pathogenic invaders . Leukocytes also release inflammatory mediators (Interferone-Y"INF-Y" , Interleukin 8 , Prostaglandins from the mast cells which are group of lipids that can cause vasodilatation , fever and pain , Tumour necrosis factor-α "TNF-α" and Interleukin 1 from the macrophages which are responsibe for fever , production of cytokines , loss of appetite and increased heart rate , in addition to nitric oxide which is potent vasodilator , relaxes smooth muscles and reduces platelets aggregation) which develop and maintain the inflammatory process as granulocytes in acute inflammation and mononuclear cells as monocytes and lymphocytes in chronic inflammation . (Eming *et al* . , 2007) .

1.5.6.1 : Causes of inflammation :

The causes of acute inflammation can be summarized as mechanical trauma eg. crushing and cutting , chemical injury eg. corrosive acids and alkalis , radiation therapy eg. heat , ultraviolet light and ionizing radiations , injury to cold and heat , injury due to micro-organisms eg. bacteria , viruses , fungi , parasites and injury due to immunological mechanism .

The causes of chronic inflammation also can be summarized as persistent infections eg. some fungi , tubercle bacilli (causing tuberculosis) and prolonged exposure to toxic agents eg. inhaled silica , elevated plasma lipids , autoimmune diseases and rheumatoid arthritis . (Mohammed , 2010)

1.5.2 : Types of inflammation :

According to the type of exudates (fibrinous , hemorrhagic , etc) and the appearance of the lesion (necrosis , ulcers , pseudomembranes) inflammation classified as catarrhal , serous , purulent – suppurative , fibrinous – pseudomembranous , hemorrhagic ,granulomatous , necrotizing and ulcerative .

Catarrhal inflammation :

Acute type of inflammation , it is inflammation of mucous membranes , tissue response is a thick , gelatinous fluids containing mucous . Goblet cells increase their secretion of mucous to protect the damaged epithelia , increased mucous enhances ciliary beating on epithelial cells which helps to clear the pathogen .

Serous inflammation :

Acute type of inflammation , transudate , plasma fluids leak from interendothelial cell junctions and hypersecretion from inflamed glands .

Suppurative inflammation :

Acute inflammation , tissue fluid is an exudates and significant numbers of polymorphonuclear cells , indicates substantial damage to capillaries and its typical response to some bacterial infections .

Fibrinous inflammation :

Acute type of inflammation , tissue surfaces are red and covered with a thick , stringy , elastic, white yellow exudate , seen in lining of body cavities . Increased vascular permeability allows large molecular weight proteins like fibrinogen to leak whick polymerizes to fibrin in tissue spaces . Tissue response is characterized as an exudates

Granulomatous inflammation :

Chronic inflammation , macrophages are dominant cell type , langerhans giant cells , three features are proceeding simultaneously active inflammation , tissue destruction and attempts at repair . Causes are persistent bacteria , prolonged exposure to toxic agents and some fungal infections .

Non-suppurative inflammation (non-granulomatous , lymphocytic) :

Chronic inflammation , lymphocytes , plasma cells , and macrophages are predominant cell types in the exudate .

Hemorrhagic inflammation :

This inflammation is characterized by formation of exudates having a large number of RBCs .

Necrotizing inflammation :

Inflammation involving mucous membranes . The necrotic mucosa and inflammatory exudateform an adherent membrane on the mucosal surface .

Eosinophilic inflammation :

Eosinophiles are present in exudates . Allergic diseases and parasites are the common causes . (Mohammed , 2010) .

1.5.3 : Cell repair and wound healing :

Repair :

is the process by which lost or destroyed cells are replaced by viable cells . There are two processes of repair . Regeneration and Replacement by connective tissue .

Regeneration is defined as the replacement of the destroyed tissue by the parenchymal cells of the same type or the replacement of destroyed cells by proliferation of surrounding undamaged cells of the same type .

Repair by connective tissue (scar formation) , the connective tissue composed of newly formed capillaries , proliferating fibroblasts and residual inflammatory cells .

Healing :

is a mechanism that occur either by first intention in which necrosis and inflammation are minimal or by second intention in which there is extensive necrosis accompanied by a phase of inflammation prior to the repair process (Mohammed , 2010) .

1.5.4 : Anti-inflammatories :

Inflammations are the most important animal health problems , which inflict heavy production losses in grazing animals particularly in developing countries (Dhar , et al. , 1982) , For example , infections , rheumatoid arthritis , allergic reactions , fever , muscular cramps , neck pain , back pain , painful conditions , sprains and arthritis . For a diversity of reasons , interest in the screening of medicinal plants for their anti-inflammatory activities attracts scientific interest despite the extensive use of synthetic chemicals in modern clinical practices all over the world .

The plant kingdom is known to provide a rich source of botanical anti-inflammatories (Nadkarni , 1954) . A number of medicinal plants have been used to treat inflammations in man and animals . (Nadkarni , 1954 , Chopra , et al. , 1956 , Said , 1969) . The use of medicinal plants may present a cheaper sustainable and an alternative if the compounds where demonstrated to work . Such herbal preparations have been used over time by smallholder farmers for treatment of their livestock .

Anti-inflammatory refers to the property of a substance to treat or that reduces inflammation . Anti-inflammatory drugs make up about half of analgesics which are of two classes opioid or morphine analgesics and non-opioid or aspirin type of analgesics . These two classes remedying pain by reducing inflammation as opposed to opioids which produce their effects by acting on specific opioid receptors . These are abundant in the CNS and other tissues and so relieve pain , Analgesia and sedation by alter both the perception and reaction to pain , it raises the pain threshold and thus increases the capacity to tolerate pain (Kumar , 2010) .

1.5.4.1 : types of anti-inflammatories :

Anti-inflammatories are classified as described by (Rang and Dale , 1991) into :

1. Steroidal anti-inflammatory drugs .
2. Non-steroidal anti-inflammatory drugs (NSAIDs) .
3. Miscellaneous drugs .

Pharmacologically they control inflammations as described by (Rang and Dale , 1991) into :

a) Preventing the release of inflammatory mediators .
b) Inhibiting their actions .
c) Treating pathophysiologic responses to them .

1. Steroidal anti-inflammatory drugs :

glucocoticoids and corticosteroids

Most frequently used and misused drugs in veterinary medicine , it needs an understanding of their actions on all body systems .

Mode of action of steroids :

Steroids reduce inflammation by binding to glucocorticoid receptors . They enter the cells by simple diffusion , bind to specific receptors in the cytoplasm

and activate them . The drug-receptor complex is then transported into the nucleus where it binds to specific sites on DNA and induce the synthesis of specific messenger RNA . By this they regulate the synthesis of new protein that bring about the hormone effect , Mary *et al.,* (1992) .

Anti-inflammatory and immunosuppressive effects :

- Glucocorticoids , suppress the development of inflammatory response to all types of stimuli like chemical , mechanical and immunological stimuli .
- They inhibit both early and late manifestations of inflammation .
  Inhibition of late response like capillary proliferation , collagen deposition , fibroplastic activity and scar formation may delay wound healing .
- They inhibit migration and depress the function of the leucocytes and macrophages and inhibit the release of chemical mediators . the ability of these cells to respond to antigen is decreased .
- Glucocorticoids – even a single dose bring about a decrease in the number of WBCs (lymphocytes , monocytes , eosinophils , basophils) decline .
- Arachidonic acid like prostaglandins and leukotrienes are important mediators of inflammation .
- Glucocoticoids induce the synthesis of a protein (lipocortil) , which inhibits phospholipase A_2 thereby decreasing the production of prostaglandins and leukotrienes . Glucocorticoids also suppress the production of cyclo-oxygenase – 2 (COX-2) in the inflammatory cells .
- They also suppress the production of cytokines (IL-6 and IL8) which play a key role in inflammation .
- Thus , they suppress cell-mediated immunity prevent manifestations of allergy and inflammation and in large doses inhibit antibody production . (Kumar , 2010) .
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Glucocorticoid preparations :

They are cassified as :

1. Short acting e.g hydrocortisone

2. Intermediate acting e.g prednisolone , prednisone and methylprednsilone .
3. Long acting e.g dexamethasone and betamethasone .

Glucocorticoids toxicity :

The glucocorticoids toxicity as mentioned by (Munk *et al* ., 1984) , diabetes mellitus and hyperlipidaemia , hypertention , pulmonary thromboembolism , polyurea and polydipsia , reversible hepatopathy , muscle weakness and muscle atrophy , susceptibility to infection , hyperadrenocorticism and adrenocortical insufficiency .

2. Non-steroidal anti-inflammatory drugs (NSAIDs) :

Non-steroidal anti-inflammatory drugs (NSAIDs) are aspirin-type or non-opioid analgesics . In addition they have anti-inflammatory , anti-pyretic and uricosuric properties without addiction liability . They are classifies as :

a) Non-selective COX inhibitors
b) Selective COX 2 inhibitors

Non-selective COX inhibitors : The non-selective COX inhibitors are classified as described by Dale and Foreman (1989)

1. Salicylic acid derivatives e.g aspirin , sodium salicylate and diflunisal .
2. Para-aminophenol derivatives e.g. paracetamol .
3. Pyrazolone derivatives e.g. phenylbutazone and azapropazone .
4. Indole acetic acid derivatives e.g indomethacin , sulindac .
5. Arylacetic acid derivatives e.g declofenac , ketorolac and tolmetin .
6. Propionic acid derivatives e.g ibuprofen , fenoprofen , naproxen and oxaprozin .
7. Anthranilic acids (Fenamates) e.g flufenamic acid , mefenamic acid , meclofenamic acid .
8. Oxicams e.g piroxicam , tenoxicam and meloxicam .
9. Alkanones e.g. nabumetone .

The selective COX-2 inhibitors : e.g nimesulide , celecoxib and etodolac

Mode of action of nonsteroidal anti-inflammatory drugs :

During inflammation , arachidonic acid liberated from membrane phospholipids converted to prostaglandins (PGs) , catalysed by the enzyme cyclo-oxygenase

(COX) . These prostaglandins produce hyperalgesia – they sensetize the nerve endings to pain caused by other mediators of inflammation like bradykinin and histamine . Lands (1985) . NSAIDs inhibit the PGs synthesis by inhibiting the enzyme cyclo-oxygenase . Lands (1985) .

Aspirin is an irreversible inhibitor of COX (by acetylation) , while the others are reversible competitive COX inhibitors . There are two forms of cyclo-oxygenase COX-1 and COX-2 . COX-1 is found in most of the normal cells and is involved in maintaining tissue homeostasis . Lands (1985)

COX-2 is induced in the inflammatory cells by cytokines and other mediators of inflammation . This COX-2 is catalyses the synthesis of prostanoids which are the mediators of inflammation . Most NSAIDs inhibit both COX-1 and COX-2 while some newer agents like , celecoxib and rofecoxib selectively inhibit only COX-2 . Lands (1985)

Toxicity of non-steroidal anti-inflammatory :

Analgesic doses are generally well tolerated but anti-inflammatory doses are usually associated with adverse effects especially when used over a long period .

- Erosive gastritis , peptic ulcer and in extreme cases can cause severe haemorrhage resulting in death . (Dani *et al* ., 2007) .
- Allergic reactions that may be manifested as rashes , urticaria and asthma (as Aspirin inhibits only cyclo-oxygenase pathway , arachidonic acid is available for conversion by lipo-oxygenase pathway into leukotrienes which are powerful bronchoconstrictors) .
- Haemolysis e.g salicylates .
- Nephrotoxicity , almost all NSAIDs can cause nephrotoxicity after long term use . Salt and water retention with hypertension and impaired renal function . (Kim and Dgl , 1998) .
- Hepatotoxicity with hepatic necrosis .
- Increase risk of myocardial infarction and stroke . (Trelle *et al* ., 2011) .

3. Miscellaneous drugs which includes :

a) Immune Selective Anti- inflammatory Derivatives (ImSAIDs) :

ImSAIDs are a class of peptides being discovered to have diverse biological properties , including anti-inflammatory properties , they work by altering the activation and migration of inflammatory cells , which are immune cells

responsible for amplifying the inflammatory response (Bao *et al* ., 2006) . The ImSAIDs represent a new category of anti-inflammatory and are unrelated to steroid hormones or non-steroidal anti-inflammatories . It is now well accepted that the immune , nervous and endocrine systems communicate and interact to control and modulate inflammation and tissue repair . One of the neuro-endocrine pathways , referred to as cervical sympathetic trunk – submandibular gland (cst-smg) axis , a regulatory system that plays a role in the systemic control of inflammation (Mathison *et al* ., 1994) .

b) Anti-inflammatory foods :

Prostaglandins are hormone like substances that affect the body in variety of ways , also regulating inflammatory mediation . An anti-inflammatory diet includes less foods that create inflammation – causing prostaglandins (PGE 2) in the body , and more foods that create anti-inflammatory prostaglandins (PGE 1 and PGE 3) , suggested diets to reduce inflammation include those rich in vegetables and low in simple carbohydrates and fats (Weil , 2012) .

Anti-inflammatory foods include most colourful fruits and vegetables , oily fish (Omega 3 fatty acids) , nuts , seeds and certain spices , such as ginger and olive oil . (www.Naturalanti-inflammatory.org.com)

1.6: Anti-inflammatory Plants :

After decades of serious obsession with the modern medicinal system , people have started looking at the ancient healing systems to treat the various types of ailments . This is because of the adverse effects associated with synthetic drugs . Herbal traditional medicines have gained considerable momentum worldwide during the past decade and play a paramount role in health care programmes specially in developing countries . (Darshan and Ved , 2003) .

