

CHAPTER FOUR

DISCUSSION

The first objective of this study was to investigate the anti-inflammatory effect of ethanolic extracts of *Aerva javanica* , *Amaranthus viridis* whole plants and *Lepidium sativum* seeds using carrageenan induced rat paw oedema .

The most promising plants should be scientifically validated through systematic experiments , using inflammatory indicators in laboratory animals and toxicity studies to screen if there are positive . Paw oedema (swelling) is one of the major methods in assessing the degree of inflammation and efficacy of the tested drugs (Begum and Sadique , 1988 ; Mizushima et al . , 1972) . *Aerva javanica* is a very useful plant , all parts of the plant are medicinal and contain a number of ingredients to treat various ailments . Paste made up of leaves is used externally to heal the wounds and inflammation of the joints . The decoctions of the whole plant are used to remove swelling and powder of the plant is applied externally to ulcers in domestic animals . (Qureshi and Folklore , 2009) .

There are several reports , which indicated the great variety of pharmacological and biological activities of *Amaranthus viridis* such as anti-microbial , anti-oxidant , analgesic , anti-pyretic , anti-cancer , hormone regulation and inhibition of enzymes . (Yosuf et al . , 1994) . It has also been used as anti-diabetic (Kesari et al . , 2005) , anti-histaminic (Yamamura et al . , 1998) , and anti-carcinogenic (Yen et al. , 2001) . The leaves used for scorpion stings and stem used as an antidote for snake bites (Obi et al. , 2006) . Powdered seeds of flower used for stomach problems (Senna , 1998) . The infusion of the plant has been used as a diuretic and anti-inflammatory agent of the urinary tract , venereal diseases , vermifuge , anti-emetic and laxative . (Quershi et al . , 2008) . The poultice of leaves used for inflammation , boils , abscesses and used for acne and skin cleansing (Bagepalli et al. , 2009) .

In India a spicy salad herb *Lepidium sativum* is an important green vegetable consumed by human-beings . (Tiwari and Kulmi , 2004) . It is considered as a 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). It is considered as a sedative , anxiolytic , myo-relaxant and analgesic . (Kulkarni , 1999) . It decreases blood sugar level (Bryan , et al., 2009) . It decreases total lipids , total cholesterol , triglycerides and the low density lipoprotein (LDL) . (Das et al., 1997) . *Lepidium sativum* has a role in hepato-protection by inhibiting the free radicles mediated damage . (Banskota et al., 2000 , Takeoka and Dao , 2003) . It has a potent diuretic activity by producing inhibition of tubular reabsorption of water and anions (Pantoja et al., 1993) , with proved anti-hypertensive action . (Gouad et al., 2001) . It has a nephro-protective effect against induced antibiotics-nephrotoxicity due to the significant anti-oxidant activities which may lead to discover a novel drug useful in treatment of drug-induced nephrotoxicity (Jain et al., 2009) .

The dose of 250 and 500 mg/kg of ethanolic extract of *Aerva javanica* whole plant was found to produce significant inhibition in rat paw oedema in a time and dose dependent manner and the maximum inhibition percentage was recorded at 3 hours post treatment as 48.30 % and 85.22 % respectively .

In addition ethanolic extract of *Aerva javanica* at dose of 500 mg/kg was close to inhibition level of the reference drug indomethacin (94.3 %) at end of the follow up period .

Phytochemical investigation on *Aerva javanica* yielded several classes of secondary metabolites such as flavonoids , steroids , alkaloids and saponins , many of which express biological activities . (Saker et al ., 2000) .

In the present study 250 and 500 mg/kg of ethanolic extract of *Amaranthus viridis* whole plant was found to produce significant inhibition in rat-paw oedema in a time and dose dependent manner and the maximum inhibition percentage was recorded at 3 hours post treatment as 40.30 % and 70.40 % respectively .

The pharmacologically active compounds of *Amaranthus viridis* are several amino-acids , lysine , arginine , histidine , cystine , phenylalanine , leucine , isoleucine , valine , thionine , methionine , tyrosine and tryptophan . (Anonymous , 1988) . Also presence of steroids , flavonoids and saponins types of compounds . (Mayer et al., 1982) .

The dose of 250 and 500 mg/kg of ethanolic extract of *lepidium sativum* seeds was found to produce significant inhibition in rat-paw oedema in a time and dose dependent manner and the maximum inhibition percentage was recorded at 3 hours post treatment as 38.60 % and 46.00 % respectively .

