Inhibition of Lipidperoxidation and Induction of Superoxide Scavenging Activity by Cynodon Dactylon In CCl_4 Induced Mice

BY

S. Jayashree

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN (DEEMED UNIVERSITY) COIMBATORE-641 043, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIO-CHEMISTRY

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Certified as Bonafide Research Work

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Signature of the Head of the Department	Signature of the Dean	Signature of the Guide

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Introduction

INTRODUCTION

Cellular hemeostasis is dependent on structural and functional integrity of cell membrane lipid bilayer. The processes that damage the membrane can interfere with signal transduction, molecular recognition and transport, maintenance of cellular metabolism and thus have the potential to initiate diseases (DIX, and Aiekens, 1993). Lipid peroxidation the undesired oxidative modification of polyunsaturated fattyacyl chain is a major contributor to membrane damage in cells and has been implicated as a cause and effect of an extraordinary range of pathological process associated with oxygen toxicity (Uchida, **etal** 1990).

In particular lipid peroxidation has been repeatedly invoked as a possible mechanism of inflammation (Das , 1993), Postischemic reperfusion (Bulkley, 1987), atherosclerosis, ethanol toxicity and cancer (Minotti, 1993).

These diseases represent only a few examples of toxicities and a complete list of lipid peroxidation dependent diseases would be probably endless. It may represent a series of non specific consequences.

Many chemical carcinogens are shown to be metabolically converted to free radical intermedates such as

super oxide anion (O_2^-), hydrogen peroxide (H_2O_2) hydroxyl radicals($OH^\cdot$), hydroperoxide(ROOH), peroxide($ROO^\cdot$) and alkoxy-radical ($RO^\cdot$) and are activated by lipid peroxidation. Thus a close relationship between free radical activity and malignancy has been well documented (Krishnamurthy and Jaya, 1986) (Masui and Kojima, 1990).

One such carcinogen which are converted to free radical(peroxyl) is carbon tetra chloride (CCl_4). This exemplifies mainly liver necrosis, then, to nodular hyperplasia and finally to cancer (Grasso, 1987).

It is pertinent to point out that inhibition of lipid peroxidation may be achieved by several antioxidants. Probably antioxidative vitamins may serve as scavengers of free radicals and thus protecting from free radical toxicity. Most important essential antioxidants are vitamin A, C and E, carotenoids and selenium. Invivo alpha tocopherol is a major chain breaking antioxidant, breaking lipid peroxidation process in membranes or LDL particles, where as ascorbate regenerates the oxidized tocopherol (Stegmayer, 1993).

The most common intermediate of oxygen metabolism is superoxide ($O_2^\cdot$). To protect cells from oxygen toxicity an oxygen defense system is present in all aerobic cells. Superoxide dismutase plays a central role in rapid dismutation of O_2 radical and hydroperoxide to hydrogen peroxide which is

subsequently converted by catalase and peroxidase to water (Lakhotia, 1994).

In addition to these antioxidants, natural products have been actively pursed as potential anticancer agents and therefore treated as antioxidant of lipid peroxidation.

India has the unique advantage of possessing a wide range of rare and precious herbs. This mainly covers tulsi, garlic and tretinoin, which have selective medicinal properties in treatment of cough, cardiac disease and Leukemia (Venkatacharya , 1994).

Curcumin the main colouring compound of turmeric exerts its effect in inhibiting of lipid peroxidation (Soudamini, et al., 1992).

The previous studies in our laboratory have shown significant influence on non enzymic antioxidants, xenobiotic phase I and phase II enzyme levels in Swiss albino mice by medicinal plants such as manathakkali, vallarai, tulsi, arugampul (Murthy et al., 1993).

Therefore the effect of different extracts of CYNODONDACTYLON leaves traditionally called as arugampul on inhibition of lipid peroxidation caused by chemical carcinogen CCl_4, on different tissues in Swiss albino mice and induction of superoxide scavenging activity were carried out

in the present investigation. Antioxidant enzyme levels such as catalase and superoxide dismutase were also evaluated.

Review of Literature

REVIEW OF LITERATURE

The review of literature pertaining to the present study " Inhibition of lipid per-oxidation and induction of Superoxide scavenging activity by CYNODONDACTYLON in CCl_4 induced mice are discussed under the following headings.

1.1 Introduction.

2.0 Mechanism of lipid peroxidation.

2.1 Initiation.

2.2 Propagation.

2.3 Termination.

3.0 Pathological basis of lipid peroxidation.

3.1 Generation of Free radicals.

3.2 Free radicals and tissue injury.

3.3 Oxidants that initiate lipid peroxidation.

4.0 Initiation of lipid peroxidation.

4.1 Mechanism of action of antioxidants.

4.2 Role of radical scavengers as antioxidant

4.3 Role of antioxidant enzymes.

4.4 Superoxide scavenging activity.

1.0 INTRODUCTION

The occurence of free radical induced lipid peroxidation had been associated with a number of pathological phenomenon such as decreased integrity of cell, decreased

cellular deformability, reduced erythrocyte survival and lipid fluidity (Mandal and Chatterjee, 1980).

Certain environmental carcinogens are converted to their active free redicals by lipid peroxidation. These radicals mainly affect DNA in two ways. DNA strands break and oxidise the bases. Unrepaired damage is potentially capable of producing mutations.

To counteract diseases caused by free radicals mediated lipid peroxidation on pathological basis, the mechanism of lipid peroxidation and antioxidants are focussed in this review.

2.0 MECHANISM OF LIPID PEROXIDATION:

Lipid peroxidation proceeds by a classic chain reaction that includes three discrete phases of initiation, propagation and termination (Dix and Aikens 1993).

It was shown that the stages of lipid peroxidation were given first by Desassure (Gutteridge and Halliwell 1990).

2.1 INITIATION:

Initiation triggers lipid peroxidation, when an unspecified oxidant gives rise to an initiating lipid peroxyl radical (Lioo) by reaction with either a lipid (LH) or a pre-existing lipid hydro peroxide (Looh).

Central mechanistic consideration of lipid peroxidation both invitro and invivo is whether LooH are participating. On the basis of this type of initiation, phases of lipid peroxidation have been classified as LooH independant and LooH-dependant (Minotti and Aust, 1992).

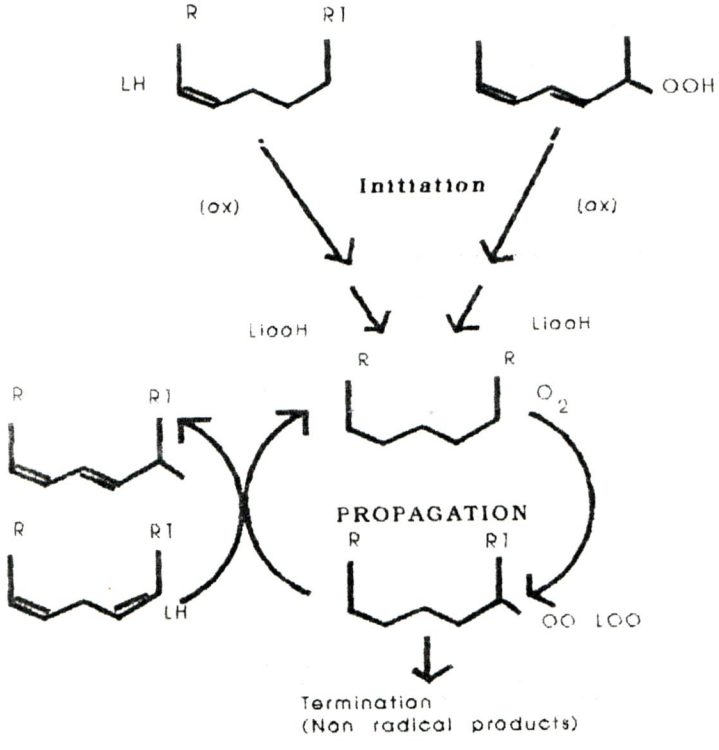
The former results from the introduction of an initiater with a lipid acyl chain, whereas latter form the interaction of an initiator with a pre-existing LooH (Slater, 1975).