In the traditional or folk medicine , many plants (with different parts , stem , leaves , flowers , seeds , roots and park) are used for their anti-inflammatory effects or activities and they are considered as a potential resource of natural anti-inflammatory compounds . (Marin *et al* ., 2012) . Different herbs / plants widely used as a source of ethnomedicines in tropical regions of the world has a potential anti-oxidants and anti-inflammatory effects . (Gbenou *et al* ., 2012) . experiments were conducted to determine anti-inflammatory effects of several medicinal plants such as *Salvadora persica* which is anti-inflammatory dental cleaning . (A.O.A.D 1988) . Different methods were used to evaluate inflammation such as rat Paw oedema model . (Winter *et al* ., 1962)

1.6.1 :Family Amaranthaceae

Characters and species :

- Herbs, leaves opposite or alternate, entire, without stipules.
- Flowers in dense spikes, heads or racemes, subtended by bracts, bisexual or unisexual .
- Fruit a circumscissile capsule,utricle or nutlet, dehiscing by a lid or indehiscent.
- Seed with embryo surrounding the endosperm.
- 65 genera, 900 species.
- The Amaranthaceae is divided into two subfamilies : the Amaranthoideae and the Gomphrenoideae on the basis of the number of locules in the anthers and the number of ovules.(Elison , 1988 , Townsend , 1993)

Important species in the family and their uses :

It is a relatively large family, having about 65 genera and 900 species. The species in this family are mostly annual or perennial herbs, although a few species are shrubs or small trees. Botanists divide Amaranthaceae into two subfamilies: the Amaranthoideae and the Gomphrenoideae, based on certain morphological characteristics of their flowers.

Most of the 900 species of Amaranthaceae are native to tropical and subtropical regions of Africa, Central America, and South America. The number of Amaranthaceae species declines as one approaches the northern and southern temperate zones. There are about 100 species of this family in North America. Many species of Amaranthaceae are considered weeds, since they invade disturbed areas, such as agricultural fields and roadsides.

Several species in the Amaranthaceae family are used by humans , some species are important horticultural plants, such as *Amaranthus caudatus,* commonly known as "love-lies-bleeding."

species of the Amaranthus genus were eaten by indigenous peoples of North and South America, and were cultivated over 5,000 years ago in the Tehuacan region of modern-day Mexico. Grain amaranths are still grown throughout Central America and Mexico, and also as a minor cash crop in the United States. Many health food stores currently sell amaranth grain, a flour-like substance made by grinding amaranth seeds. Amaranth grain can be used with wheat to make bread, or can be cooked with water to make a side dish. (Bolanle , *et al* 2012) .

1.6.1.1 : *Aerva javanica :*

1.6.1.1.1 : Classification

Kingdom :	Plantae
Subkingdom :	Tracheobionta
Superdivision :	Spermatophyta
Division :	Magnoliophyta
Class :	Magnoliopsida
Subclass :	Caryophyllidae
Order :	Caryophyllales
Family :	Amaranthaceae
Genus :	Aerva Forsk
Species :	*Aerva javanica*

Schult

Common name : Arabic : Ras Elshaieb . English : Schult

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1.6.1.1.2 : Distribution :

Aerva javanica plant is a perinneal herb belongs to the Genus Aerva from the Family Amaranthaceae and represented by 20 species in Pakistan . (Sharif et al ., 2011) . It is distributed in various parts of the world . It is native to Africa in Algeria ; Egypt ; Libya ; Morocco ; Chad ; Somalia ; Sudan ; Kenya ; Tanzania ; Uganda ; Cameron ; Mali; Mauritania ; Niger ; Nigeria ; Senegal ; Malawi ;

Mozambique ; Botswana and Madagascar . And some Asian countries : Saudi Arabia ; Afghanistan ; Egypt – Sinai ; Iran ; Israel ; Jordan ; India; Pakistan ; Srilanka and Myanmar . (Neuwinger , 2000)

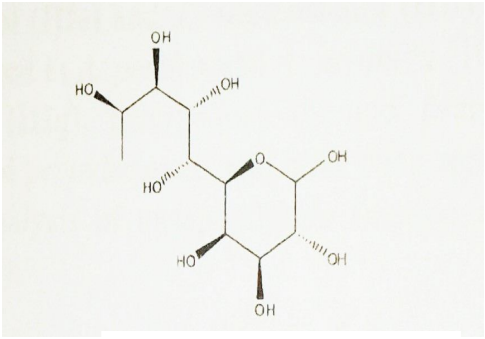
1.6.1.1.3 : Botanical description :

Hoary – white erect or sub- erect under shrub 50 cm in height , occurring in arid localities ; stems densely covered with stellate hairs . Leaves lanceolate or sometimes almost linear (10 – 50 × 8 – 15 mm) , flowers in a terminal part , leafless , with long cylindric spikes , fruit 1 – seeded . (Jessop and Toelken ,1986).

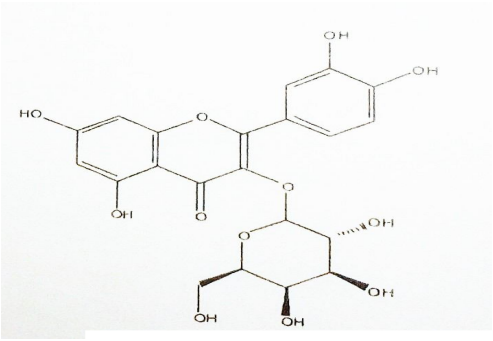
1.6.1.1.4 : Active Constituents :

Various chemical constituents including steroids , triterpenes , lipids , flavonoids , tannins , saponins , alkaloids , sulphates , glycosides , carbohydrates and triterpenoids have isolated from this plant . (Emam , 1999 , Khan *et al* ., 1982 , Reddy and Reddy , 2009) .

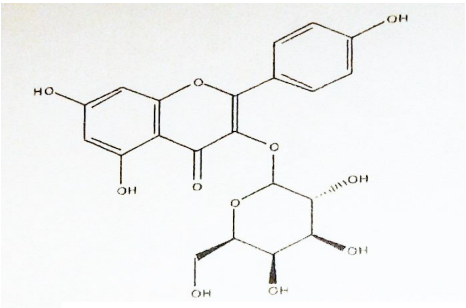
Flavonol glycosides *



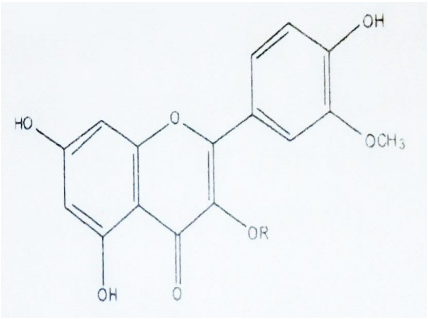
3-rhamnogalactoside



Quercetin-3-galactoside



Kaempferol-3-galactoside



Isorhamnetin-3-galactoside

*Garg *et al* ., 1980

1.6.1.1.5 : Medicinal Folk – uses :

In traditional medicine , this herb is used as diabetic and demulcent (Chopra *et al.*,1956),. The decoctions of *Aerva javanica* are used to remove swelling and powder of this plant is applied externally to ulcers in domestic animals (Baquar ,1989). The seeds are used to relief headache (Chopra *et al* .,1956) . Flowers and roots of *Aerva javanica* possess medicinal properties against kidney problems, diuretic and rheumatism (Kirtikar and Basu , 1918) . Paste made up of leaves is used externally to heal the wounds and inflammation of joints . (Qureshi and Folklore , 2009) . The whole *Aerva javanica* plant is used for the purpose of chest pain , ascaris and diarrhoea with blood . (Teklchaymanot and Giday , 2010)

1.6.1.1.6 : Pharmacological studies :

Aerva javanica shows anti microbial (Sharif *et al* ., 2011) , anti-hyperglycemic (Reddy and Reddy , 2009) , and antidiabetic (Srinivas and Reddy , 2009), cytogenetical (Soliman , 2006) , cytotoxic (Al-Fatimi *et al* ., 2007) anti-plasmodial (Ahmed *et al* ., 2010 and Elhadi *et al* ., 2010) , antidiarrheal (Joanofarc and Vamsadhara , 2003) activities . The plant has been widely used for its therapeutic effects in relieving the swelling and pain due to kidney stones (Deshmekh *et al* ., 2008) , dysentery , gonorrhoea and cutaneous infections (Gorg *et al* ., 1980) . *Aerva javanica* showed anti-oxidant (Singh *et al* ., 2010) , antiviral (Baltina *et al* ., 2003) activities .

1.6.1.2.: *Amaranthus viridis* :

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1.6.1.2.1.: classification :

Kingdom :	Plantae
Subkingdom :	Tracheobionta
Superdivision :	Spermatophyta
Division :	Magnoliophyta
Class :	Magnoliopsida
Subclass :	Caryophyllidae
Order :	Caryophyllales
Family :	Amaranthaceae
Genus :	Amaranthus L.
Species *:*	*Amaranthus viridis*
	Slender amaranthus

Common name : Arabic : Lissan elTair Kabir . English : Slender amaranthus and Green amaranthus

1.6.1.2.2: Botanical description :

Green amaranth is an annual herb with stems erect or occasionally ascending 10 – 80 cm long . Stems are sparingly two densely branched channelled . leaves are tringlular – ovate to narrowly rhombic 2-7 cm long , 1.5 – 5.5 cm wide , hairless , tip usually narrow and with a small narrow notch , stalks 1 – 10 cm long . Flowers are green , inslender , paniculate spikes , in leaf axis or at the end of branch , both sexes are mixed throughout the spikes , but female flowers are more numerous , sepals are 3 , flowers narrowly spoon-shaped to oblong , 1.3 – 1.8 mm long . Fruit is neary round .*Amaranthus viridis* //http: www.heor.org)

1.6.1.2.3: Distribution :

The genus Amaranthus , a widely distributed genus of short-lived herbs , occurring mostly in temperate and tropical regions , Egypt ;Palestine ; : Saudi Arabia ; Iraq ; Iran ; Pakistan ; Afghanistan ; India; Sudan ; Ethiopia ; Djibouti ; and Somalia . Although there remains some confusion over the detailed taxonomy , there are about 60 amaranthus species . Several of them are cultivated as leaf vegetables as *Amaranthus blitum* , *Amaranthus dubius* and *Amaranthus tricolor* (Costea 2003) , cereals as *Amaranthus cruentus* (Tucker , 1986) , and ornamental

plants as *Amaranthus caudatus* , *Amaranthus hypochondriacus* (*Amaranthus viridis* / http://www.heor.org) .

1.6.1.2.4 : Active constituents :

The phytochemical analysis of aqueous extract of *Amaranthus viridis* indicated the presence of steroids , flavanoids and saponins types of compounds (Mayer *et al* ., 1982) . It was found to contain aminoacids , lysine , arginine , histidine , cystin , phenylalanine , leucine , isoleucine , valine , thionine , methionine , tyrosine and tryptophan . (Anonymous ,1988) .

1.6.1.2.5 : Medicinal Folk- uses :

Traditionally *Amaranthus viridis* is an edible plant which is grow all regions of India , stem used as antidote for snake bites (Obi *et al* ., 2006) , leaves used for scorpion stings . Traditionally used for constipation , inflammation , eczema , bronchitis , anaemia and leprosy , an infusion of powdered seeds of flowers used for stomach problems (Sena ., 1998) .

Seeds also used in pregnant women to lessen labour pain . Infusion of plant has ben used as a diuretic and anti-inflammatory agent of the urinary tract , venereal diseases , vermifuge , anti-emetic and laxative , (Quershi *et al* ., 2008) . Decoction of the plant used for dysentery and inflammation , (Kumar , 2010) . Poultice and boils of leaves are used for absesses and skin cleansing , anti-diabetic (Kesari *et al* ., 2005) , antihistaminic (Yamamura et al ., 1998) and anti-carcinogenic (Yen *et al* ., 2001) .

1.6.1.2.6 : Pharmacological studies :

Study yielded flavonoids which possesses biochemical effects , inhibition for enzymes , hormone regulation , antimicrobial , anti-oxidant and anti-cancer activities among other , and the whole plant possesses analgesic and anti-pyretic properties and it is used for treatment of pain and fever respectively in traditional systems of medicine . (Yosuf *et al* ., 1994) . The phenolic compounds of *Amaranthus viridis* have been found to be beneficial in controlling diabetes suggesting that this compounds may show insulinomimetic activity improved glucose utilization mechanism . (Vasco *et al* ., 2008) .

1.6.2 : **Family Brassicaceae :**

Characters of family Brassicaceae :

The Brassicaceae, a medium-sized and economically important family of flowering plants (Angiosperms), are informally known as the mustards, mustard flowers, the crucifers or the cabbage family.

The name Brassicaceae is derived from the included genus Brassica. Cruciferae, an older name, meaning "cross-bearing", describes the four petals of mustard flowers, which are reminiscent of a cross; it is one of eight plant family names without the suffix -aceae that are authorized alternative names (according to ICBN Art. 18.5 and 18.6 (Vienna Code)), and thus both Cruciferae and Brassicaceae are used.

The family contains over 330 genera and about 3,700 species, according to the (Angiosperm , 2009). The largest genera are Draba (365 species), Cardamine (200 species, but its definition is controversial), Erysimum (225 species), Lepidium (230 species), and Alyssum (195 species).