The pharmacologically active compounds of *lepidium sativum* are saponins , tannins , steroids , cardiac glycosides and flavonoids in the various organic and inorganic seed extract (Bajpai et al., 2005) . Also presence of alkaloids , linolenic , oleic acids and tocopherols . (Bryan et al., 2009) . Amino-acids as glutamine and cysteine and glycine (Kirtkar and Basu , 2005) . In addition to the presence of triterpens and cumarins. (Panskota et al., 2000 and Takeoka and Dao , 2003) . Leaves and seeds extracts were found to have anti-inflammatory effect . The presence of flavonoides , alkaloids , cyanogenic glycosides , tannins , glucosinolates , sterols and triterpens contribute this effect (Sheel and Nidhi ., 2011 and Najeeb – ur-Rehmana *et al.*, 2011). Chemical analysis of *L.sativium* seeds showed that they contain high percentage of protein content which explained the role of the plant seeds in healing process (Komesu *et al.*, 2003) . Diet rich in protein provide amino acid precursor for synthesis of different types of substances needed for cellular activities repair or healing processes (Pollak *et al.*, 1986 ; Delvin ,1992) .

It can be concluded that the extract of *Aerva javanica* , *Amaranthus viridis* and *lepidium sativum* posses anti-inflammatory activity .

The active principles that induced the observed anti-inflammatory activity might be found in these classes of chemicals . This was supported by Debella (2002) who stated that , medicinal plants showed good anti-inflammatory activity have secondary metabolites like alkaloids and flavonoids and these classes of plant secondary metabolites are considered as the sources of chemicals responsible for wide therapeutic activities of several plants .

Acute inflammation caused by chemicals such as carrageenan-induced oedema involves the synthesis or release of mediators at the injured site , their inhibition will normally ameliorate inflammation and other symptoms . The ethanolic extracts from the *Aerva javanica* , *Amaranthus viridis* whole plant , and *Lepidium sativum* seeds posses a significant anti-oedematogenic effect on paw oedema

induced by carrageenan , which was dose and time dependent comparable to that of the reference drug , indomethacin . Reduction in inflammation is also enhanced through the stimulation of the immune system to release white blood cells (Steenkamp and Stewart , 2007) . It therefore , could be that the immune cells were initiated already such that a sub-class of cytokines called leukotriens or interleukins insured that the immune response is checked before it destroys outlining healthy cells and tissue called off the inflammatory response .

Carrageenan-induced rat paw is a suitable experimental animal model for evaluating the anti-oedematous effect of natural products and is believed to be biphasic . The first phase (1 h) involve the release of serotonin and histamine and the second phase (over 1 h) is mediated by prostaglandins , the cyclo-oxygenase products and the continuity between the two phases is provided by kinins (Perianayagam et al . , 2006) . Development of oedema induced by carrageenan is commonly correlated with early exudative stages of inflammation . (Adedapo et al . , 2008) . Emam , 1999 , Khan et al . , 1982 , Reddy and Reddy , 2009 reported that the *Aerva javanica* plant contained tannins , these compounds are known to be potent cyclo-oxygenase-1 (Cox-1) inhibitors , through their binding nature with proteins . Since the carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agent acting by the mediators of acute inflammation . (Sawadogo et al . , 2006) , these results are an indication that *Aerva javanica* whole plant , *Amaranthus viridis* and *Lepidium sativum* can be effective in acute inflammatory disorders . The extract may have exhibited its anti-inflammatory actions by means of either inhibiting the synthesis , release or action of inflammatory mediators such as histamine , serotonin and prostaglandins . Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin act by the reduction of sensitization of pain receptors caused by prostaglandins at the inflammation site (Dhara et al . , 2000) . The different triterpenoids , polyphenolics and other chemical constituents of the plant extract may be involved in the obsererved anti-inflammatory effects of the plant extract and may be having actions similar to NSAIDs . It should be noted that the anti-inflammatory activities of many plants have been attributed to their high sterol / triterpene (Silva *et al .* , 2005) .

The second objective of the present study was to investigate the effect of the ethanolic extracts of *Aerva javanica* , *Amaranthus viridis* whole plants and

Lepidium sativum seeds on selected isolated tissue preparations on rabbit jejunum strip .

The result of the pharmacological screening of *Aerva javanica* whole plant ethanolic extract showed that contraction when tested on isolated rabbit jejunum on different doses (1 , 2 , 4 , 8 mg /ml) . This contraction activity was blocked completely by atropine (5 µg/ml) . This result express the muscarinic stimulatory effects of the plant due to the presence of cholinomimetic constituents and/or acetylcholine-esterase inhibitors , that indicated Genus *Aerva* traditional uses as pugnate and emetic for hoarses and camels (Payal *et al.*, 2012) .