2.2 PROPAGATION:

Lioo's enter the propagation phase by abstraction of the comparitively labile bis methylene hydrogen atom of a polyunsaturated Fatty acyl chain

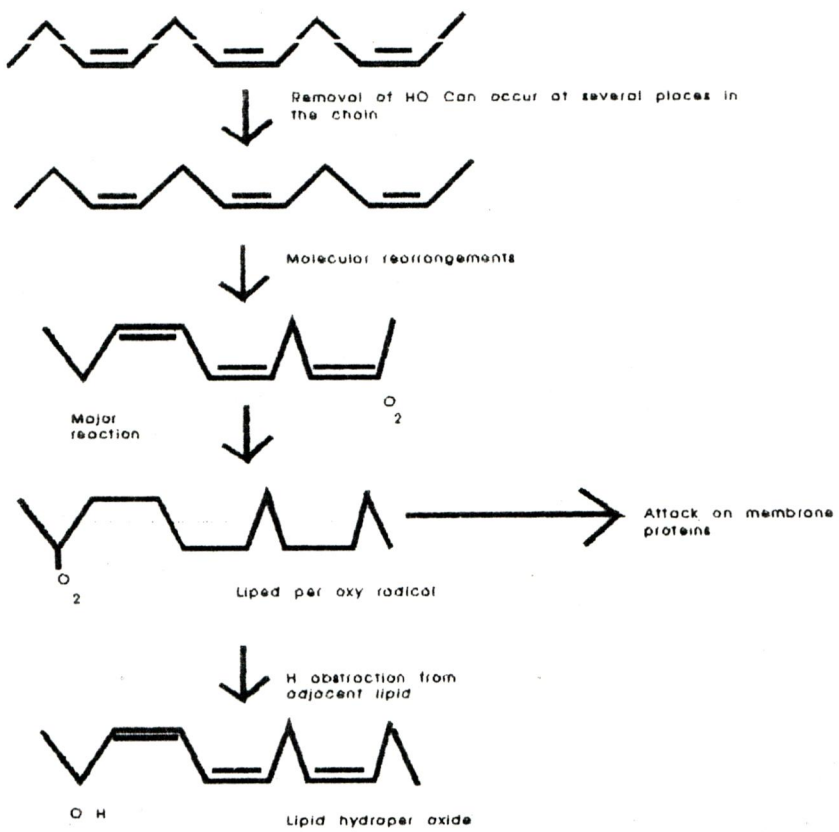
to yield a resonance stabilised pentadienyl radical (Porter et al., 1984). Diffusion controlled addition of oxygen generates a lipid peroxyl radical, the chain carrying species of lipid peroxidation. Propagation is cycled through rounds of LOO abstraction of bisallylic hydrogen atoms to generate new Loo's which results in net conversion of lipids to LooHs. Many propagation reactions can occur per each initiation (Porter et al 1984).

Diagram:



CHEMICAL TRANSFORMATION OF THREE PHASES OF LIPID PEROXIDATION

Diagram :



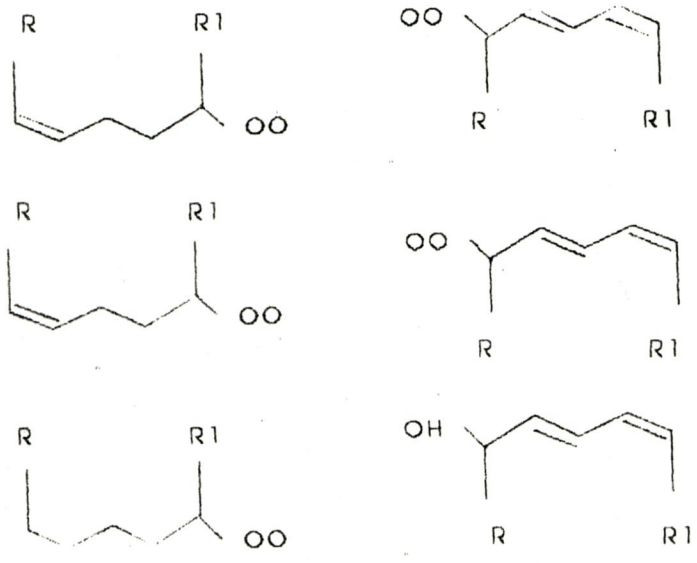
PROPAGATION

2.3 TERMINATION:

Lipid peroxidation termination involves the reaction of two LOO's to form non radical products or reaction of one LOO with another terminating radical to generate non propagating radical species which then self quench by various path ways (Russell, 1957).

Quenching of Loo's by antioxidants must be the primary mechanism of termination.

Diagram:



3.0 PATHOLOGICAL BASIS OF LIPID PEROXIDATION:

The net result of lipid peroxidation is the conversion of di and polyunsaturated lipidacyl chains to LooH and destabilization of hydrophobic membrane lipid bilayer integrity and function by the need to accomodate polar hydroperoxide groups (Jaya et al., 1993 and Richter, 1987).

In addition a number of toxic compounds are generated during lipid peroxidation and propagating LOO's can react with proteins, nucleic acids and endogenous or exogenous molecule and thus lipid peroxidation products are known to interact with biological material and cause cellular damage (Dhuley et al., 1993).

As a result of this lipid peroxidation have been implicated in the pathogenesis of a number of disease like atherosclerosis, myocardial infarction and emotional stress (Kumari et al., 1993).

There is also proof that oxygen radicals play a role in tumour promotion . These radicals able to damage DNA, show a mutagenic effect of elevated oxygen concentration. Some chemical carcinogens such as nitrosureas, nitroso guanidine or nitrogen mustard are highly reactive and can attack DNA directly (Slaga et al, 1984).

3.1 GENERATION OF FREE RADICALS:

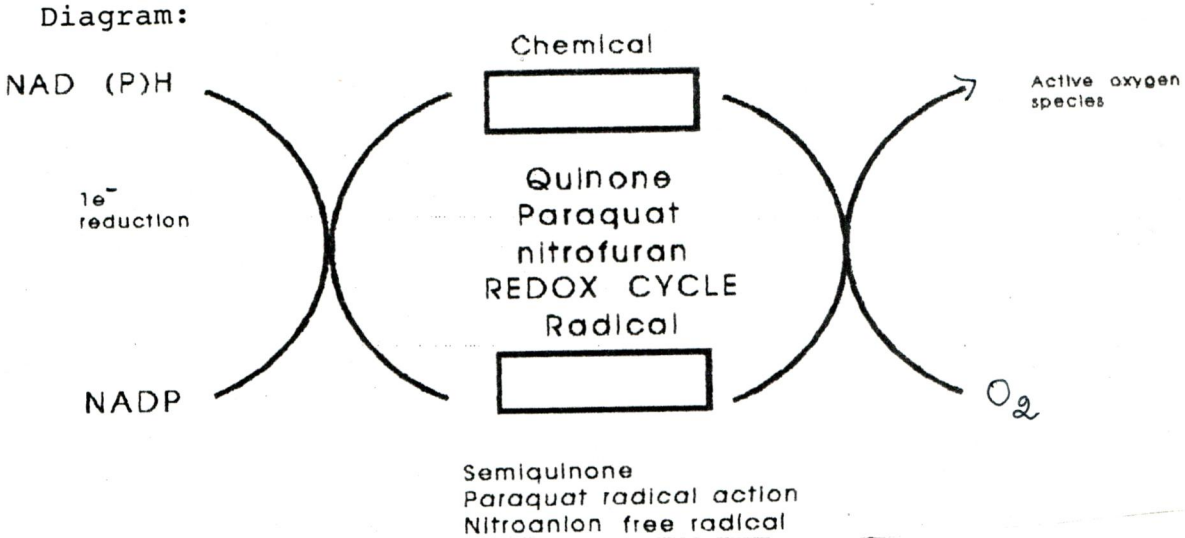
A free radical is defined as any atom or group of atoms or molecules in a particular state with one unpaired electron occupying an outer orbit NO, NO_2, O_2, O_2^- (superoxide) H_2O_2, OH, ROOH, ROO, RO are examples of free radicals.

The complete reduction of oxygen by the univalent pathway results in the formation of superoxide anion, H_2O_2

and OH radical as the intermediates. These intermediates are too reactive and hence are not tolerated by living tissues (Borek, 1986).

Free radicals can be generated mainly by two ways (a) Radiation (b) Redox reactions mostly calalyzed by transition metals or by enzyme catalysis (Slater, 1987).

Diagram:



compounds can undergo a one electron reduction to form radical species which then can react with oxygen forming superoxide (O_2^-) and regenerating the parent molecule. This process is called redox cycling (Cohen and Doerty, 1987).

Paraquat, CCl_4, chemotherapeutic drugs such as adriamycin and vincristine, nitrofurantoin, benzo(a)pyrene are helpful in more generation of free radicals (Das, 1993).

3.2 FREE RADICALS AND TISSUE INJURY:

One of the examples of free radical disturbances in relation to tissue injury is CCl_4. CCl_4 is metabolized mainly in the liver probably to a free radical product. These studies on metabolic activity opened up a new field of study in biochemical pathway (Patel et al., 1993).