The family contains well-known species such as *Brassica oleracea* (broccoli, cabbage, cauliflower, etc.), *Brassica rapa* (turnip, Chinese cabbage, etc.), *Brassica napus* (rapeseed, etc.), *Raphanus sativus* (common radish), *Armoracia rusticana* (horseradish), *Stock matthiola*, *Arabidopsis thaliana* (thale cress) (model organism) and many others. (Angiosperm , 2009) .

Important species of the family and their uses :

The importance of this family for food crops has led to its selective breeding throughout history. Some examples of cruciferous food plants are the cabbage, broccoli, cauliflower, turnip, rapeseed, mustard, radish, horseradish, cress, wasabi, and watercress.

1.6.2.1 : *Lepidium sativum* :

Lepidium sativum Plant:

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Lepidium sativum seeds :

1.6.2.1.1 : Classification :

Kingdom :	Plantae
Subkingdom :	Tracheobionta
Superdivision :	Spermatophyta
Division :	Magnoliophyta
Class :	Magnoliopsida
Subclass :	Dilleniidae
Order :	Capparles
Family :	Brassicaceae
Genus :	Lepidium L.
Species :	*Lepidium sativum* Garden cress

Common name : Arabic : Hab El-Rashad . English : Garden cress

1.6.2.1.2 : Botanical description :

Lepidium sativum is an annual herb , belonging to Brassicaceae family ,it is a fast-growing , edible plant botanically related to water cress and mustard sharing their peppery , tangy flavour and aroma . Seeds , leaves and roots are economically important , however , the crop is mainly cultivated from seeds . The seeds are reddish in colour , oblong , somewhat angular and curved slightly on one side with rugous surface . Near the point of attachment there is a white scar , from which a small channel extends to $1/3^{th}$ length of the seeds . Seeds are odourless and taste is pungent and mucilaginous . (Khori , 1999. It is an important medicinal crop in India and important green vegetable consumed by human beings . (Tiwari and Kulmi , 2004) .

1.6.2.1.3 : Distribution :

Lepidium sativum is grown world-wide as a spicy salad herb . Its origin is not known , but possibly from Ethiopia or Iran .(Kloos , 1979) .

1.6.2.1.4: Active constituents :

The active phytochemical components of seeds of *Lepidium sativum* were saponins , tannins , steroids , cardiac glycosides and flavonoids in the various organic and inorganic seed extracts . (Bajpai *et al* ., 2005) . Also Phytochemical studies of L.sativum showed the presence of flavonoids , coumarins , sulphur glycosides , triterpenes , sterols and various imidazole alkaloids (Patel *et al*., 2009) and glucosinolates (Gill and Macleod , 1980). L.sativium is found to contain significant amounts of iron , calcium and folic acid in addition vitamin A and C . It contains higher amount of protein 25% : glutamic acid 19.3% , Leucine 8.21% , Methionin 0.97% . The major fatty acid is linolenic acid 30.2% with low amount of erucic acid 3.9% . The major secondary compound of this plant are glucosinolates (Wadhwal et al., 2012). L.sativium leaves have the following composition : Protein 5.8% , Fats 1.0% , Carbohydrates 87% . Mineral matter 2.2% , Calcium 0.36% , Phosphorous 0.11% , Trace Elements Iron 20.6 mg/ 100 gm , Nickel 40ug/Kg , Cobalt 12ug/Kg and Iodine 1.6ug/Kg . Vitamin A , thiamine , riboflavin , niacin and ascorbic acid have also estimated (Wadhwal et al., 2012) .

The plans seeds contains mainly Alkaloides eg : glucotropeaolin , lepidine , sinapic acid and its choline ester (sinapin) , also contain calcium iron , carotene , riboflavin , uric acid , cellulose , phosphorus , thiamine and niacin .Seed oil found to contain stearic ,palmitic , linoleic , behenic , oleic , arachidic , lignoceric acids , benzyle isothiocyanate , benzyle cyanide , sterol and sitosterol , which mainly can be used in treating dysentery and diarrhea (Sheel and Nidhi ., 2011).

1.6.2.1.5 : Medicinal Folk-uses :

Lepidium sativum is widely used in folk medicine for treatment of asthma , bronchitis and cough (Kloos , 1976) . *Lepidium sativum* L. seeds are used as aperients , diuretic , good anti-inflammatory , demulcant , aphrodisiac , carminative , galactagogue , anti-asthmatic , anti-scurbutic , and stimulant (Welbourne 1979 , Kirtkar 2005) . The seeds were found as tonics , demulcants and emmenagogue (Nadkarni , 1954) . The seeds are traditionally used in the diet of lactating woman to induce milk secretion (Sahsrabudde and De , 1943) and in the treatment of some inflammatory conditions like asthma , skin disease and diabetes (Gill and Macleod , 1980; Maier *et al*., 1998).

1.6.2.1.6 : **Pharmacological studies :**

Numerous studies have shown that *Lepidium sativum* as a medicinal plant is as source of diverse nutrients and non-nutrient molecules , many of which display anti-oxidant and anti-microbial properties which can protect the human body against both cellular oxidation reactions and pathogens . (Mothana and Lindequist , 2005) . *Lepidium sativum* alkaloid from seeds have a neuron – behavioural effects exhibited as sedative , anxiolytic , myorelaxant and analgesic activities . (Kulkarni , 1999) . *Lepidium sativum* seed extract was investigated on the blood glucose and lipid profile to found that *Lepidium sativum* decreases blood sugar level due to the presence of linolenic , oleic acids as primary fatty acids and high concentrations of tocopherols . *Lepidium sativum* affect the lipid profile by decreasing total lipid , total cholesterol , triglycerides and the low density lipoprotein (LDL) , (Das *et al* ., 1997) . This due to the presence of glycosides , alkaloids , tannin (phenolic compounds) , flavonoids , and aminoacids like glutamine , cysteine , glycine (Kirtkar and Basu , 2005) . *Lepidium sativum* characterized by hepatoprotective effects , due to its ability to inhibit lipid peroxidation in the liver . The presence of flavonoids , triterpens , alkaloids , tannins and cumarins in *lepidium sativum* explain its role in hepatoprotection by inhibiting the free radicles mediated damage . (Banskota *et al* ., 2000) and (Takeoka and Dao , 2003) . *Lepidium sativum* is Known to possess varied medicinal properties with a potent diuretic activity for its stimulation of regional blood flow or initial vasodilatation (Stamic and Samorzija , 1993) or by producing inhibition of tubular reabsorption of water and anions (Pantoja *et al* ., 1993) . The increased sodium and water excretion activity also provide strong basis for its proved anti-hypertensive action (Jouad *et al* ., 2001) . *Lepidium sativum* was found significantly protect induced-antibiotics nephrotoxicity by decreasing levels of creatinine and urea which could explain the nephroprotective effect due to the significant anti-oxidant activities reported from *Lepidium sativum* and that may lead to discover a novel drug which will be useful in treatment of drug-induced nephrotoxicity (Jain *et al* ., 2009) .

1.7 : Neurotransmitters , receptors , stimulants , blockers and actions :

Acetylcholine (ACh) : is the neurotransmitter of the parasympathetic system . The nerves that synthesize , store and release ACh are called cholinergic nerves , (Rang , *et al.*, 2000) .

Cholinesterases : acetylcholine is hydrolized to choline and acetic acid by the enzymes cholinesterases . Two types of AChE are present : True cholinesterase - at neurons , ganglia and neuromuscular junction and Pseudocholinesterase - in plasma , liver and other organs .

Acetylcholine –cholinergic receptors : there are two classes of cholinergic receptors – muscarinic and nicotinic . Muscarinic receptors are present in the heart , smooth muscles , glands , eyes and CNS . Muscarinic receptors are G protein coupled receptors . Five subtypes of muscarinic receptors M1-M5 are recognized . (Rang , *et al.*, 2000) . Nicotinic receptors are present in the neuromuscular junction , autonomic ganglia and adrenal medulla . Nicotinic receptors are ion channels (Rang , *et al.*, 2000) .

Cholinergic drugs : are chemicals that act at the same site as acetylcholine and thereby mimic its actions . They are , therefore , called parasympathomimetics or cholinomimetics . (Rang , *et al.*, 2000) .

Cholinergic drugs may be classified as :

1. Esters of choline for example , acetylcholine , carbachol
2. Cholinomimetic alkaloids ,for example , muscarine , pilocarpine
3. Anticholiesterases and are divided into :
    a. Reversible e.g. neostigmine , edrophonium .
    b. Irreversible e.g. organophosphorus compounds .

Actions of acetylcholine :

Acetylcholine is the proto type of parasympathomimetic drugs and it has both muscarinic and nicotinic actions . The muscarinic actions result from the stimulation of the muscarinic receptors by acetylcholine . It affects the smooth muscles by increasing the tone of the smooth muscles . Gastrointestinal tract – tone and peristalsis is enhanced . Sphincters are relaxed , resulting in rapid forward propulsion of intestinal contents , (Rang , *et al.*, 2000) .

Anticholinesterases :

Anticholinesterases or choline-esterase inhibitors are drugs which inhibit the enzyme cholinesterase or inactivate it . Thus acetylcholine is not hydrolized and it accumulates . Hence , the actions are similar to cholinergic agonists .

Anticholinergic drugs :

They are agents which block the effects of acetylcholine on cholinergic receptors . Conventionally anti-muscarinic drugs are reffered to as anticholinergic drugs . They are also called cholinergic blocking or parasympatholytic drugs .

Drugs that block the nicotinic receptors are ganglion blockers and neuromuscular blockers , (Rang , *et al.*, 2000) .

Anticholinergic drugs include atropine and related drugs – atropine is the prototype .

1 - Atropine :

Is obtained from the plant *Atropa belladonna* . It compete with acetylcholine for muscarinic receptors and block these receptors – they are muscarinic antagonists .

Actions :

The actions of atropine on the smooth muscles :

Gastrointestinal tract : it reduces tone and motility and relieves spasm , (Rang , *et al.*, 2000) .

2- 5-Hydroxytryptamine - 5HT (Serotonin) :

Was isolated in 1948 and it was found in various plant and animal tissues in human body. 5HT is present in the intestines , platelets and the brain . It is synthesized from the aminoacid tryptophan and stored in granules . It is degraded mainly by MAO (Kumar , 2010) .

5-HT receptors : the actions of serotonin are mediated through its receptors . Seven types of 5-HT receptors are known . Many receptor –selective agonists and antagonists are being developed .

Actions :

Gastrointestinal tract , it increases motility and contraction resulting in diarrhoea . 5-HT is postulated to be having a role in peristalsis . Vomiting , platelet aggregation , homeostasis and imflammation .

Drugs acting on 5-HT receptors :

Serotonin has no therapeutic uses . Howevere , its receptor agonists and antagonists have been used in various conditions .

Serotonin antagonists :

Cyproheptadine :

Blocks 5-HT 2, H1 histamine and cholinergic receptors (muscarinic receptors) , (Kumar , 2010) .

CHAPTER TWO

MATERIALS AND METHODS

2.1 : Materials :

2.1.1 : Plants :

The plants used in the present study were mainly the whole plant (root , stem , branches , leaves and flower) of *Aerva javanica* , *Amaranthus viridis* and the seeds of *Lepidium sativum* . The first two plants were obtained from banks of river Nile and the third was obtained from Omdurman local market **.**These plants authenticated by Dr. Haider Abdelgader , Herparium of the Medicinal and Aromatic Plant Research Institute .

2.1.2 : Samples collection :

2.1.2.1 : Plants collection :

Aerva javanica and *Amaranthus viridis* whole plants were collected from Nile river banks in the month of November 2012 , dried under sun-rays and after complete dryness removed for extraction . *Lepidium sativum* seeds were obtained in the month of November 2012 . Seeds of the plant were dried and granulated by huns and muller to be extracted .

2.1.3 : Chemicals

2.1.3.1 : : Physiological Salts :

1. Sodium chloride (BDH , UK) .
2. D-Glucose (BDH , UK) .
3. Potassium chloride (Riedel-Dehaeen AG) .
4. Sodium dihydrogen phosphate (BDH , UK) .
5. Sodium hydrogen carbonate (BDH , UK)
6. Magnesium chloride (BDH , UK) .
7. Calcium chloride (Riedel-Dehaen AG) .

2.1.3.2 : Solvents :

1. Ethanol 80% (Avondale Laboratories , England)

2.1.3.3 : Standard drugs :

All drugs used were of the high commercially available purity and were purchased from Sigma-Aldrich , Germany . Indomethacine , Carrageenan , Normal saline , Atropine sulphate , Cyproheptadine HCL .

2.1.4 : Apparatus :

1. Harvard organ bath (Eden bridge , Kent . UK)
2. Harvard universal oscillograph (Eden bridge , Kent , UK)
3. Water bath (Fisher Scientific , Pittsburgh , PA ,USA)
4. Rotavapor apparatus (Buchi Laboratoriums – Technik AG , Switzerland)
5. Analytical balance (A and D Company Ltd , Japan)
6. Air pump (BioScience , Shreemess , Kent , UK)
7. Langendorff apparatus (BioScience , Shreemess , Kent , UK)
8. Oven (B and T , Searle Company , England)
9. Dessicator (Glaswerk , Wertheim , Germany)
10. SYSMEX KX-21 (Automated haematology analyzer manual , Eden bridge , Kent . UK.

2.1.5 : Laboratory animals :

Hundred and sixty eight of both sexes , albino rats , weighing 100-200 grams were used for anti-inflammatory and sub-chronic toxicity studies . All rats were obtained from the Faculty of Pharmacy ; University of Khartoum and from the Veterinary Research Center / Soba , divided into groups each of five rats , each group was kept in a pen , fed with standard food pellets and water *ad libitum* , with day and night lightening .