In pharmacological screening , the ethanolic extract of *Amaranthus viridis* whole plant and *Lepidium sativum* seeds produced a dose dependent contraction when tested on isolated rabbit jejunum at different doses (1 , 2 , 4 mg/ml) . This contraction activity was blocked partially by atropine (5 µg/ml) implicating a non acetylcholine induced contraction , instead the antagonist cyproheptadine (non-selective 5-HT blocker) at dose of 40 mg/ml effectively blocked the contraction induced by the extract . The small intestine was found to be less sensitive to 5-HT than the uterine muscle and the fundus strip . (Cohen *et al.*, 1985) , but it was also found to be blocked by a non-selective 5-HT blockers . 5-HT might have ultimately exerted its stimulant effect on the intestinal smooth muscle by enhancing calcium influx . (Mark *et al.*, 1982) , or by activation of 5-Hydroxytryptamine (5-HT₄) receptors in this system causes increased acetylcholine release and there by mediate a motility-enhancing or prokinetic effect of selective serotonin agonists . (Craig and his team , 1990) . This finding of a 5-HT like activity give a strong support to the folkloric use of the plant extract for different inflammatory disorders . (Braun and massey , 1992) .

These findings are correlated to the results of Kulkarni , 1999 as sedative , analgesic , relieve headache and anxiolytic .

Lepidium sativum seeds contains amucilaginous matter which consist of mixture cellulose 18.3% and uronic acid containing poly saccharides which in the presence of water in the GI track swell . This swelling is due to the poly uranide chains that contain ionisable carboxyl groups that with water present become hydrated and swell , and the cellulose micelles become dispersed .The size of the cellulose

micelles , chain length and the proportion of hydrated poly urinides all determine the extend of mucilaginous matter dispersion . This property shows that *L.sativum* seeds can be used for constipation as a laxative , which was proved on mice using aqueous methanolic extract of lepidium seeds at 30 and 100 mg/Kg . Also , another test on isolated gut preparations of mouse and guinea pig using a dose of 0.1 mg/ml showed stimulatory effect both in jejunum and ileum that depend on concentration (Sheel and Nidhi ., 2011) and (Najeeb –ur- Rehmana *et al.*, 2011).

Although , the *Aerva javanica* whole plant , *Amaranthus viridis* and *Lepidium sativum* were used in traditional medicine of several countries in Africa and Asia for the treatment of various diseases , little research had been done to investigate the safety of *Aerva javanica* plant , *Amaranthus viridis* and *Lepidium sativum* on rodents and other species of livestock .

The third objective of the present study was to elucidate effects of ethanolic extracts of the three plants on some vital organs of albino rats .

In the present study , the haematological values obtained for (Hb , PCV , MCV , MCHC , RBCs and WBCs and the plasma level of AST, ALT , ALP , Total protein , albumin , globulin , bilirubin , cholesterol , urea , sodium and potassium recorded results nearly to that of the control group .

From the literature there are approximately 28 species of *Aerva* genus , but only a few species are medicinal of which *A.persica* , *A.lanata* , *A.javanica* are of great value . *Aerva* plants are used to cure ulcer , lithiasis , dropsical affections , eye affections , toothache , headache , in disorders of abdomen and inflammation of internal organs . Roots and flowers are reported to possess hypoglycemic , anti-oxidant , anthelmintic , analgesic , antimalarial , antivenin activities and medicinal properties against rheumatism and kidney troubles (Payal *et al.*, 2012) .

It was found that dried alcoholic extract of root and leaf of *A.lanata* at dose of 600 mg/kg body weight showed significant hepatoprotective activity by restoring the elevated activities of liver marker enzymes and enhance the anti-oxidant enzyme activities . (Majmudar *et al.* , 1999) . Flowers of *A.lanata* were found to be the most effective in inducing diuresis at the dose of 50 g/l (Goonaratna *et al.*, 1993 and Uduphille and Jiffry 1981) . Alcoholic extract of *A.lanata* at 800 mg/kg act as diuretic (Vetrichelvan *et al.*, 2000) . The ethanolic extract of the entire plant of

A.lanata at the dose of levels of 75 , 150 and 300 mg/kg showed dose dependent reduction in the elevated blood urea and serum creatinine (Shirwaikar *et al .*, 2004) . *A.lanata* was found to increase the urine volume and thereby helped in the reduction of the solubility product with respect to the calcium oxalate and other crystallizing salts . The efficacy of the plant suggests their role to be used as the antilithic agent (Selvan *et al .*, 2001) . The aqueous extract of *Aerva sanguinolenta* showed significant diuretic activity by increasing secretion of sodium and potassium ions (Srinivas *et al .*, 2011) .