Tissue injury (mainly Hepatotoxicity) due to CCl_4 has been shown to occur via cytochome P-450 mediated free radical generation and lipid peroxidation in rat liver cell which consequently results in destruction of membranes (Slater, 1987).

These reactive metabolites (free radicals) covalently bind to cellular macromolecules resulting in disruption of hepatocellular function ultimately leading to degeneration of liver cells. (Singh et al., 1992).

Thus the bisactivation of CCl_4 and the initiation of self propagating lipid peroxidation working in tandemm destroy the cellular membrane leading to liver cell death (Teufel et al., 1990).

CCl_4 is rapidly absorbed by all routes and is excreted by the lungs. Metabolism is mainly carried out by the mixed function oxidase systems. In addition to hepatotoxicity it is also a causative agent for cirrhosis and other dysfunction of liver.

3.3 OXIDANTS THAT INITIATE LIPID PEROXIDATION:

Mechanisms of various Oxidants that intiate lipid peroxidation are given under this section.

Superoxide radical (O_2) is capable of bis allylic hydrogen atom abstraction whereas perhydroxy radical(HOO) performs the abstraction like pulse radiolysis experiment with the help of xanthine oxidase. HOO can initiate lipid peroxidation in the absence of transition metals or other co-oxidants (Bielski & Gebick 1982, Forni, 1990; Britigan et al., 1990, Fridovich 1982)

Hydroxyl Radical

$OH^{\bullet}$ is usually assumed to be the initiating oxidant in the vast majority of lipidperoxidation studies both invitro and invivo. High energy irradiation of aqueous solution produces highly reactive hydroxyl radicals that can attack all biological molecules including membrane lipids and this attack can result in the initiation of lipid peroxidation (Buxton et al., 1988).

$OH^{\bullet}$ attacks where not the bis allylic hydrogen atom, rather, attack occurred at other locations along the acyl chain of bisunsaturated fattyacylchain (King et al., 1992).

Although OH-radicals are almost detectable in metal dependent peroxidation system, addition of OH-scavengers or of catalase, rarely inhibits the peroxidation observed (Boivin et al., 1990).

PEROXYLRADICAL

Peroxyl radicals initiate lipid peroxidation by bis allylic hydrogen atom and are formed in a number of metal catalyzed process and in the metabolic activation of CCl_4 which was an important link between free radicals and pathology (Poli et al., 1987) and Cooper, 1980)

4.0 INHIBITION OF LIPID PEROXIDATION :

Organisms capable of handling oxygen including all eukaryotes and many prokaryotes are steadily exposed to rective oxygen species (free radicals) which are caused by lipid peroxidation. This lipidperoxidation involves oxidative damage to proteins and DNA especially mitochondrial DNA which acts as a molecular basis for oxy radical mediated toxicity, carcinogenicity and ageing (Ernster, 1993).

To meet this challenge, aerobic organisms have developed a battery of protective mechanisms, collectively referred to as antioxidant defences.

In biochemical terms antioxidants can be divided into two categories:

(1) Antioxidant enzymes and (2) Antioxidant Compounds.

ANTIOXIDANT ENZYMES :

These are enzymes that can remove or prevent the formation of reactive oxygen species in a catalytic fashion.

ANTIOXIDANT COMPOUNDS :

(ie) Compounds of non protein nature, (eg) Ascorbic acid, tocopherols, carotenoids, glutathione and a large number of other chemicals of both biological and xenobiotic origin which can remove or prevent the formation of reactive oxygen species non enzymatically and have to be regenerated enzymimatically of non enzymatically in order to function in a catalytic fashion (Ernster , 1993).

4.1 MECHANISM OF ACTION OF ANTIOXIDANTS :

The antioxidants scavenge active radicals before the radicals attack lipids to inhibit the chain initiation and or chain carrying lipid peroxyl radicals to break the chain propagation, usually by electron transfer followed by deprotonation or by a direct hydrogen atom transfer in one step (Sies et al., 1992). (Numan et. al., 1990)

The potency and relative importance of radical scavenging antioxidant is determined by the rate of reaction with the radical and rate of radical derived from the antioxidant . They are dependent on physical factors such as location, concentration and mobility of antioxidants in a micro environment as well as their inherent chemical reactions towards free radicals (Simic et al, 1992)

4.2 ROLE OF RADICAL SCAVENGERS AS ANTIOXIDANT :

Vitamin C, E, ubiquinol and uric acid are known to act as an antioxidants by donating hydrogen atoms while carotenoids react with radicals by addition to double bond. These can function as hydrophilic oxygen radical scavengers. Vitamin E suppresses oxidation stress by scavenging chain carrying peroxylradical.

Ascorbate being the first line of defense against lipid peroxidation in plasma, greatly inhibits the formation of nitrosamines and prevents development or growth of tumours induced by chemical carcinogen (Frei, 1991).

Nordihydroguiaretic acid (NDGA) a plant lignin derived from harreadivaricate is a lipid soluble antioxidant and inhibition of lipooxygenase from a wide variety of sources (Nakayama et al., 1992).

4.3 ROLE OF ANTIOXIDANT ENZYMES :

Free radical mediated toxicity is moderated by enzymes such as superoxide dismutase, catalase, peroxidase, selenium dependent glutathione peroxidase (Gabryelak and Tawfek, 1991).

Evidence suggest that maintaining the activity of glutathione transferase, glutathlone peroxidase and reduce hydroperoxides can conceivably protect membranes from lipid peroxidation by eliminating hydro peroxide which further support lipid peroxidation.

Vitamin E is required to form hydroperoxides by reacting with peroxylradicals in glutathione dependent Vitamin E radical reductase (Nath, et al., 1993).

Catalases and peroxidases catalytically decompose hydrogen peroxide. Catalase also scavenges electrons besides hydroxyl radicals (Das, 1993).

Superoxide dismutase (SOD) inhibits the toxicity of superoxide radicals in biological system. SOD catalyes damage in cells which involve oxidation of SH groups to thyl radicals.

$$RSH + O_2^- \text{ ---- } RS + H_2O_2$$

This progress of radiation damage in cells may involve lipid peroxidation which in turn is inhibited by superoxide dismutase (Petkau, 1987, Nandhi and Chattergee 1988).

4.4 SUPEROXIDE SCAVENGING ACTIVITY :

O_2 is essential for aerobic life, but physiologists have known for years that exposure of aerobic organisms to concentrations of O_2 above those seen normally produced toxic effect.

The superoxide theory of oxygen toxicity states that the damaging effects of elevated oxygenase is due to an increase in the formation of superoxide radical (O_2) within cells, O_2 being the one electron reduction product of O_2 (Mc Cord et al., 1978).

This suppression of superoxide radicals are done mainly by certain scavengers, probably non enzymatic antioxidant and enzymatic antioxidant (Simic et al., 1992). Especially Vitamins and enzymes mainly superoxide dismutase is involved in this process (Palozza, et al., 1992).

Thus sources and chemical nature of various species of radicals and oxidants which perturb biological system is generally well understood. Additionally there is a growing body of evidence on the specific actions of these substances on the metabolic, regulator and genetic activities of

living organisms. It is clear that antioxidant and repair mechanisms must play an important role in protecting human populations against free radical and oxidant damage.

Anticarcinogenic and antimutagenic properties possessed by traditional plants such as manathakkali , arugampul, tulsi, vallari might prove to be effective agents in preventing carcinogenesis and mutagenesis in the experiments undertaken so far in our laboratory.

Thus an attempt is made to detect antioxidative mechanisms of arugampul in CCl_4 induced mice, thereby evaluating superoxide scavenging activity a contradictory mechanism of lipid peroxidation and levels of enzymes such as peroxidase, superoxide dismutase and catalase in this thesis.

Experimental Procedure

EXPERIMENTAL PROCEDURE

Experiments related to the present study,"Inhibition of lipid peroxidation and induction of superoxide scavenging activity by CYNODONDACTYLON in CCl_4 induced mice" were progressed under the following heads.

1. Collection and preparation of the extracts.
2. Selection and grouping of animals
3. Preparation of tissue homogenates
4. Biochmical evaluation of different extract treated tissue homogenates against CCl_4 induced mice on
   - 4.1 Lipid peroxidation
   - 4.2 Super oxide scavenging activity
   - 4.3 Protein content
   - 4.4 Catalase
   - 4.5 Peroxidase
   - 4.6 Superoxide dismutase
5. Histo pathological studies.