Nine healthy rabbits (local breed) , weighing 1-3 kilograms were obtained from Khartoum-Mayo local market , put in different cages and fed with green vegetables , carrots and water *ad libitum* with day and night lightening . Rabbits were used for pharmacological studies .

2.2 : Methods :

2.2.1 :Method of extraction :

2.2.1.1 : preparation of the ethanolic extract :

Each of the plants (*Aerva javanica* , *Amaranthus viridis* and seeds of *Lepidium sativum*) was studied as ethanolic extract . The ethanolic extracts of the plants were performed at the Medicinal and Aromatic Plants Research Institute (MAPRI) – National Center for Researches (Khartoum) according to the method of Harborne (1984) .

500 gram of *Aerva javanica* plant sample , 260 gram of *Amaranthus viridis* plant sample and 500 gram of *Lepidium sativum* seeds were soaked in 2500 ml of 80 % ethanol for about 3 days with daily filtration and evaporation . The solvent used under reduced pressure using rotary evaporator apparatus . Final extract residues allowed to air in petri-dishes till complete dryness , and the yield percentage (Table 2.1) was calculated as follows :

$$\frac{\text{Weight of extract obtained} \times 100}{\text{Weight of plant sample}}$$

Table 2.1 :

Sample	Sample weight (gram)	Extract weight (gram)	Yeild %
Aerva javanica	500	120.212	24.042
Amaranthus viridis	260	131.910	50.735
Lepidium sativum	500	120.071	24.014

Working solution :

From each extract yield of each plant , 250 mg and 500 mg were dissolved in 10 ml D.W and served as a fresh daily preparation . According to the average weight for each group , the dose calculated in mg/kg then calculated in ml/kg .

2.3 : Experiments

Anti-inflammatory activity :

Three experiments were conducted to determine anti-inflammatory effects of ethanolic extracts of the three plants (*Aerva javanica* , *Amaranthus viridis* and *Lepidium sativum*)

Experimental animals :

A total of 60 of both sexes , Albino rats , weighing 100-200 grams were used in this study .

Experimental design :

Procedure :

Acute inflammation was produced by the sub-plantar injection of 0.1 ml of 1 % w/v carrageenan in normal saline in the right hind paw of the rats . The paw volume was measured at 0,1,2 and 3 hour after the carrageenan injection using a micrometer screw gauge (A and D Company Ltd , Japan) . The increase in the linear diameter of the right hind paws were taken as an indication of paw oedema . The percentage inhibition of the inflammation was calculated from the formula

$$\% \text{ inhibition} = \frac{D_0 - D_t \times 100}{D_0}$$

D_0 is the average inflammation (hind paw oedema) of the control group of rats at a given time .

D_t is the average inflammation of the treated extract or the reference indomethacin (Winter *et al* ., 1962) .

Experiment 1 :

2.3.1 : Anti-inflammatory activity of ethanolic extract of *Aerva javanica* in albino rats using rat paw oedema model :

Twenty Albino rats were used in this experiment divided into four groups each of 5 . Each group was kept in a separate pen .

Group 1 : each rat in this group was injected at sub-planter region with the standard inflammatory inducer (0.1 ml carrageenan in 0.9 ml normal saline in a dose of 2 ml/kg body weight) and served as negative control

Group 2 : each rat in this group was injected intra-peritonealy (IP) with the standard anti-inflammatory (indomethacin in a dose of 10 mg/kg body weight) and served as positive control .

Group 3 : each rat in this group was injected with plant extract of *Aerva javanica* at dose level of 250 mg/kg body weight intra-peritonealy (IP)

Group 4 : each rat in this group was injected plant extract of *Aerva javanica* at dose level of 500 mg/kg body weight intra-peritonealy .

Experiment 2

Amaranthus viridis :

2.3.2 : Anti-inflammatory activity of ethanolic extract of *Amaranthus viridis* in albino rats using rat paw oedema model :

Twenty Albino rats were used in this experiment divided into four groups each of 5 . Each group was kept in a separate pen

Group 1 : each rat in this group was injected at sub-planter region with the standard inflammatory inducer (0.1 ml carrageenan in 0.9 ml normal saline in a dose of 2 ml/kg body weight) and served as negative control

Group 2 : each rat in this group was injected intra-peritonealy (IP) with the standard anti-inflammatory (indomethacin in a dose of 10 mg/kg body weight) and served as positive control .

Group 3 : each rat in this group was injected with plant extract of *Amaranathus viridis* at dose level of 250 mg/kg body weight intra-peritonealy (IP) .

Group 4 : each rat in this group was injected plant extract of *Amaranthus viridis* at dose level of 500 mg/kg body weight intra-peritonealy .

Experiment 3

Lepidium sativum

2.3.3 : Anti-inflammatory activity of ethanolic extract of *lepidium sativum* seeds in Albino rats using rat paw oedema model :

Twenty Albino rats were used in this experiment divided into four groups each of 5 . Each group was kept in a separate pen .

Group 1 : each rat in this group was injected at sub-planter regon with the standard inflammatory inducer (0.1 ml carrageenan in 0.9 ml normal saline in a dose of 2 ml/kg body weight) and served as negative control

Group 2 : each rat in this group was injected intra-peritonealy (IP) with the standard anti-inflammatory (indomethacin in a dose of 10 mg/kg body weight) and served as positive control .

Group 3 : each rat in this group was injected with plant extract of *lepidium sativum* at dose level of 250 mg/kg body weight intra-peritonealy (IP) .

Group 4 : each rat in this group was injected plant extract of *lepidium sativum* at dose level of 500 mg/kg body weight intra-peritonealy .

Pharmacological studies :

Three experiments were conducted to investigate the pharmacological effects of the three ethanolic extracts (*Aerva javanica* , *Amaranthus viridis* and *Lepidium sativum*) on isolated rabbit jejunum strip .

Apparatus :

The glass jar bath apparatus connected to an ink-writing recorder (Harvard Universal Oscillograph, Eden bridge , Kent , UK) was used as described by (Ian Kitechen , 1984).

Procedure :

A rabbit was killed by dislocating the neck , exsanguinated , abdomen opened and jejunal part of the intestine was located (this is the proximal part were the mesenteric supply is perfused) . Jejunum was removed and cut into segments (4 cm in length) , a section of intestine was transferred to petri-dish containing Tyrode solution which consist of Sodium chloride (8 g) , Sodium hydrogen carbonate (1 g) , D-Glucose (1 g) , Sodium dihydrogen phosphate (0.5 g) , Potassium chloride (0.2 g) , Magnesium chloride (0.1 g) and Calcium chloride (0.264 g) . The mesentry and fat surrounding the muscles were trimmed away and a thread was passed through one wall of the jejunum at both top and bottom . The bottom thread was attached to the tissue holder then the mounted tissue was transferred to the organ bath and attached to isotonic transducer . The tissue was led for 15 minutes to adapt to the new environment and washed several times by out-flow. Then the extract of the plants were added to determine the response . Different known antagonists were used to determine the mechanism of response , atropine in a dose of 5 μg/ml and cyproheptidine in a dose of 40 μg/ml .

Experiment 1

Aerva javanica

***2.3.4 : The* effect of the *Aerva javanica* plant extract on the isolated rabbit jejunum strip motility :**

For investigation of the effects of different concentrations of *Aerva javanica* (1,2,4 and 8 mg/ml) on the intestinal motility , three rabbits were used .

Experiment 2

Amaranthus viridis :

2.3.5 : *The* effect of the *Amaranthus viridis* plant extract on the isolated rabbit jejunum strip motility :

For investigation of the effects of different concentrations of *Amaranthus viridis* (1,2,4 mg/ml) on the intestinal motility , three rabbits were used .

Experiment 3

Lepidium sativum :

2.3.6. : *The* effect of the *lepidium sativum* seeds extract on the isolated rabbit jejunum strip motility :

For investigation of the effects of different concentrations of *lepidium sativum* (1,2,4 mg/ml) on the intestinal motility , three rabbits were used .

Sub-chronic toxicity studies :

Three experiments were conducted to investigate the toxic effects of the plants ethanolic extract as follows :

Experimental animals :

A total of 108 of both sexes , Albino rats were used in this study , divided into 9 groups , each group consist of 12 rats .

Working solution :

From each yield plant extract , 250 mg and 500 mg were dissolved in 10 ml DW and served as a fresh daily preparation . According to the average body weight for each group , the dose calculated in mg/kg then calculated in ml/kg .

Experimental design :

Albino rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute (MAPRI) , National Center for Research , Khartoum . Rats were fed with standard food pellets and water provided *ad libitum* . Each experiment consist of three groups each of 12 rats . One group served as control untreated while the other two groups were treated with two different dosage of plant extract . After two weeks of extract treatment , half of the rat in each group were slaughtered and underwent post-mortem and lesions were recorded . Specimens of the different organ (liver , intestines , kidneys , spleen and heart)

were taken and fixed in 10% neutral buffered formalin , embedded in paraffin wax , sectioned at five μm and stained with haematoxylin and eosin (H and E) , using Mayers haemalum and processed for histopathology . Blood samples for each rat in the different groups were taken in two vacutainer , one containing EDTA as anticoagulant and used for haematology investigations and the other without anticoagulant and used for plasma collection . Plasma were analyzed for different biochemical parameters to investigate liver and renal functions . Same was done after 4 weeks post treatment for the rats in the different groups .

Experiment 1

Aerva javanica :

2.3.7: The toxic effects of ethanolic extract of *Aerva javanica* in albino rats :

Thirty six Albino rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute (MAPRI) , National Center for Research , Khartoum . Rats were fed with standard food pellets and water provided *ad libitum* . The rats were allotted into 3 groups each of 12 rats . Group 1 fed the normal diet and served as control group . Each group (2 and 3) was kept separately and were given the ethanolic extract of the *Aerva javanica* at doses of 250 and 500 mg/kg/day via the oral route respectively , for 14 and 28 days .

After two weeks post treatment half of the rats from each group were slaughtered . The remaining continue the treatment for another two weeks then were slaughtered . Average body weight and body weight gain were measured , after each slaughtered .

Blood samples were collected from the cervical blood vessels of each rat for plasma analysis and haematology , rats from each group underwent post mortem and lesions were recorded , and specimens of the liver , intestines , kidneys , spleen and heart were immedietly fixed in 10 % neutral buffered formalin , embedded in paraffin wax , sectioned at 5 μm , and stained with haematoxylin and eosin (H and E) , using Mayer's haemalum .and processed for histopathology .

Experiment 2

Amaranthus viridis :

2.3.8 : The toxic effects of ethanolic extract of *Amaranthus viridis* in albino rats :

Thirty six Albino rats of both sexes weighing 100-200 g were housed within the premises of the Medicinal and Aromatic Plants Research Institute (MAPRI) , National Center for Research , Khartoum . Rats were fed with standard food pellets and water provided ad libitum . The rats were allotted into 3 groups each of 12 . Each group was kept in a pen separately . Group 1 fed the normal diet and served as control group . Groups 2 and 3 were given the ethanolic extract of the *Amaranthus viridis* at doses of 250 and 500 mg/kg/day via the oral route respectively , for 14 and 28 days .

After two weeks post treatment half of the rats from each group were slaughtered . The remaining continue the treatment for another two weeks then were slaughtered . Average body weight and body weight gain were measured , after each slaughtered .

Blood samples were collected from the cervical blood vessels of each rat for plasma analysis and haematology . Rats from each group underwent post mortem and lesions were recorded and specimens of the liver , intestines , kidneys , spleen and heart were immedietly fixed in 10 % neutral buffered formalin , embedded in paraffin wax , sectioned at 5 μm , and stained with haematoxylin and eosin (H and E) , using Mayer's haemalum .and processed for histopathology .

Experiment 3

Lepidium sativum :

2.3.9 : The toxic effects of ethanolic extract of *lepidium sativum* in albino rats :

Thirty six Albino rats of both sexes weighing 100-200 g were housed within the premises of the Medicinal and Aromatic Plants Research Institute (MAPRI) , National Center for Research , Khartoum . Rats were fed with standard food pellets and water provided *ad libitum* . The rats were allotted into 3 groups each of 12 rats , each group was kept in a separate pen . Group 1 fed the normal diet and served as

control group . Groups 2 and 3 were given the ethanolic extract of the *lepidium sativum* at doses of 250 and 500 mg/kg/day via the oral route respectively , for 14 and 28 days .

After two weeks post treatment half of the rats from each group were slaughtered . The remaining continue the treatment for another two weeks then were slaughtered . Average body weight and body weight gain were measured , after each slaughtered .

Blood samples were collected from the cervical blood vessels of each rat for plasma analysis and haematology , rats from each group underwent post mortem and lesions were recorded, and specimens of the liver , intestines , kidneys , spleen and heart were immedietly fixed in 10 % neutral buffered formalin , embedded in paraffin wax , sectioned at 5 μm , and stained with haematoxylin and eosin (H and E) , using Mayer's haemalum .and processed for histopathology .

2.3.10 : Blood samples :

Blood samples were taken in EDTA coated vacutainer tubes , from the cervical blood vessels for haematological examinations , Haemoglobin (Hb) concentration , Red Blood Cell (RBC) counts , Packed Cell Volume (PCV) , Mean Corpuscular Volume (MCV) , Mean Corpuscular Haemoglobin (MCH) , Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell (WBC) counts were determined by the automatic multi-parameter blood cell counter , Sysmex KX-21 .(Eden bridge , Kent . UK) .