There are very few reports in the literature regarding the toxicity of *Lepidium sativum* . In one of the study , investigators have shown that seeds of *Lepidium sativum* in Wistar albino rats treated for 6 weeks did not cause any toxicity at 2%, but caused toxicity at 10 % (w/w) (Adam , 1999) .

Lepidium sativum showed slight toxic effect on liver parenchyma represented by focal necrosis , hepatocytes , apoptosis and inflammatory cell infiltrate the portal areas. These changes may be due to isothiocyanates reported to be one of *L.sativum* constituents (Burow et al., 2007). From a human health perspective , isothiocyanates are quite important because they are major inducers of carcinogendetoxifying enzymes with marked anti proliferative activity (O'Hare *et al.*,2007). Apoptosis seen in liver parenchymatous cells could be a part of anti-neoplastic effect of isothiocyanates constituent of *L.sativum* taken in high dose . *Lepidium sativum* seed extracts has proved hepato-protective effects against CC14 induced liver damage and the reason behind this effect is due to the presence of flavonoides , tannin , alkaloid , cuomarine and triterpenes which induce antioxidant effect and a decrease in free radical formation from CC14 , which is the main trigger of hepatotoxicity (Sheel and Nidhi ., 2011 and Wadhwal *et al.*, 2012) .

Despite the therapeutic uses of the plants in traditional medicine , their toxicity for vital organs have not been fully evaluated .

It is clear from the results of the present investigation that the liver and kidneys are the sensitive organs to the toxic action of the active constituents of the plant products utilized .

Although , there were no significant changes observed in the plasma biochemical parameters measured ,there were an increase in the values of ALP activity and also decreases in the total protein which in some times accompanied with decreases in the albumin concentration . These results in concomitant with the histopathological findings in the intestines which might explain interference of the plant constituents on the process of absorption and the hepatobiliary functions . Morag (2002) mentioned that intestinal dysfunction is accompanied by an increase in non-specific ALP and reduction in the total protein and albumin values . The same author also mentioned that ALT increased when hepatic parenchyma was involved specially in monogastric animals .

The renal histopathological changes which consisted of disappearance and / or lobulation of some glomeruli , fragmentation of glomerular basement membrane and the vacuolation and dilation of some renal tubules with the slight increase in the urea concentration and slight decrease in potassium concentration indicate that plant constituents had a nephropathy consequence . The presence of hemosidrin deposits in the spleen might be due to the haemolysing effect of the phenolic compounds of the plants specially tannins .

The mechanism were by these plants constituents injured the body tissues cannot be elucidated from the present studies but the injury to these organs probably contributed to the fluctuating serum AST , ALT and ALP activity , cholesterol , urea and albumin concentrations .

The decrease in the Hemoglobin concentration and in the RBCs count observed in the present study indicated anemia which might be due to interference in the mechanism of the hematopoiesis , hemolysis of RBCs and / or intestinal malabsorption .

The decreases in the values of the WBCs count observed in the present study might be attributed to the anti-inflammatory effects of some plants constituents (Sawadogo *et al.*, 2006), while the increase in the WBCs count attributed to the inflammatory responses of the vital organs towards plants constituents .

CONCLUSION :

In the current study , ethanolic extracts of *Aerva javanica* , *Amaranthus viridis* and *Lepidium sativum* posses appreciable anti-inflammtory effects depending on the dose size and time after dosing .

The crude ethanolic extract of *Aerva javanica* blocked the contraction of the smooth muscle of rabbit jejunum strip completely by atropine .

The crude ethanolic extract of *Amaranthus viridis* and *Lepidium sativum* blocked the contraction of the smooth muscle of rabbit jejunum strip completely by cyproheptidine .

Entero-hepatorenal changes is a sequel of the plants ethanolic extracts administered specially at the dose of 500 mg/kg for 4 weeks .

Recommendations :

The use of the crude ethanolic extracts as an anti-inflammatory preparation , specially *Aerva javanica* .

The use of the plants as a remedy for the different ailments for short periods beyond 14 days .

Further studies are needed to investigate the phytochemical/s responsible for the anti-inflammatory effect and further studies to determine plants constituents responsible for the toxico or immunopharmacological effect in different animal species .