1. Collection and preparation of extracts :

To test the efficiency of CYNODONDACTYLON in lipid peroxidation only the leaves of CYNODONDACTLON which were supposed to be anticarcinogenic were taken as a drug in our experiment.

1.1 **PREPARATION OF WATER EXTRACT :**

10g of fresh leaves were washed and were dried by blotting. They were cut into small piece and homogenized in sterile water. The homogenate was stored in the dark at 4 ^{0}C for 72 hours with occasional shaking to facilitate complete extraction. After 72 hours, the contents were filtered through cotton guaze and freeze dried in a lyophilizer. The residue was weighed and used for the experiment. The residue was weighed and used for the experiment.

1.2 **ACID EXTRACT :**

It is well known that some mutagens are converted to their active mutagenic form in the stomach, under the acidic conditions. In the present study we also explored such a possibility by preparing an acid extract of the plants.

100g of washed plant leaves which was wiped dry was cut into small pieces and then homogenized using 0.1N hydrochloric acid (pH 1.8). The whole of the homogenate was carefully transferred into a flask and stored in dark at 4 four degrees for 72 hours with occasional shaking of the contents. After 72 hours, the contents were filtered through cotton guaze. The result-ant filterate was neutralized with 0.1N sterile sodium

bicarbonate and the extract was freeze dried in a lyophilizer. The residue was weighed and used for the experiments.

1.3 PREPARATION OF SALINE FRESH JUICE:

Two grams of CYNODONDACTYLON were cut into small pieces and minced with 100ml of 0.9% saline. This was squeezed and the juice obtained was used for the experiments .

1.4 PREPARATION OF FRESH JUICE:

Similar to the above method sterile water was used instead of 0.9 percent Saline.

2.0 SELECTION AND GROUPING OF ANIMALS:

7-8 weeks old swiss albino male mice weighing 24-26g purchased from Pasteur Institute of India, Coonoor were acclimatized for fifteen days before the start of the experiments. Throughout the experimental protocols the mice were fed the standard diet of boiled wheat flour containing milk and mineral supplements.

To find out the role of extracts on acute and chronic doses of CCl_4 (0.5ml/Kg bodyweight) the experiments were done in two phases. In each phase the animals were divided into 11 groups of 3 mice in each group.

The groupings were as follows.

Water control - (1), Saline control - (2), water extract - (3), acid extract - (4), Fresh Juice - (5), Saline Fresh - (6), CCl_4 - (7), CCl_4 + acid - (8), CCl_4 + water - (9), CCl_4 + Fresh juice - (10), CCl_4 + Saline Fresh juice, and the above were administered intraperitoneally and the animals were sacrificed after 24 hours in case of phase I and seven days in phase II.

3.0 PREPARATION OF TISSUE HOMOGENATE:

Lipid peroxidation level was estimated in various organs such as liver, kidney, spleen, lungs, heart and brain.

The organs were individually washed in normal saline blotted between a filter paper and weighed. A 20 percent homogenate (using a teflan homogenizer) was prepared and used for the assay.

One part of the liver was used for the lipid peroxidation by preparing a 20 percent homogenate in 0.2M Tris HCl buffer of pH 7.0. The other part of it was used for the assay of superoxide scavenging activity and various enzymes such as catalase, superoxide dismutase and peroxidase by preparing 20 percent homogenate

in 1.15 percent isotonic KCl using teflon homogenizer.

4 BIO-CHEMICAL EVALUATION OF DIFFERENT EXTRACT TREATED TISSUE HOMOGENATES AGAINST CCl_4 INDUCED MICE:

4 .1 Lipid peroxidation :

The levels of tissue peroxides were determined in the selected organs of the experimental animals by the method of Bisharyee et al., 1971. The details of this procedure is given in Appendix I.

4.2 Evaluation of superoxide scavenging activity:

The percentage inhibition of superoxide radical formation by CYNODONDACTYLON was determined in liver samples of mice (Winter Bourn et al., 1975). The detailed procedure is given in Appendix II.

4.3 Estimation of protein:

To express the enzyme activity in terms of mg protein, the protein content of the liver homogenates were estimated by the method of Lowry et al., (1951) as in Appendix III.

4.4 Assay of Catalase:

Catalase, one of the interrelated enzyme of lipid peroxidation was estimated by the method of Luck

(1974) as in Appendix IV.

4.5 Assay of Peroxidase:

Peroxidase activity was measured by the method of Addy and Goodman (1972) as given in Appendix V.

4.6 Estimation of superoxide dismutase (SOD) :

Superoxide dismutase a suppressing enzyme of superoxide radical was estimated in CCl_4 treated mice by the method of Misra and Fridovich (1972) as given in Appendix VI.

5.0 **HISTOPATHOLOGICAL STUDIES:**

After cervical dislocation of the mice, the livers from normal, extract treated, CCl_4 treated and CCl_4 and extract treated mice were removed for histopathological studies, inorder to reveal the chemopreventive effect of extracts and no toxic side effect of the extracts on liver also carried out by the method of Culling 1979 (Appendix VII).

Results and Discussion

RESULTS AND DISCUSSION

The present study "Inhibition of lipid peroxidation and induction of superoxide scavenging activity by CYNODONDACTYLON in CCl_4 induced mice" was undertaken to reveal the role of CYNODONDACTYLON extracts (arugampul) on lipid peroxidation in CCl_4 induced mice by inducing superoxide dismutase, catalase and by reducing the activity of peroxidase in different organs.

The lipid peroxidation effect was followed in the different organs (kidney, brain, liver, lungs, spleen, heart) of mice treated with CCl_4. The enzymes were assayed in the liver homogenates of 7 days CCl_4 treated mice. CCl_4 was selected because it is one of the good inducers of lipid peroxidation.

The results of the study are discussed as follows.

1. INHIBITION OF LIPID PEROXIDATION BY CYNODON DACTYLON EXTRACTS IN DIFFERENT ORGANS OF CCl_4 INDUCED MICE:-

Acute and chronic doses of CCl_4 significantly induced lipid peroxidation in all the organs studied. This induced lipid peroxidation was the highest in brain followed by Kidney, liver, heart, spleen and lungs. The lipid peroxide was expressed as nmoles of malonaldehyde per gram weight of tissue.

TABLE I

Inhibition of Lipid peroxidation by Saline Fresh Juice in ccl$_4$ Induced Mice

(Oneday)

Treatment	Lipid peroxidation (nmoles of malonaldehyde/g tissues)					
	Organs					
	Liver	Brain	Kidney	Heart	Lungs	Spleen
Normal	171.0000	243.3333	170.6667	122.0000	81.3333	80.0000
ccl$_4$	205.3333	336.0000	274.6667	196.6667	159.0000	169.0000
ccl$_4$ & Saline Fresh Juice	97.6667	204.0000	183.6667	122.3333	113.3333	107.0000
Saline Fresh Juice	106.6667	203.6667	177.0000	110.0000	79.0000	81.6667
S E D : 5.32421			C.D : 10.56848			

TABLE II

Inhibition of Lipid Peroxidation by Saline Fresh Juice in ccl_4 Induced Mice (seven day)

Treatment	Lipid Peroxidation (n moles of malonaldehyde /g tissue)					
	Organs					
	Liver	Brain	Kidney	Heart	Lungs	Spleen
Normal	171.0000	243.3333	170.6667	122.0000	81.3333	80.0000
ccl_4	360.0000	474.0000	347.3333	381.0000	280.6667	271.0000
ccl_4 + Saline Fresh Juice	202.0000	212.3333	202.0000	143.3333	99.0000	167.3333
Saline Fresh Juice	158.3333	232.3333	165.6667	119.0000	81.0000	76.0000

SED : 5.32421 CD : 10.56848

Effect of hepato protective ayurvedic drugs (Kumari assav, KumariKalp,. Arogyavardhini and Tamra) on lipid peroxidation in longterm CCl_4 induced rat reported by Patel et al., (1993) and inhibition of Lipid peroxidation by curcumin in the short term and longterm CCl_4, induced mice studied by Subramanian and Selvam (1993) made us, to select CYNODONDACTYLON (arugampul) for our work which was reported as antimutagenic and anticarcienogenic agent in our laboratory findings (Murthy, et al., 1993).

1.1 EFFECT OF INHIBITION OF LIPID PEROXIDATION BY SALINE FRESH JUICE in CCl_4 INDUCED MICE:-

Table I and Table II and figure I depict the effect of saline fresh juice on lipid peroxidation in one day and seven day CCl_4 treated mice. The modulatory influence of Saline Fresh Juice on different organs of CCl_4 induced mice in inhibiting lipid peroxidation was found to be significant at 5 percent level.