2.3. 11 : Preparation of the plasma :

Samples were collected in EDTA coated vacutainer tubes and after mild shaking were centrifuged at 3000 revolutions/minute (rpm) for 15 minutes . The fluid part (plasma) was separated from the cellular part using a dropper and the plasma was placed in a new plane sample container for each rat labelled according to the study group , time and date of collection and stored at – 20 ˚C for the analysis of the activity of aspartate aminotransferase (AST) , alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and for concentrations of total proteins , albumin , globulins , total bilirubins , cholesterol , urea , sodium and potassium .

2.3.12 : Blood and biochemical analysis :

Sysmex KX-21, (Eden bridge , Kent ,UK) is an automatic multi-parameter blood cell counter , this instrument performs blood cell count by DC detection .

Procedure :

DC detection method : blood sample is aspirated , measured to determine volume , diluted at specific ratio , then field into tranducer . The tranducer chamber has minute whole called the aperture . On side of the aperture there are electrodes between which direct current flows . Blood cells suspended and diluted sample pass through the aperture , causing direct current resistance to change between the electrode . as direct resistance changes , the blood size is detected as electric pulses .

Equipment and material :

1. Reagents :
   a. Diluent , cell pack
   b. WBC/Hb lyser (stromatolyser) .
2. Detergent : Cell clean
3. Mixture

Preparation of the test sample :

Specified amount of sample , corresponding to the amount of EDTA anticoagulant , is collected from the vein .

- Use tube up to 80 mm in heighest
- The volume that can be aspirated as follows , volume of sample aspirated APP KX 50 µl

Procedure :

Press (Enter) key , this will fix the sample number and the status becomes ready , namely , ready for analysis .

1. Press sample No. Key in the ready status . In the system status area on the LCD screen , the next sample number turn to the reverse display and the system is wauting for sample No input (in the not ready status)

2. The cursor appears under sample No input sample No using the numeric keys
3. Inputting sample No , input from the panel keyboard
4. The buzzer sounds two times (beep – beep) and when the LCD screen displays analysing remove the tube after that the unit excute automatic analysis and display the result on LCD screen .

(Automated haematology analyser manual)

Biochemical analysis :

2.3.12.1: Glutamate Oxalate Transaminase (GOT)

Aspartate Transaminase (AST)

The activity of GOT was determined by the colorimetric method as described by (Reitman- Frankel 1957) , in an automatic analyzer .

Principle of the method :

The glutamic transaminase enzyme , serum glutamic oxalate (GOT) , catalyse the transfers of the amino group of glutamic acid to oxalacetic acid in reversible reactions . The transaminase activity is proportional to the amount of oxalate formed over a definite period of time and is measured by a reaction with 2,4 – Dinitrophenylhydrazine (DNPH) in alkaline solution . Absorbance of sample was read at wave length 505 nm , from absorbance , read unites of GOT from the corresponding calibration curves . Results were expressed as a international unit IU/L .

2.3.12.2: Glutamate Pyruvate Transaminase (GPT)

Alanine Transaminase (ALT)

The activity of GPT was determined by the colorimetric method as described by (Reitman – Frankel 1957) , in an automatic analyzer .

Principle of the method :

The glutamic transaminase enzyme , serum glutamic pyruvic (GPT) , catalyse the transfers of the amino group of glutamic acid to pyruvic acid in a

reversible reaction . The transaminase activity is proportional to the amount of pyruvate formed over a definite period of time and is measured by a reaction with 2 , 4 – Dinitrophenylhydrazine (DNPH) in alkaline solution . Absorbance of sample was read at wave length 505 nm , from absorbance , read units of GPT from the corresponding calibration curves . Results were expressed as a international unit IU/L .

2.3.12.3 : Alkaline Phosphatase (ALP)

The activity of ALP was determined by P-Nitrophenyl phosphate . Kinetic. In an automatic analyzer as described by (Wenger *et al* ., 1984 and Kaplan *et al* , 1984 , Rosalki *et al* ., 1993) .

Principle of the method :

Alkaline Phosphatase (ALP) catalyses the hydrolysis of P-Nitrophenyl phosphate at PH 10.4 , liberating P-nitrophenol and phosphate according to the following reaction :

P-Nitrophenyl phosphate + H_2o___> P-Nitrophenol + phosphate

The rate of P-Nitrophenol formation , measured photo metrically , is proportional to the catalytic concentration of Alkaline Phosphatase present in the sample . Results were expressed as a international unit IU/L .

2.3.12.4 : Total Protein :

The concentration of total protein was determined by Biuret colorimetric as described by (Koller and Kaplan et al 1984 , Burtis et al . , 1999) automatic Analyzer .

Principle of the method :

Proteins give an intensive Violet-blue complex with copper salts in an alkaline medium . Iodide is inclouded as an anti-oxidant . The intensity of the colour formed is proportional to the total protein conceneration in the sample . Results were expressed as g/dl .

2.3.12.5 : Albumin

The concentration of Albumin was determined by Bromcresol green colorimetric as described by (Gendler and Kaplan et al 1984 , Rodkey , 1965 , Webster , 1974 , Doumas, 1971) .

Principle of the method :

Albumin in the presence of bromocresol green at a slightly acid PH , produces a colour change of the indicator from Yellow – Green to Green –Blue . The intensity of the colour formed is proportional to the Albumin concentration in the sample . Results were expressed as g/dl .

2.3.12.6 : Globulin : (total protein – albumin)

Results were expressed as g/dl .

2.3.12.7 : Bilirubin :

The concentration of bilirubin was determined by DMSO . Colorimetric as described by (Kaplan *et al* 1984 , Malloy *et al* ., 1937)

Principle of the method :

Bilirubin is converted to coloured azobilirubin by diazotized sulfanilic acid and measured photometrically . Of the two fractions present in the serum , bilirubin glucuronide and free bilirubin loosely bound to albumin , only the former reacts directly in aqueous solution (bilirubin direct) . while free bilirubin requires solubilization with dimethyl-sulfoxide (DMSO) to react (bilirubin indirect) . In the determination of indirect bilirubin the direct is also determined . The results corresponds to total bilirubin . The intensity of the colour formed is proportional to the bilirubin concentration in the sample . Results were expressed as a mg/dl .

2.3.12.8 : Urea :

The concentration of urea was determined by Kinetic and end point (Lyophilized) as described by (Bablock *et al* ., 1988) .

Principle of the method :

Urea is hydrolysed in presence of urease to produce ammonia and CO_2 . The ammonia produced is combines with 2-Oxoglutarata and NADH in the presence and GLDH to yield glutamate and NAD

$$\text{Urea} + H_2O + 2H^+ \xrightarrow{\text{urease}} 2\ NH_4^+ + CO_2$$

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} H_2o + \text{NAD} + \text{glutamate}$$

The decrease in absorbance due to the decrease of NADH concentration in unit time is proportional to urea concentration . Ammonia which is produced by different decomposition processes is also determined by this method . Results were expressed as mg/dl .

2.3.12.9 : Cholesterol :

The concentration of cholesterol was determined by CHOD-PAP as described by (Richmond 1973 , NIH publication , 1990) in an automated analyser .

Principle of the method :

Cholesterol is present in serum as cholesterol esters and three cholesterol . The cholesterol esters present in serum are hydrolysed by cholesterol esterase and the cholesterol is then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide , the hydrogen peroxide in turn reaeacts with phenol and 4-aminoantipyrine present to form the red quinoneimine dye .The intensity of the dye fromed is directly proportional to the level of cholesterol present in the sample Results were expressed as mg/dl .

2.3.12.10 : Sodium Determination :

Sodium was determined by flame emission spectro-photometer at 589 nm . Results were expressed as mmol/l (Rodkey , 1965 , Doumas , 1971 , Webster , 1974) , and (Vogel , 1989).

2.3.12.11 : Potassium Determination :

Potassium was determined by flame emission spectro-photometer at 768 nm . Results were expressed as mmol/l . (Rodkey , 1965 , Doumas , 1971 , Webster , 1974) , and (Vogel , 1989).

2.3.13 : Histopathology examination :

Specimens of liver , intestine ,kidney , spleen and heart were immedietly fixed in 10 % neutral buffered formalin , embedded in paraffin wax , sectioned at 5μm and stained with haematoxylin and eosin (H and E) , using Mayer's haemalum .and processed for histopathology .

2.3.14 : Statistical analysis :

Anti-inflammatory experiment :

In each table :

1. Due to the small sample size , Mann-Whitney test is used .
2. Each cell shows the percentage , mean±SD , and P-value 0.5 respectively .
3. * means there is significant difference between the control positive and the plant extract at the specified time . (Snedecor and Cochan , 1989)

Sub-chronic toxicity experiment :

Study objective :

The study aims to test whether different doses (low and high dose) of the three plants : *Aerva javanica* , *Amaranthus viridis* and *Lepidium sativum* , have a toxic effect on the rats over different periods of time .

Data description :

The data consist of 108 rats , 36 rats represent the control group , each 72 rats from the remaining 108 rats were taken one of the three plants *Aerva javanica* , *Amaranthus viridis* and *Lepidium sativum* .

After two weeks , 36 of the 72 rats (with the high or low dose) were killed and the different measures were taken and the same is done for the remaining 36 rats after four weeks .

Analysis description :

Since the data are not normally distributed the non-parametric test , Mann-Whitney U was used to test whether each one of the different groups of each plant , are significantly different from the control group at significant level ≤ 0.05 (Snedecor and Cochan , 1989) .

CHAPTER THREE

RESULTS

Aerva javanica :

3.1.1: Anti-inflammatory experiment :

Anti-inflammatory effects of crude ethanolic extract (CEE) of *Aerva javanica* were tested in two different doses .

3.1.1.1 : Anti-inflammatory activity at 250 and 500 mg/kg of plant extract :

The experiment was conducted to determine anti-inflammatory effects of ethanolic extract of *Aerva javanica* on carrageenan-induced rat paw oedema .

The CEE of *Aerva javanica* (250 mg/kg) when compared with the control (Carrageenan) and the reference drug (Indomethacin) , produced significant inhibition ($p \leq 0.05$) in rat paw oedema of 48.30 % at 3 hours post treatment (Table 3.1) .

The CEE of *Aerva javanica* (500 mg/kg) when compared with the control (Carrageenan) and the reference drug (Indomethacin) , produced significant inhibition ($p \leq 0.05$) in rat paw oedema of 85.22% at 3 hours post treatment (Table 3.1) .

Table 3.1 : The anti-inflammatory effect of crude ethanolic extract of *Aerva javanica* on the rat paw oedema at dose of 250 and 500 mg/kg

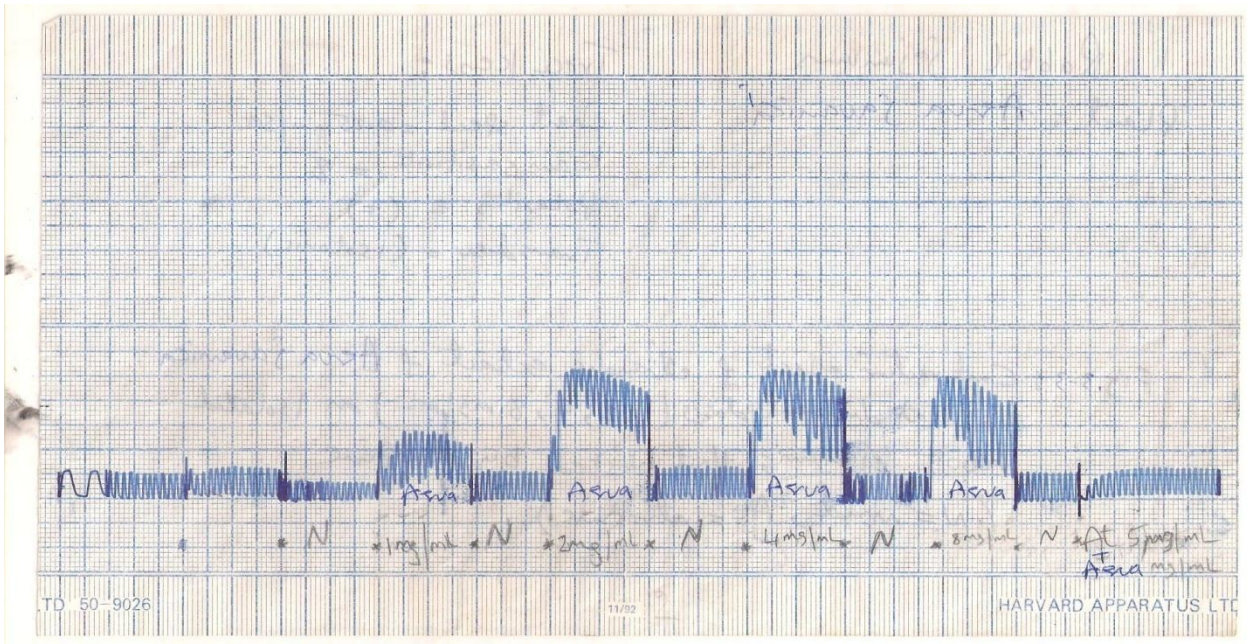
TREATMENT / TIME	Hour 1	Hour 2	Hour 3
Control negative (Carrageenan) 2ml/Kg BWt	1.95	1.89	1.76
Control positive (Indomethacin) 10mg/Kg BWt	1.16 ± 0.37 40.51 %	0.47 ± 0.24 75.13%	0.1 ± 0.06 94.32%
Aerva javanica 250 mg/kg	1.92 ± 0.44 1.54 % 0.009*	1.66 ± 0.44 12.17% 0.009*	0.91 ± 0.42 48.30% 0.009*
Aerva javanica 500 mg/kg	0.86 ± 0.25 55.89% 0.009*	0.57 ± 0.09 69.84% 0.009*	0.26 ±0.1 85.22% 0.009*

In each table :
1.Due to the small sample size , Mann-Whitney test is used
2.Each cell shows the percentage , mean±SD , and P-value (≤ 0.05) respectively
3.*means there is significant difference between the control positive and the plant extract at specified time .