The lipid peroxidation of liver in both longterm (7 day) and short term (one day) treatment was augmented mainly by Saline Fresh Juice. The percentage of inhibition was found to be 52.6 and 43.8 in one day and seven day treated liver homogenates respectively.

The inhibition in all the other organs due to Saline Fresh Juice was marked when compared to other extracts. The inhibitory percentage in the brain, heart, spleen, kidney and lungs of one day treated mice showed values of 39,37,36.6,33 and 28.7 respectively.

Heart and lungs depicted maximum inhibition in seven day treated mice and the percentage was 62.3-65.

Lipid peroxide Formation in brain was counteracted mainly by saline Fresh juice when compared to other extracts. Percent inhibition in the brain of one day treated mice gave a value of 39 percent and for seven day treated mice it showed a value of 55.2 percent.

The range of inhibition in kidney, spleen and liver were found to be 38-43 percent in seven day treated animals.

1.2 INHIBITION OF LIPID PEROXIDATION BY FRESH JUICE IN CCl_4 INDUCED MICE :-

The results obtained in Fresh Juice as given in tables III and IV and in figure II.

The significant inhibition of Lipid peroxidation by Fresh Juice in the organs of 7 day treated mice was significantly higher than that of one day treated mice.

TABLE III

Inhibition of Lipid Peroxidation by Fresh Juice in ccl_4 Induced Mice (one day)

Treatment	Lipid Peroxidation (n moles of malonaldehyde /g tissue)					
	Organs					
	Liver	Brain	Kidney	Heart	Lungs	Spleen
Normal	171.0000	243.3333	170.6667	122.0000	81.3333	80.0000
ccl_4	205.3333	336.0000	274.6667	196.6667	159.0000	169.0000
ccl_4 + Fresh Juice	173.0000	221.6667	211.6667	121.0000	94.3333	86.6667
Fresh Juice	142.3333	189.6667	169.0000	104.3333	82.0000	86.0000

S E D : 2.99266

C D : 5.94039

TABLE IV

Inhibition of Lipid Peroxidation by Fresh Juice in ccl_4 Induced Mice (seven day)

Treatment	Lipid Peroxidation (n moles of malonaldehyde /g tissues)					
			Organs			
	Liver	Brain	Kidney	Heart	Lungs	Spleen
Normal	171.0000	243.3333	170.6667	122.0000	81.3333	80.0000
ccl_4	360.0000	474.0000	380.6667	381.0000	280.6667	271.0000
ccl_4 + Fresh Juice	330.0000	280.0000	301.6667	263.3333	141.6667	112.3333
Fresh Juice	161.6667	222.6667	140.0000	123.0000	91.3333	74.3333

S E D : 2.99266 C.D : 5.94039

Both in acute and chronic doses of CCl_4 spleen has got its highest inhibitory effect. The percentage may be 48.7 and 58.7.

One of the distinct features of Fresh Juice was its marked inhibitory effect on brain of seven day treated mice (40.9 percent inhibition). This property is not performed by any other extracts other than Saline Fresh Juice.

The maximum inhibition was noticed in the heart and lungs and the minimum inhibition in the case of Kidney and liver.

1.3 INHIBITION OF LIPID PEROXIDATION BY WATER EXTRACT IN CCl_4 INDUCED MICE :-

CCl_4 + Water extract showed a significant inhibition of lipid peroxidation at 5 percent level when compared to tissue homogenates of mice treated with CCl_4 alone.

In the short term assay, the order of inhibitory effect of water extract was as follows. Spleen (53.6 percent), heart (46.6 percent), Kidney (38.1 percent), brain (36 percent), lungs and liver (26 percent).

TABLE VI

Inhibition of Lipid Peroxidation by Water extract in ccl_4 Induced Mice (seven day)

Treatment	Lipid Peroxidation (n moles of malonaldehyde /g tissue)					
	Organs					
	Liver	Brain	Kidney	Heart	Lungs	Spleen
Normal	170.6667	81.3333	170.6667	122.0000	81.3333	80.0000
ccl_4	360.0000	474.0000	380.6667	381.0000	280.6667	271.0000
ccl_4 + Water extract	173.3333	303.3333	151.6667	244.6667	238.0000	117.3333
Water extract	96.0000	165.6667	106.0000	244.6667	89.0000	78.3333

S E D : 8.65223 C D : 17.17455

As per the table V and VI the efficiency of water extract in the liver of 7 day treated mice (51.85 percent) more than that of one day treated mice.

Maximum efficiency was seen in kidney (60.2 percent) followed by spleen (56.7 percent). In addition, water extract showed a better inhibition in brain and heart.

Finally it can be concluded that both in short and long term treatments water extract showed its inhibitory effect in all the organs.

1.4 INHIBITION OF LIPID PEROXIDATION BY ACID EXTRACT IN CCl_4 INDUCED MICE :-

Acid extract counteracted lipid peroxidation more in liver of both one day (42.4 percent) and seven day (51.8 percent) treated mice. Inhibition in brain of seven day treated was negligible but the brain of one day treated mice gave a value of 33.3 percent which was significant at 5 percent level.

The order of inhibition of lipid peroxidation by acid extract according to the data shown in tables VII and VIII was as follows: spleen > kidney > lungs > liver > Brain > Heart in case of acute inhibition and heart > liver > lungs > kidney > spleen > Brain in

TABLE VII

Inhibition of Lipid Peroxidation by Acid extract in ccl_4 Induced Mice (one day)

Treatment	Lipid Peroxidation (n moles of malonaldehyde /g tissues)					
	Organs					
	Liver	Brain	Kidney	Heart	Lungs	Spleen
Normal	171.0000	243.3333	170.6667	122.0000	81.3333	80.0000
ccl_4	205.3333	336.0000	274.6667	196.6667	159.0000	169.0000
ccl_4 + Acid Extract	118.3333	224.0000	153.3333	149.0000	90.0000	82.3333
Acid Extract	115.0000	179.3333	146.0000	109.6667	96.3333	75.3333

S E D : 5.02701 C.D : 9.97854

TABLE VIII

Inhibition of Lipid Peroxidation by Acid extract in ccl_4 Induced Mice (seven day)

Treatment	Lipid Peroxidation (n moles of malonaldehyde /g tissue)					
	Organs					
	Liver	Brain	Kidney	Heart	Lungs	Spleen
Normal	171.0000	243.3333	170.6667	122.0000	81.3333	80.0000
ccl_4	360.0000	474.0000	380.6667	381.0000	280.6667	271.0000
ccl_4 + Acid extract	173.3333	366.0000	241.0000	125.0000	150.6667	183.3333
Acid Extract	96.0000	216.0000	139.0000	117.6667	105.6667	114.3333

S E D : 5.02701 C.D : 9.97854

chronic inhibition.

1.5 COMPARISION OF EFFECT OF VARIOUS EXTRACTS OF CYNODONDACTYLON ON LIPID PEROXIDATION:-

The overall comparison of effect of lipid peroxidation by various extracts of CYNODONDACDYLON such as Saline Fresh Juice, Fresh Juice, Water extract, acid extract in CCl_4 treated organ system both in short term (One day) and long term (7 day) study is highlighted in Table IX. and In figures 1, 2, 3, 4, 5 and 6 and 7.

It can be clearly understood from the table that saline fresh juice counteracts lipid peroxidation more than water and acid extracts. Fresh juice has got less influence in augmenting lipid peroxidation than water and acid extracts. Equal influence was exhibited by both acid and water extracts.

The mean of percentage inhibition of Saline Fresh Juice, water extract, acid extract, and fresh juice were 194.5, 171.72, 171.36 and 154.5 respectively.

Individual administration of extract recorded significant (5 percent level) counteraction of lipid peroxidation.