3.1.2 : Pharmacological studies :

3.1.2.1 : Effect of *Aerva javanica* extract on isolated rabbit jejunum :

The effect of ethanolic extract of *Aerva javanica* was tested using isolated rabbit jejunum tissue . The ethanolic extract produced a dose dependent contraction on isolated rabbit jejunum at different doses (1 , 2 , 4 , 8 mg/ml) (Figure 3.1) . Atropine as antagonist was used to determine the mechanism of this contraction response . The result revealed that the contraction activity was blocked by atropine (5 µg/ml) (Figure 3.2) . This blockade is directly proportional to the dose which is competitive type of antagonism .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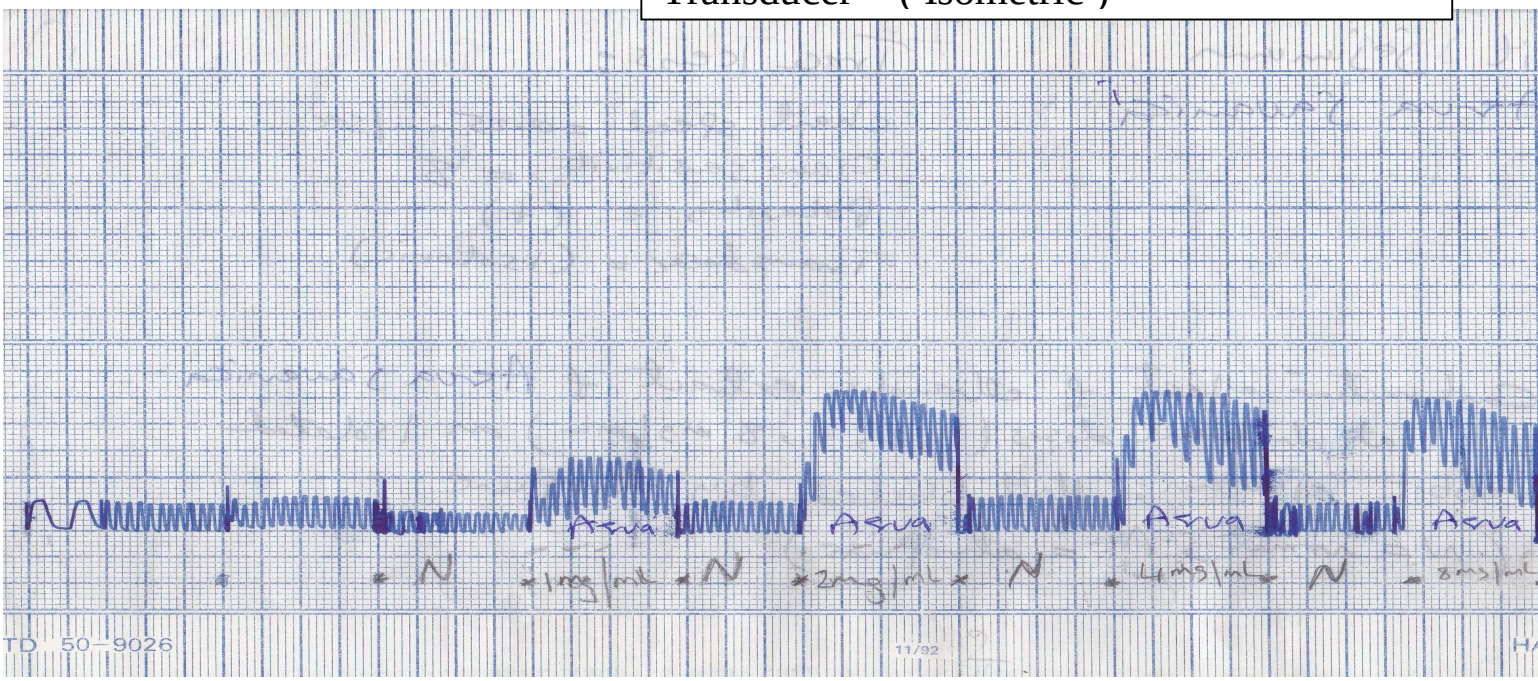

3.1.3 : Sub-chronic toxicity study :

igure 3.2: Contraction effect of ethanolic extract of *Aerva javanica* at different doses (1 , 2 , 4 g/ml) on isolated rabbit jejunum ; that blocked by atropine (5 µg/ml) .

= Normal

3.1.3.1 : Clinical signs and body weight :

No apparent clinical signs and no significant change in body weight

findings were observed at the two dose level study of the experiment .

Table 3.2 : The effect on the body weight of rats given daily oral doses of *Aerva javanica* ethanolic extracts at 250 and 500 mg/kg/day doses for 2 and 4 weeks .

Treatment groups	Body weight(g) (0 week)	Body weight(g) (2 weeks)	Body weight(g) (4 weeks)
Control	106	116	120
Aerva javanica 250 mg/kg/day	111.2± 0.453	120.5± 0.463	130± 0.093
Aerva javanica 500 mg/kg/day	116.7± 0.459	120± 0.518	126.7± 0.116

Results were expressed as mean ± SD

*means significant at P-value ≤ 0.05 .

3.1.3.2 Haematological findings :

Haematological changes for rats given daily oral doses of *Aerva javanica* whole plant ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks were presented in Table 3.3 . The values of Hb , RBC , PCV , MCV , MCH , MCHC and WBC in the treated groups were not significantly changed compared to the control group .

Table 3.3 : Effects of *Aerva javanica* ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks on haematological parameters .

Parameters	Control	2 weeks		4 weeks	
		250 mg/kg/day	500 mg/kg/day	250 mg/kg/day	500 mg/kg/day
Hb (g/dl)	13.33 ± 0.67	13.10 ± 0.20	13.00 ± 0.10	15.87 ± 0.45	13.77 ± 0.23
RBC (10^6 /µl	8.06 ± 0.58	7.12 ± 0.28	7.97 ± 0.16	7.23 ± 0.25	7.49 ± 0.20
PCV (%)	38.53 ± 2.80	38.47 ± 1.12	38.30 ± 0.53	45.67 ± 1.68	40.30 ± 0.89
MCV (fl)	47.80 ± 0.65	54.03 ± 0.45	48.05 ± 0.46	63.16 ± 0.46	53.80 ± 0.30
MCH (pg)	16.53 ± 7.50	18.39 ± 0.42	16.31 ± 3.02	21.94 ± 0.15	18.38 ± 0.05
MCHC (g/dl)	34.59 ± 0.83	34.05 ± 0.46	33.94 ± 0.70	34.47 ± 0.26	34.16 ± 0.71
WBC (10^3 /µl)	11.03 ± 2.45	9.07 ± 1.21	9.27 ± 1.20	9.67 ± 2.96	9.47 ± 1.81

Values are expressed as means ± SD

*means significant at P-value ≤ 0.05

3.1.3.3 Plasma biochemical findings :

Plasma biochemical changes of rats given daily oral doses of *Aerva javanica* whole plant ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks are presented in Table 3.4 . The activity of AST , ALT , ALP and the concentration of total proteins, globulins , bilirubin , urea , cholesterol , potassium and sodium in the treated groups were not significantly changed compared to the control group .

Table 3.4 : Effect of *Aerva javanica* ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks on plasma biochemical parameters .

Parameters	Control	2 weeks		4 weeks	
		250 mg/kg/day	500 mg/kg/day	250 mg/kg/day	500 mg/kg/day
AST (IU/l)	83.50 ± 18.50	56.00 ± 18.00	74.50 ± 22.50	76.50 ± 23.50	83.50 ± 13.50
ALT (IU/l)	67.00 ± 5.00	69.00 ± 1.00	74.00 ± 14.00	71.50 ± 4.50	73.00 ± 7.00
ALP (IU/l)	271.33 ± 1.53	280.50 ± 1.50	295.00 ± 3.00	514.00± 8.00	570.00 ± 15.00
Total proteins (g/dl)	7.33 ± 0.65	7.17 ± 0.55	7.17 ± 0.45	6.50 ± 0.30	6.75 ± 0.25
Albumin (g/dl)	3.67 ± 0.25	3.63 ± 0.15	3.60 ± 0.17	2.60 ± 0.40	3.00 ± 0.40
Globulins (g/dl)	3.70 ± 0.40	3.53 ± 0.70	3.26 ± 0.60	3.90 ± 0.10	3.75 ± 0.25
Bilirubin (mg /dl)	0.65 ± 0.05	0.50 ± 0.10	0.40 ± 0.10	0.60 ± 0.10	0.65 ± 0.05
Urea (mg/dl)	25.00 ± 1.00	19.00 ± 1.00	20.50 ± 1.50	38.50 ± 1.50	39.67 ± 3.51
Cholesterol (mg/dl)	49.50 ± 0.50	53.50 ± 0.50	46.00 ± 1.00	67.00 ± 12.00	61.50 ± 3.50
K (mmol/l)	3.60 ± 0.10	3.63 ± 0.15	3.45 ± 0.15	3.45 ± 0.05	3.20 ± 0.20
Na (mmol/l)	136.50 ± 1.50	138.50 ± 1.50	138.17 ± 59.91	139.00 ±1.00	140.50 ± 1.50

Values are expressed as means ± SD

*means significant at P-value ≤ 0.05

3.1.3.4 : Histopathological findings :

No histopathological changes were observed in the spleen and heart tissue for 250 mg/kg/day for 2 weeks , but some of the hepatic blood vessels and sinusoids were congested and slightly scattered hemorrhagic foci were observed . While 500

mg/kg/day for 4 weeks showed focal swollen vacuolated hepatocytes and the spleen red pulb showed hemosidrin deposits .

The renal lesions which observed in the two dose levels during the experimental period consisted of some empty and lobulated glomeruli while some of the renal tubular epithelial cells showed vacuoles and their basement membrane were interrupted . Also there were congestion and dilatation of some renal tubules with focal scattered foci of mononuclear cells .

The intestinal lesions observed for the two dose levels during the experimental period showed desquamation of the intestinal mucosal epithelium and the lumen contain RBCs and shreads of epithelial cells and WBCs .

***Amaranthus viridis* :**

3.2.1 : Anti-inflammatory experiment

3.2.1.1 : Anti-inflammatory activity at 250 and 500 mg/kg of plant extract :

The experiment was conducted to determine anti-inflammatory effects of ethanolic extract of *Amaranthus viridis* on carrageenan-induced rat paw oedema .

The CEE of *Amaranthus viridis* (250 mg/kg) when compared with the control (Carrageenan) and the reference drug (Indomethacin) , produced significant inhibition ($p \leq 0.05$) in rat paw oedema of 40.30 % at 3 hours post treatment (Table 3.5) .

Table 3.5 : The anti-inflammatory effect of crude ethanolic extract of *Amaranthus viridis* on the rat paw oedema at dose of 250 and 500 mg/kg :

TIME / TREATMENT	Hour 1	Hour 2	Hour 3
Control negative (Carrageenan) 2ml/Kg BWt	1.95	1.89	1.76
Control positive (Indomethacin) 10mg/Kg BWt	1.16 ± 0.37 40.51 %	0.47 ± 0.24 75.13%	0.10 ± 0.06 94.32%
Amaranthus viridis 250mg/kg	1.96 ± 0.11 -0.51% 0.009*	1.66 ± 0.35 12.17% 0.009*	1.05 ± 0.32 40.34% 0.009*
Amaranthus viridis 500mg/kg	1.25 ± 0.41 35.89% 0.117	1.10 ± 0.28 41.79% 0.009*	0.52 ± 0.22 70.45% 0.012*

In each table :
1.Due to the small sample size , Mann-Whitney test is used
2.Each cell shows the percentage , mean ± SD , and P-value (≤ 0.05) respectively
3.*means there is significant difference between the control positive and the plant extract at specified time .

The CEE of *Amaranthus viridis* (500 mg/kg) when compared with control (Carrageenan) and the reference drug (Indomethacin) , produced significant inhibition ($p \leq 0.05$) in rat paw oedema of 70.45% at 3 hours post treatment , greater than CEE of *Amaranthus viridis* at dose of (250 mg/kg) . (Table 3.5) .

3.2.2 : Pharmacological studies :

3.2.2.1 : Effect of *Amaranthus viridis* extract on isolated rabbit jejunum strip :

The effect of ethanolic extract of *Amaranthus viridis* was tested using isolated rabbit jejunum strip . The ethanolic extract produced a dose dependent contraction on isolated rabbit jejunum strip at different doses (1 , 2 , 4 mg/ml) (Figure 3.3) . Atropine as antagonist was used to determine the mechanism of this contraction response . The result revealed that the contraction activity was blocked partially by atropine (5 µg/ml) (Figure 3.4) . The antagonist cyproheptadine was used to determine the mechanism of this contraction response . The result revealed that the contraction activity was completely blocked by cyproheptadine (40 µg/ml) (Figure 3.5) .

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Trace keys :

Chart speed = 0.1 mm/sec

Gain sensitivity = 5

Polarity = (+)

Transducer = (Isometric)

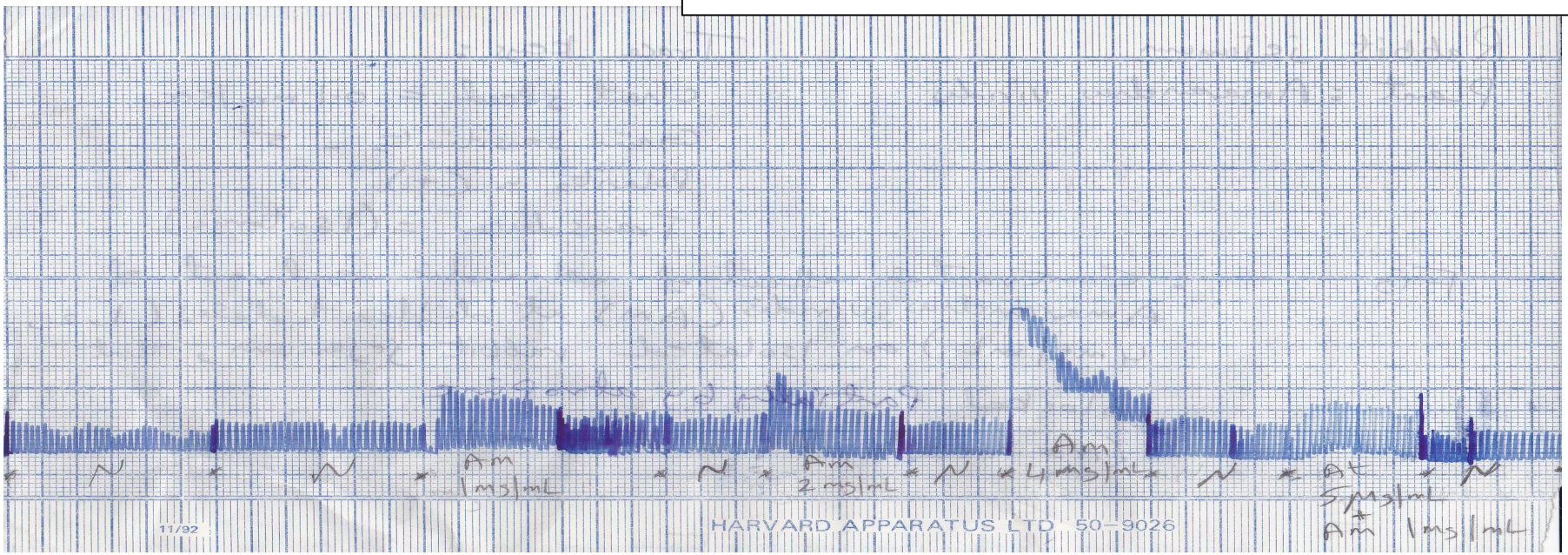

Figure (3. 4) : Contraction effect of ethanolic extract of Amaranthus viridis (Am) at different doses (1 , 2 , 4 mg/ml) on isolated rabbit jejunum ; that blocked partially by Atropine (5 µg/ml)

N= Normal , At = Atropine

Trace keys :

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Transducer (isometric)

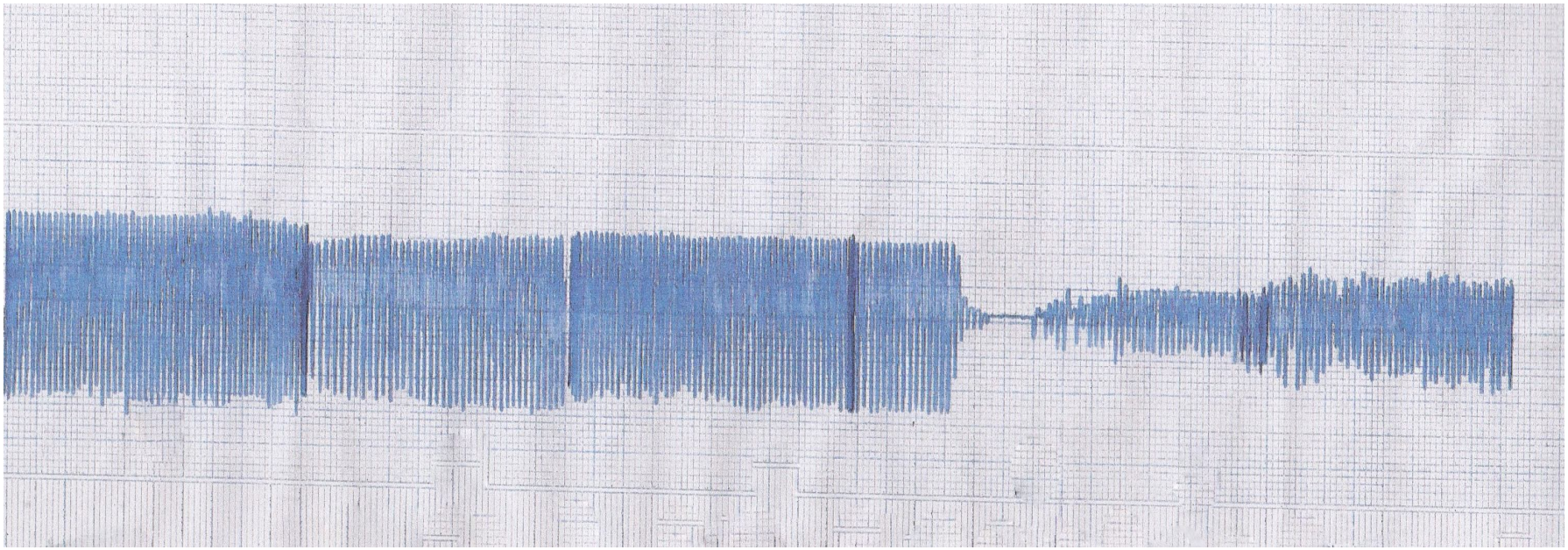

Figure 3.5 : Contraction effect of ethanolic extract of Amaranthus viridis (Am) at different doses (1 , 2 , 4 mg/ml) on isolated rabbit jejunum ; that blocked completely by cyproheptadine 40 µg/ml

N=Normal , Cp = Cyproheptadine

3.2.3 : Sub-chronic toxicity study :

3.2.3.1 : Effect of *Amaranthus viridis* whole plant ethanolic extract on clinical signs and body weight findings at 250 and 500 mg/kg/day doses for 2 and 4 weeks :

No apparent clinical signs and no significant change in body weight findings were observed at the two dose level study of the experiment , but there was significant increase in body weight for the 500 mg/kg at 2 and 4 weeks .

Table 3.6 : The effect on the body weight of rats given daily oral doses of *Amaranthus viridis* ethanolic extracts at 250 and 500 mg/kg/day for 2 and 4 weeks .

Treatment groups	Body weight(g) (0 week)	Body weight(g) (2 weeks)	Body weight(g) (4 weeks)
Control	106	116	120
Amaranthus viridis 250 mg/kg/day	102± 0.518	138.3± 0.1	145.5± 0.082
Amaranthus viridis 500 mg/kg/day	129.2± 0.097	166.7± 0.006*	170± 0.006*

Results were expressed as mean ± SD

*means significant at P-value ≤ 0.05

3.2.3.2 : Haematological findings :

Haematological changes for rats given daily oral doses of *Amaranthus viridis* whole plant ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks were presented in Table 3.7 . The values of Hb , RBC , PCV , MCV , MCH , MCHC and WBC in the treated groups were not significantly changed compared to the control group .

Table 3.7 : Effects of *Amaranthus viridis* whole plant ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks on haematological parameters :

Parameters	Control	2 weeks		4 weeks	
		250 mg/kg/day	500 mg/kg/day	250 mg/kg/day	500 mg/kg/day
Hb (g/dl)	13.33 ± 0.67	13.47± 0.25	11.80 ± 0.56	14.80 ± 2.97	13.95 ± 0.21
RBC (10^6 /µl	8.06 ± 0.58	7.21 ± 0.07	7.00 ± 0.10	7.38 ± 1.44	7.40 ± 0.57
PCV (%)	38.53 ± 2.80	36.30 ± 0.61	32.90 ± 1.42	35.35 ± 9.83	40.75 ± 0.94
MCV (fl)	57.80 ± 0.65	50.34 ± 1.04	47.00 ± 1.44	47.08 ± 2.97	55.06 ± 3.54
MCH (pg)	16.53 ± 7.50	18.68 ± 0.46	16.85 ± 0.55	20.05 ± 0.14	18.85 ± 1.13
MCHC (g/dl)	34.59 ± 0.83	34.10 ± 0.17	35.86 ± 0.20	34.45 ± 1.77	34.23 ± 0.07
WBC (10^3 /µl)	11.03 ± 2.45	9.35 ± 0.64	9.27 ± 4.10	9.25 ± 3.18	8.75 ± 0.07

Values are expressed as means ± SD

*means significant at P-value ≤ 0.05

3.2.3.3 : Plasma biochemical findings :

Plasma biochemical changes of rats given daily oral doses of *Amaranthus viridis* whole plant ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks were presented in Table 3.8 . The activity of AST , ALT and ALP and the concentration of total proteins , albumin , globulins , bilirubin , urea , cholesterol , potassium and sodium in the treated groups were not significantly changed compared to the control group .

Table 3.8 : Effect of *Amaranthus viridis* whole plant ethanolic extract at 250 and 500 mg/kg for 2 and 4 weeks on plasma biochemical parameters .

Parameters	Control	2 weeks		4 weeks	
		250 mg/kg/day	500 mg/kg/day	250 mg/kg/day	500 mg/kg/day
AST (IU/l)	83.50 ± 18.50	69.50 ± 7.50	76.00 ± 19.00	71.00 ± 1.41	82.00 ± 0.00
ALT (IU/l)	67.00 ± 5.00	69.50 ± 13.50	71.00 ± 15.00	61.50 ± 2.12	63.00 ± 0.00
ALP (IU/l)	271.33 ± 1.53	282.00 ± 6.00	298.00 ± 3.00	492.50 ± 17.68	597.00 ± 0.00
Total proteins (g/dl)	7.33 ± 0.65	7.15 ± 0.05	7.05 ± 0.25	6.05 ± 0.49	6.10 ± 0.00
Albumin (g/dl)	3.67 ± 0.25	3.60 ± 0.10	3.45 ± 0.45	2.60 ± 0.42	2.80 ± 0.00
Globulins (g/dl)	3.70 ± 0.40	3.55 ± 0.15	3.60 ± 0.20	3.45 ± 0.92	3.30 ± 0.00
Bilirubin (mg /dl)	0.65 ± 0.05	0.60 ± 0.00	0.50 ± 0.20	0.60 ± 0.14	0.80 ± 0.00
Urea (mg/dl)	25.00 ± 1.00	22.00 ± 1.00	20.00 ± 3.00	29.50 ± 3.54	29.00 ± 0.00
Cholesterol (mg/dl)	49.50 ± 0.50	42.50 ± 1.50	50.50 ± 2.50	64.00 ± 15.56	57.00 ± 0.00
K (mmol/l)	3.60 ± 0.10	3.55 ± 0.05	3.55 ± 0.05	3.45 ± 0.21	3.30 ± 0.00
Na (mmol/l)	136.50 ± 1.50	136.50 ± 0.50	136.00 ± 1.00	136.50 ±2.12	138.00 ± 0.00

Values are expressed as means ± SD

*means significant at P-value ≤ 0.05

3.2.3.4 : Histopathological findings :

No histopathological changes were observed in the spleen and heart tissue for 250 mg/kg/day for 2 weeks , but some of the hepatic sinusoids were dilated . While 500 mg/kg/day for 4 weeks showed swollen vacuolated hepatocytes , dilated sinusoids and the spleen red pulb showed hemosidrin deposits .

The renal lesions which observed in the two dose levels during the experimental period consisted of variations of some glomeruli size while some of the renal tubules were vacuolated and fragmented with dilated bowman's spaces .

The intestinal lesions observed for the two dose levels during the experimental period showed desquamation of the intestinal mucosal epithelium .

***Lepidium sativum* :**

3.3.1: Anti-inflammatory experiment

3.3.1.1 : Anti-inflammatory activity at 250 and 500 mg/kg of plant extract :

The experiment was conducted to determine anti-inflammatory effects of ethanolic extract of *Lepidium sativum* on carrageenan-induced rat paw oedema .

The CEE of *Lepidium sativum* (250 mg/kg) when compared with the control (Carrageenan) and the reference drug (Indomethacin) , produced significant inhibition ($p \leq 0.05$) in rat paw oedema of 38.60% at 3 hours post treatment (Table 3.9) .

Table 3.9 : The anti-inflammatory effect of crude ethanolic extract of *Lepidium sativum* seeds on the rat paw oedema at dose of 250 and 500 mg/kg :

TIME / TREATMENT	Hour 1	Hour 2	Hour 3
Control negative (Carrageenan) 2ml/Kg BWt	1.95	1.89	1.76
Control positive (Indomethacin) 10mg/Kg BWt	1.16 ± 0.37 40.51%	0.47 ± 0.24 75.13%	0.10 ±06 94.30%
Lepidum sativum 250 mg/kg	2.03 ± 0.47 -4.10% 0.009*	1.73 ± 0.21 8.47% 0.009*	1.08 ± 0.3 38.64% 0.028*
Lepidium sativum 500 mg/kg	1.33 ± 0.27 31.79% 0.016*	1.15 ± 0.29 39.15% 0.009*	0.95 ± 0.37 46.02% 0.009*

In each table :
1.Due to the small sample size , Mann-Whitney test is used
2.Each cell shows the percentage , mean ±SD , and P-value (≤ 0.05) respectively
3.*means there is significant difference between the control positive and the plant extract at specified time .