TABLE IX

Overall comparison of Inhibitory effect of Lipid peroxidation due to various extracts of yarododactylon

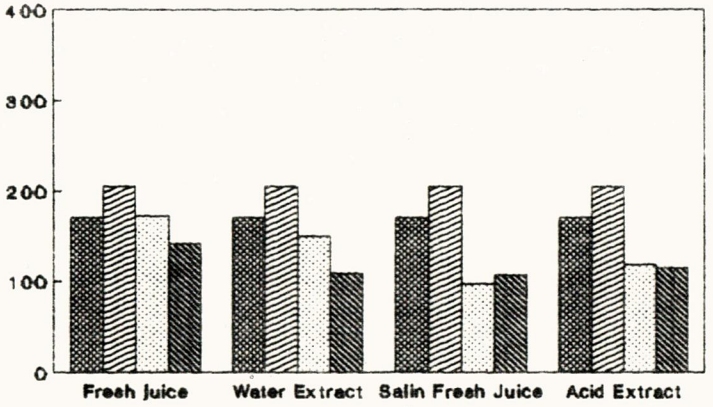
Treatment	Lipid peroxidation (n moles of malonaldehyde/g tissue)					
Extract	Normal	ccl_4	ccl_4 +Extract	Extract	S.E.D	C.D
Water extract Day 1	144.7222	223.4444	138.7222	127.0556	8.65223	17.17455
Day 7	144.7222	357.8889	204.7222	129.9444		
Acid Extract Day 1	144.7222	223.4444	136.1667	120.2778	5.02701	9.97854
Day 7	144.7222	357.8889	206.555	131.4444		
Saline Fresh Juice Day 1	144.7222	223.4444	138.0000	126.3333	5.32421	10.56848
Day 7	144.7222	357.8889	171.0000	138.7222		
Fresh Juice Day 1	144.7222	223.4444	151.3889	128.8889		
Day 7	144.7222	357.8889	238.1667	135.5000	2.99266	5.94039

nonamoles of malonaldehyde/gram weight of the tissue.

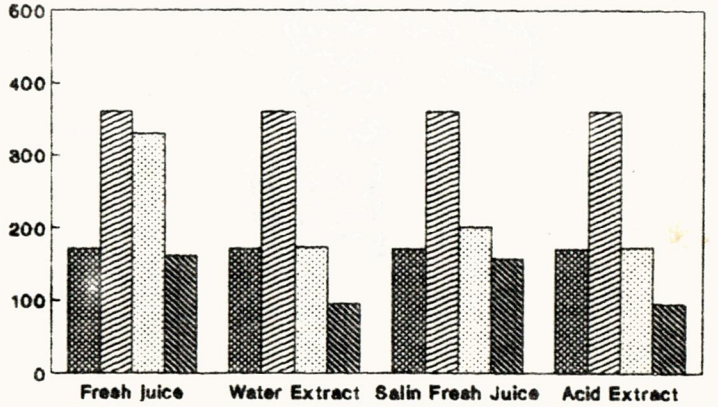
Fig 1(a)

INHIBITION OF LIPID PEROXIDATION IN LIVER

Fig 1(b)



ONE DAY



SEVEN DAY

INHIBITION OF LIPID PEROXIDATION IN BRAIN

Fig 2(a)

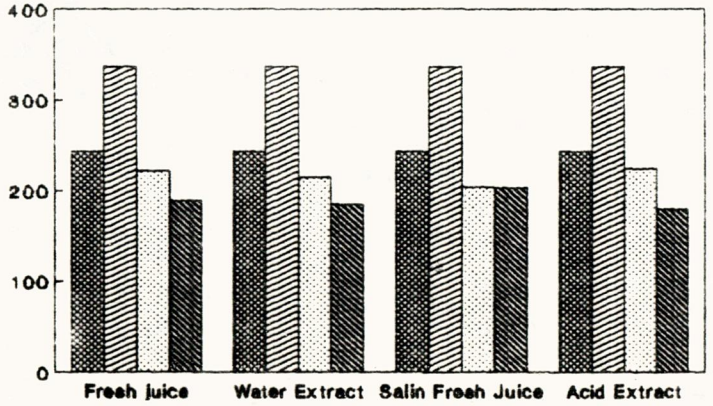
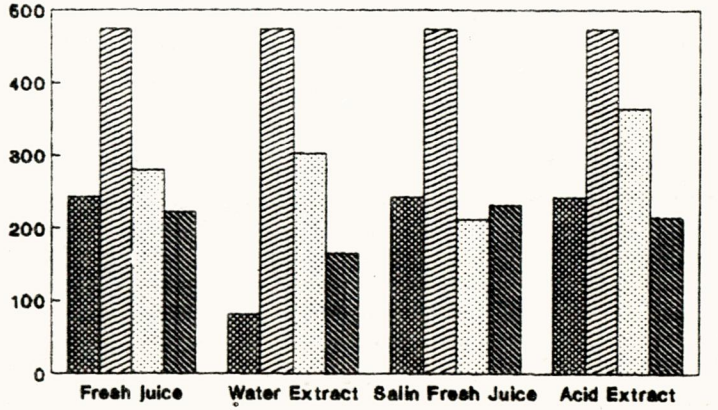


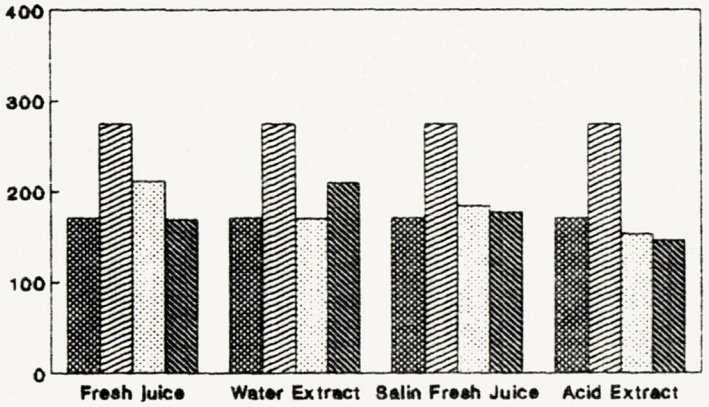
Fig 2(b)



Normal CCl_4 CCl_4 + extract extract

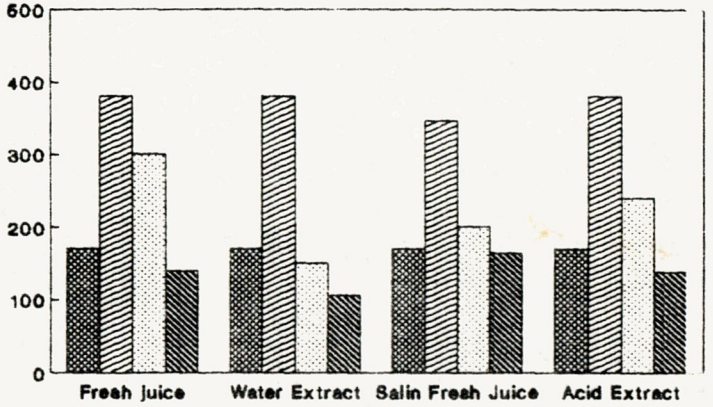
INHIBITION OF LIPID PEROXIDATION IN KIDNEY

Fig 3(a)



One day

Fig 3(b)



Seven day

nonamoles of malonaldehyde/gm wt of the tissue.

INHIBITION OF LIPID PEROXIDATION IN HEART

Fig 4(a)

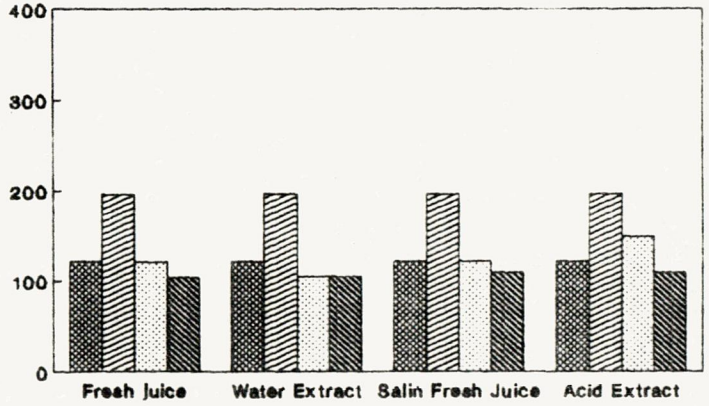
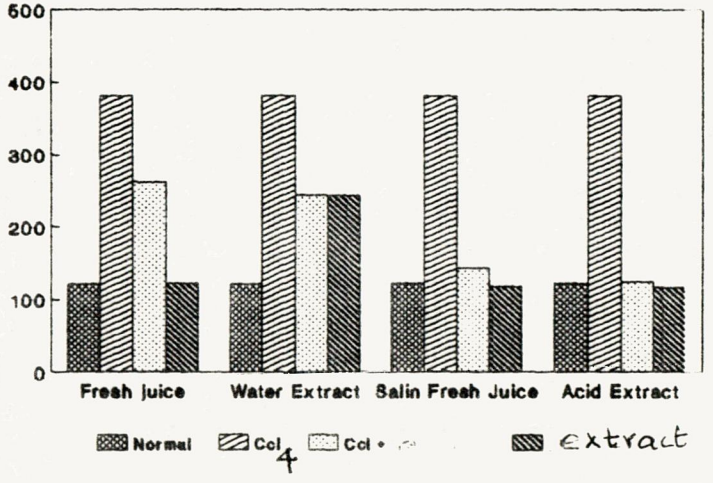