The CEE of *Lepidium sativum* (500 mg/kg) when compared with control (Crrageenan) and the reference drug (Indomethacin) , produced significant inhibition ($p \leq 0.05$) in rat paw oedema of 46.02% at 3 hours post treatment , greater than CEE of *Lepidium sativum* at dose of (250 mg/kg) . (Table 3.9) .

3.3.2 : Pharmacological studies :

3.3.2.1 : Effect of *Lepidium sativum* extract on isolated rabbit jejunum strip :

The effect of ethanolic extract of *Lepidium sativum* was tested using isolated rabbit jejunum strip . The ethanolic extract produced a dose dependent contraction on isolated rabbit jejunum strip at different doses (1 , 2 , 4 mg/ml) (Figure 3.6). Atropine as antagonist was used to determine the mechanism of this contraction response . The result revealed that the contraction activity was blocked partially by

atropine (5 µg/ml) (Figure 3.7) . The antagonist cyproheptadine was used to determine the mechanism of this contraction response . The result revealed that the contraction activity was completely blocked by cyproheptadine (40 µg/ml) (Figure 3.8) .

Trace keys :

Chart speed = 0.25 mm/sec

Gain sensitivity = 8

Polarity = (+)

Transducer = (Isometric)

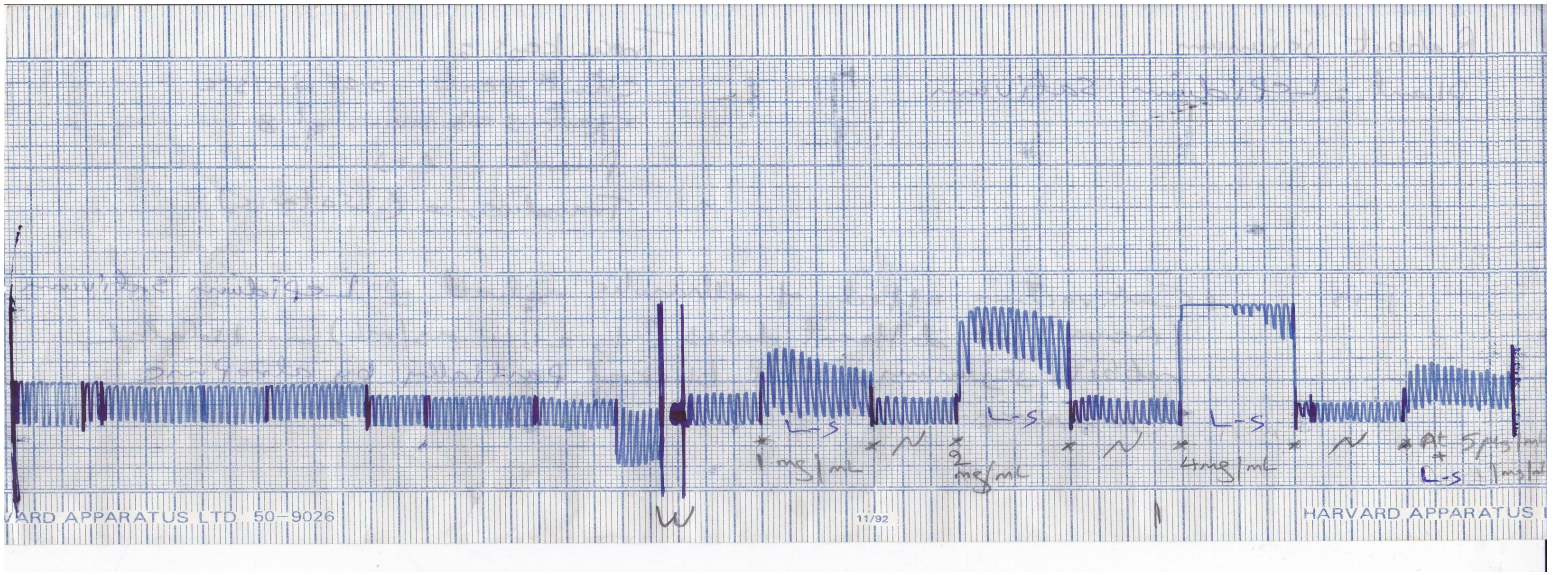

Figure 3.7 : Contraction effect of ethanolic extract of Lepidium sativum (Ls) at different doses (1 , 2 , 4 mg/ml) on isolated rabbit jejunum ; that blocked partially by Atropine

N= Normal , At = Atropine , W= Wash

Trace keys :

Chart speed = 0.25 mm/sec

Gain sensitivity = 8

Polarity = (+)

Tansducer (Isometric)

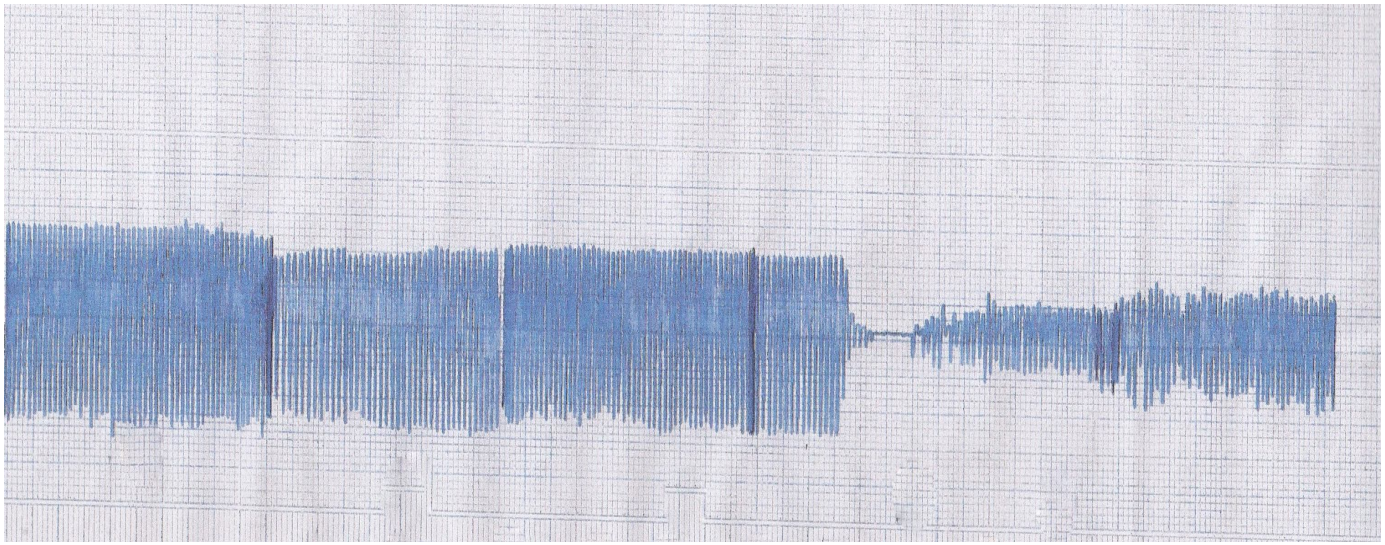

Figure 3.8: Contraction effect of ethanolic extract of Lepidium sativum (Ls) at different doses (1 , 2 , 4 mg/ml) on isolated rabbit jejunum ; that blocked completely by cyproheptadine (Cp) 40 mg/ml

N=Normal , Cp = Cyproheptadine

3.3.3 : Sub-chronic toxicity study :

3.3.3.1 : Effect of *Lepidum sativum* seeds ethanolic extract on clinical signs and body weight findings at 250 and 500 mg/kg/day doses for 2 and 4 weeks :

No apparent clinical signs and no significant change in body weight findings were observed at the two dose level study of the experiment , but there was significant increase in body weight for the 500 mg/kg at 2 and 4 weeks .

Table 3.10 : The effect on the body weight of rats given daily oral doses of *Lepidum sativum* ethaolic extracts at 250 and 500 mg/kg/day doses for 2 and 4 weeks :

Treatment groups	Body weight(g) (0 week)	Body weight(g) (2 weeks)	Body weight(g) (4 weeks)
Control	106	116	120
Lepidum sativum 250 mg/kg/day	115.8± 0.646	145± 0.054	160± 0.009*
Lepidum sativum 500 mg/kg/day	149.2± 0.035*	166.7± 0.006 *	173± 0.006*

Results were expressed as mean ± SD

* means significant at P-value ≤ 0.05

3.3.3.2 : Haematological findings :

Haematological changes for rats given daily oral doses of *Lepidium sativum* seeds ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks were presented in (Table 3.11) . The values of Hb, RBC , PCV , MCV , MCH , MCHC and WBC in the treated groups were not significantly changed compared to the control group .

Table 3.11 : Effects of *Lepidium sativum* seeds ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks on haematological parameters :

Parameters	Control	2 weeks		4 weeks	
		250 mg/kg/day	500 mg/kg/day	250 mg/kg/day	500 mg/kg/day
Hb (g/dl)	13.33 ± 0.67	12.90± 0.14	12.00 ± 0.44	12.70 ± 0.86	13.33 ± 1.14
RBC (10^6 /µl)	8.06 ± 0.58	7.70 ± 0.28	7.64 ± 0.50	6.76 ± 1.86	6.96 ± 0.71
PCV (%)	38.53 ± 2.80	37.70 ± 1.13	32.20 ± 3.16	32.10 ± 9.13	38.07 ± 4.10
MCV (fl)	47.80 ± 0.65	48.96 ± 0.28	42.14 ± 1.04	47.48 ± 0.84	54.69 ± 0.42
MCH (pg)	16.53 ± 7.50	16.75 ± 0.28	15.70 ± 0.67	18.78 ± 0.92	19.15 ± 0.35
MCHC (g/dl)	34.59 ± 0.83	34.21 ± 0.21	37.26 ± 1.36	39.56 ± 1.25	35.01 ± 1.00
WBC (10^3 /µl)	11.03 ± 2.45	9.75 ± 2.05	9.13 ± 1.96	8.77 ± 1.50	8.37 ± 0.04

Values are expressed as means ± SD

*means significant at P-value ≤ 0.05

3.3.3.3 : Plasma biochemical changes :

Plasma biochemical changes of rats given daily oral doses of *Lepidium sativum* seeds ethanolic extract at 250 and 500 mg/kg/day doses for 2 and 4 weeks were presented in (Table 3.12) . The activity of AST , ALT and ALP and the concentration of total proteins , albumin , globulins , bilirubin , urea , cholesterol , potassium and sodium in the treated groups were not significantly changed compared to the control group .

Table 3.12 : Effects of *Lepidium sativum* seeds ethanolic extract at 250 and 500 mg/kg/day for 2 and 4 weeks on plasma biochemical parameters :

Parameters	Control	2 weeks		4 weeks	
		250 mg/kg/day	500 mg/kg/day	250 mg/kg/day	500 mg/kg/day
AST (IU/l)	83.50 ± 18.50	60.00 ± 4.24	62.00 ± 1.00	71.00 ± 20.00	78.50 ± 31.50
ALT (IU/l)	67.00 ± 5.00	61.50 ± 14.85	45.50 ± 4.50	40.50 ± 9.50	48.00 ± 22.00
ALP (IU/l)	271.33 ± 1.53	278.50 ± 13.44	281.00 ± 4.00	599.00 ± 25.00	599.00 ± 94.00
Total proteins (g/dl)	7.33 ± 0.65	6.60 ± 0.28	6.50 ± 0.10	5.20 ± 0.70	5.15 ± 0.55
Albumin (g/dl)	3.67 ± 0.25	3.45 ± 0.21	3.20 ± 0.20	2.35 ± 0.05	2.40 ± 0.20
Globulins (g/dl)	3.70 ± 0.40	3.15 ± 0.49	3.30 ± 0.10	2.85 ± 0.65	2.75 ± 0.35
Bilirubin (mg /dl)	0.65 ± 0.05	0.50 ± 0.14	0.70 ± 0.00	0.75 ± 0.15	0.60 ± 0.20
Urea (mg/dl)	25.00 ± 1.00	21.00 ± 1.41	20.50 ± 1.50	30.00 ± 8.00	32.00 ± 7.00
Cholesterol (mg/dl)	49.50 ± 0.50	41.50 ± 2.12	42.00 ± 4.00	49.00 ± 1.00	59.00 ± 14.00
K (mmol/l)	3.60 ± 0.10	3.45 ± 0.07	3.45 ± 0.25	3.00 ± 0.10	3.10 ± 0.10
Na (mmol/l)	136.50 ± 1.50	137.00 ± 1.41	136.00 ± 2.00	138.50 ±1.50	139.00 ± 1.00

Values are expressed as means ± SD

*means significant at P-value ≤ 0.05

3.3.3.4 : Histopathological findings :

No histopathological changes were observed in the spleen and heart tissue for 250 mg/kg/day for 2 weeks , but some of the hepatic sinusoids were dilated . While 500 mg/kg/day for 4 weeks showed dilated liver sinusoids and dilated blood vessels with perilobular and some centrilobular hepatocytic necrosis .

The renal lesions which observed in the two dose levels during the experimental period consisted of many lobulated glomeruli tufts , swollen cortical tubules , dilated bowman’s space and one focus of macrophage collection .

The intestinal lesions observed for the two dose levels during the experimental period showed desquamation of the intestinal mucosal epithelium and the intestinal villi were tall and blunt with elongated crypts .