Fig 4(b)



4

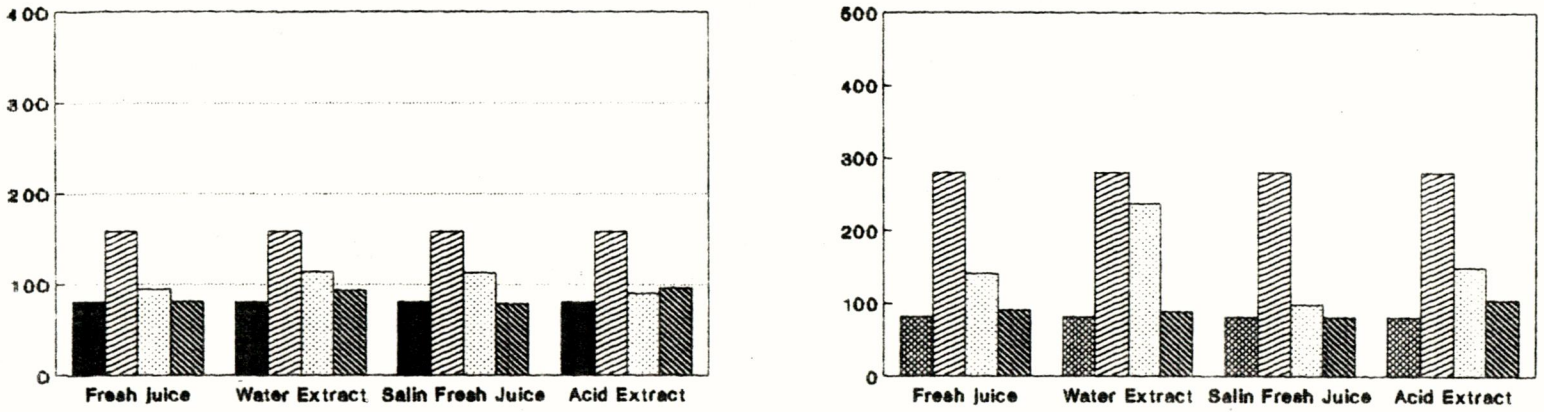
43

nonamoles of malonaldehyde/gram weight of the tissue.

Fig 5(a)

INHIBITION OF LIPID PEROXIDATION IN LUNGS

Fig 5(b)



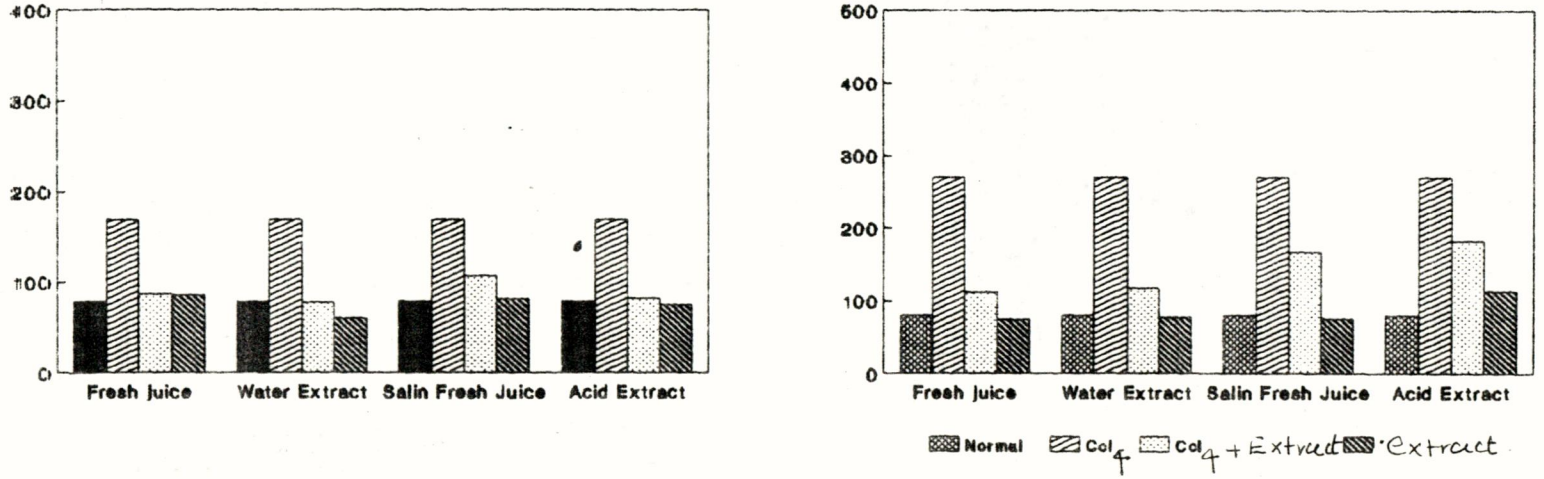
ONE DAY

SEVEN DAY

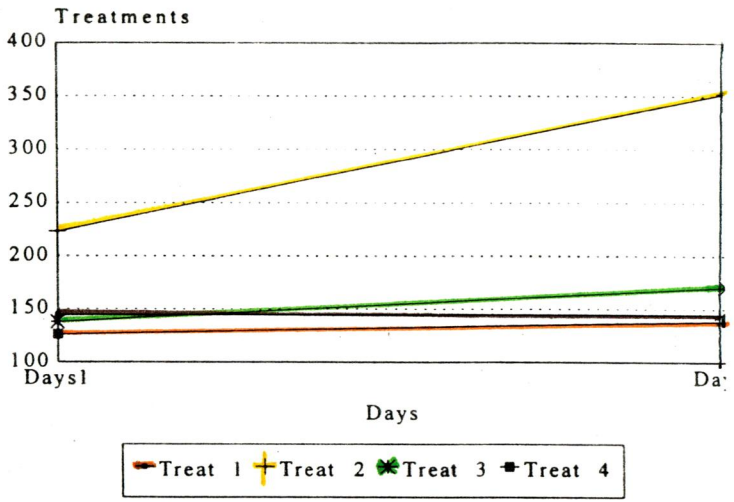
INHIBITION OF LIPID PEROXIDATION IN SPLEEN

Fig 6(a)

Fig 6(b)

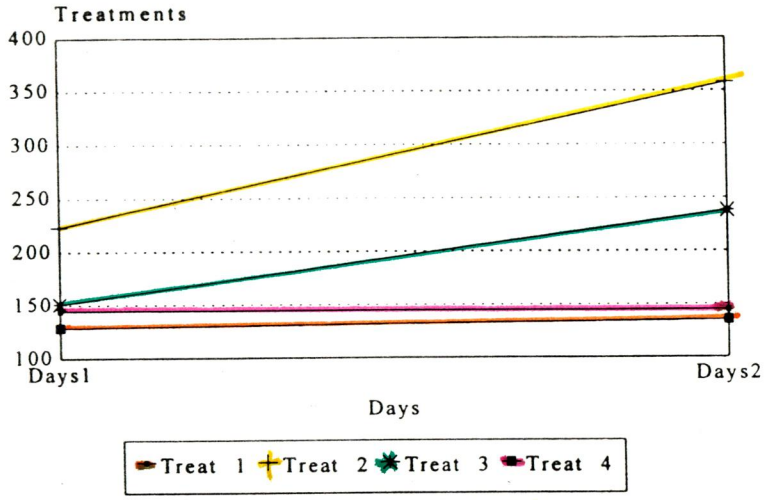


Fig(a) Saline fresh



Treat1- Normal Treat2- ccl4 Treat3-ccl4+saline
Treat4-Saline fresh

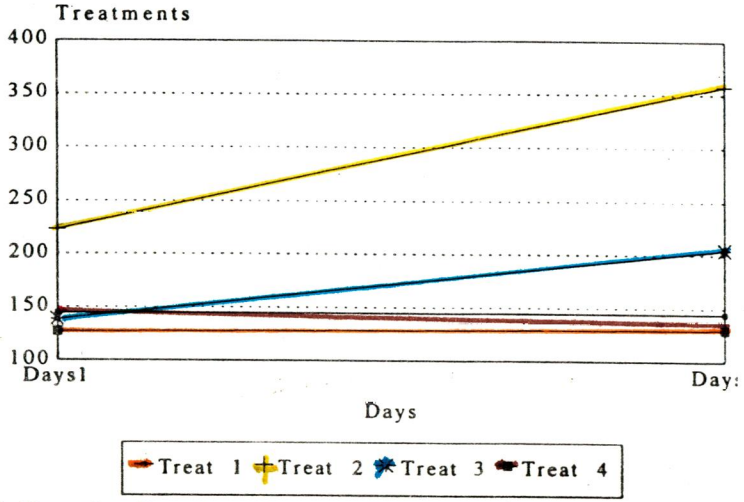
Fig.(b) Fresh juice



Treat1- Normal Treat2- ccl4 Treat3- ccl4+fresh juice
Treat4-fresh juice

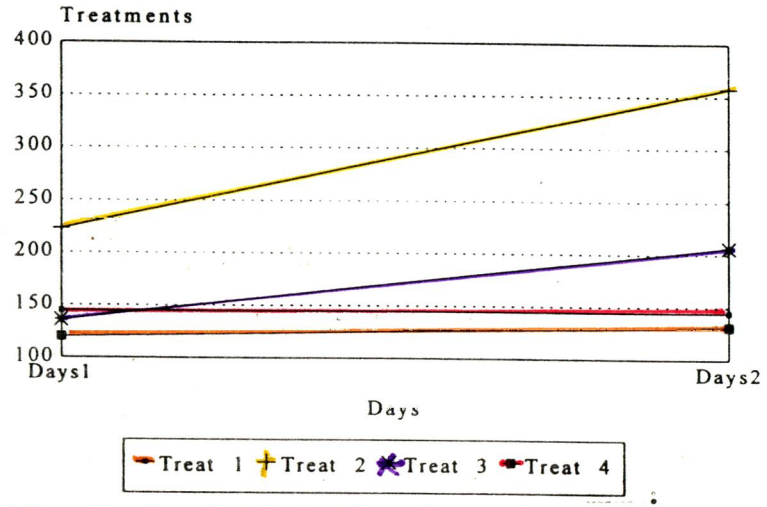
Fig(7) OVERALL INHIBITORY EFFECT OF LIPID PEROXIDATION BY VARIOUS EXTRACTS OF CYNODON DACTYLON.

Fig(c) Water extract



Treat1-Normal Treat2- ccl4 Treat3- ccl4+Water extract
Treat4- Water extract

Fig(d) Effect of acid extract



Treat1-Normal Treat2-ccl4 Treat3 -ccl4+ acid extract
Treat4-acid extract

Rao and Mehendale (1992) also studied the effect of food additives on the damage caused by toxic chemicals including CCl_4, Paraquat and aflatoxin.

2.0 SUPER OXIDE SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF CYNODONDACTYLON ON CCl_4 INDUCED MICE:

The percentage inhibition of superoxide scavenging activity is tabulated in table X.

Superoxide scavenging activity, the contradictive reaction of lipid peroxidation is able to prevent diseases caused by lipid peroxidation.

To support this, the effect of different extracts of cynodon dactylon on liver homogenates of mice induced with CCl_4 for seven days were evaluated for superoxide scavenging activity.

Saline Fresh Juice treated animals induced superoxide scavenging activity (66 percent) when compared to the other three extracts. Water extract (55 percent) and acid extract (48 percent) exhibited superoxide scavenging activity almost equally. Fresh Juice was also effective but not like the other three extracts.

Krishnakantha and Lokesh (1993) reported that spice principles and cuminaldehyde can scavenge superoxide

TABLE X

SUPEROXIDE SCAVENGING ACTIVITY (SOSA) OF
Different extracts of *Cynodondactylon* in CCl_4 induced mice

Treatment	Percentage Induction of SOSA
CCl_4 + Water extract	52.0
Water extract	54.0
CCl_4 + Acid Extract	49.0
Acid Extract	51.0
CCl_4 + fresh juice	32.0
fresh juice	33.0
CCl_4 + saline fresh juice	66.6
saline fresh juice	60.0

radicals.

Research work done by Wood et al., (1982) showed that the superoxide scavenging capacity mainly due to antioxidant properties in trapping the proximate carcinogens that are formed as epoxides during enzymatic activation.

In addition Zhao et al., (1989) studied the effect of green tea extracts on active oxygen species using a spin trapping method.

3.0 ANTIPEROXIDATIVE ENZYMATIC STATUS OF EXTRACTS OF CYNODONDACTYLON :-

To prove the role of antioxidative enzymes on the observed induction by CCl_4 and inhibition of lipid peroxidation by different extracts of CYNODONDACTYLON the biological evaluation of liver homogenate of seven day treated mice (CCl_4 , CCl_4 & different extracts and extracts alone) was carried out.

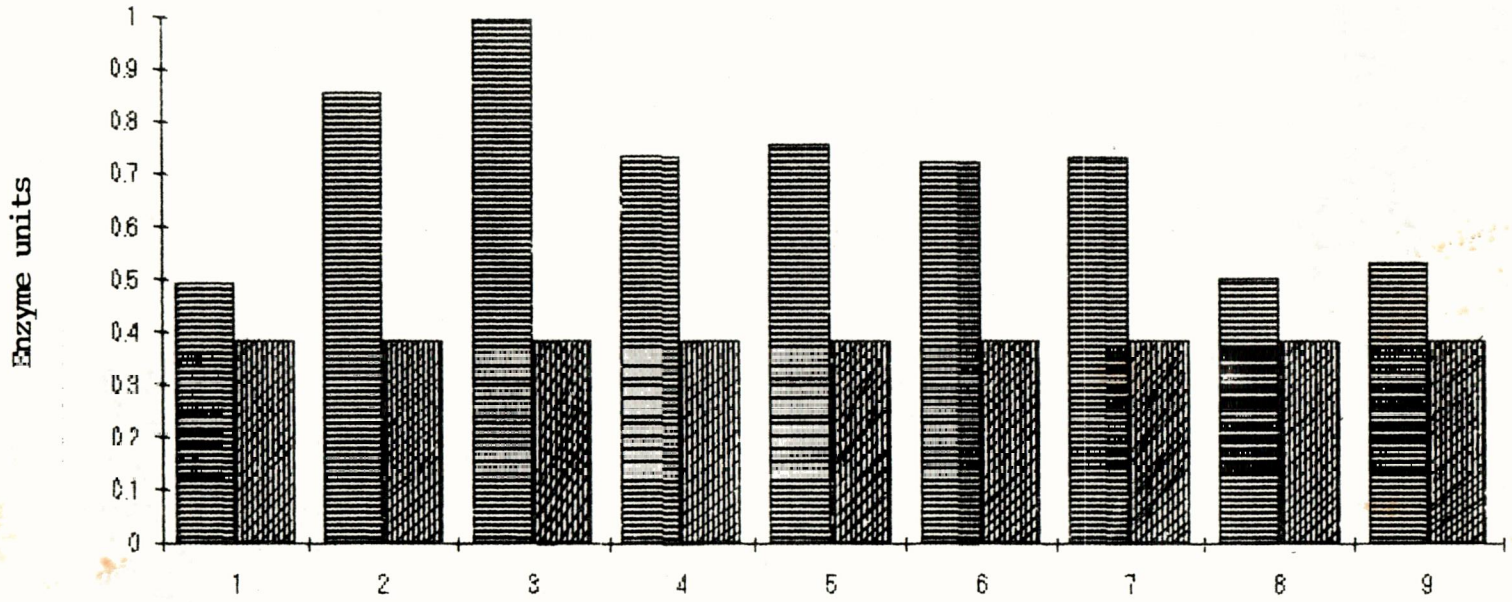
Lipid peroxidation increasingly registered a marked depletion of antiperoxidative enzymes such as catalase and superoxide dismutase and concomitant increase in peroxidase in CCl_4 fed mice compared to controls.

TABLE XI

Effect of the various extracts of CYNODON DACTYLON in the liver of ccl_4 induced mice on Antiperoxidative enzymatic status

Treatment	Superoxide Dis utase (SOD) Enzyme unit/mg of proten	Catalase enzyme unit/mg of protein * 10^{-3}
Normal	0.4900	62.48
ccl_4	0.3835	45.48
ccl_4 + SALINE FRESH JUICE	0.8535	70.88
SALINE FRESH Juice	0.9939	90.48
ccl_4 + Water extract	0.7333	71.88
Water extract	0.7555	87.88
ccl_4 + Acid extract	0.7200	71.80
Acid extract	0.7300	85.10
ccl_4 + Fresh Juice	0.5000	50.48
Fresh Juice	0.5300	82.48
SED = 0.0080	CD = 0.0167	SED = 1.1636 CD = 2.6833

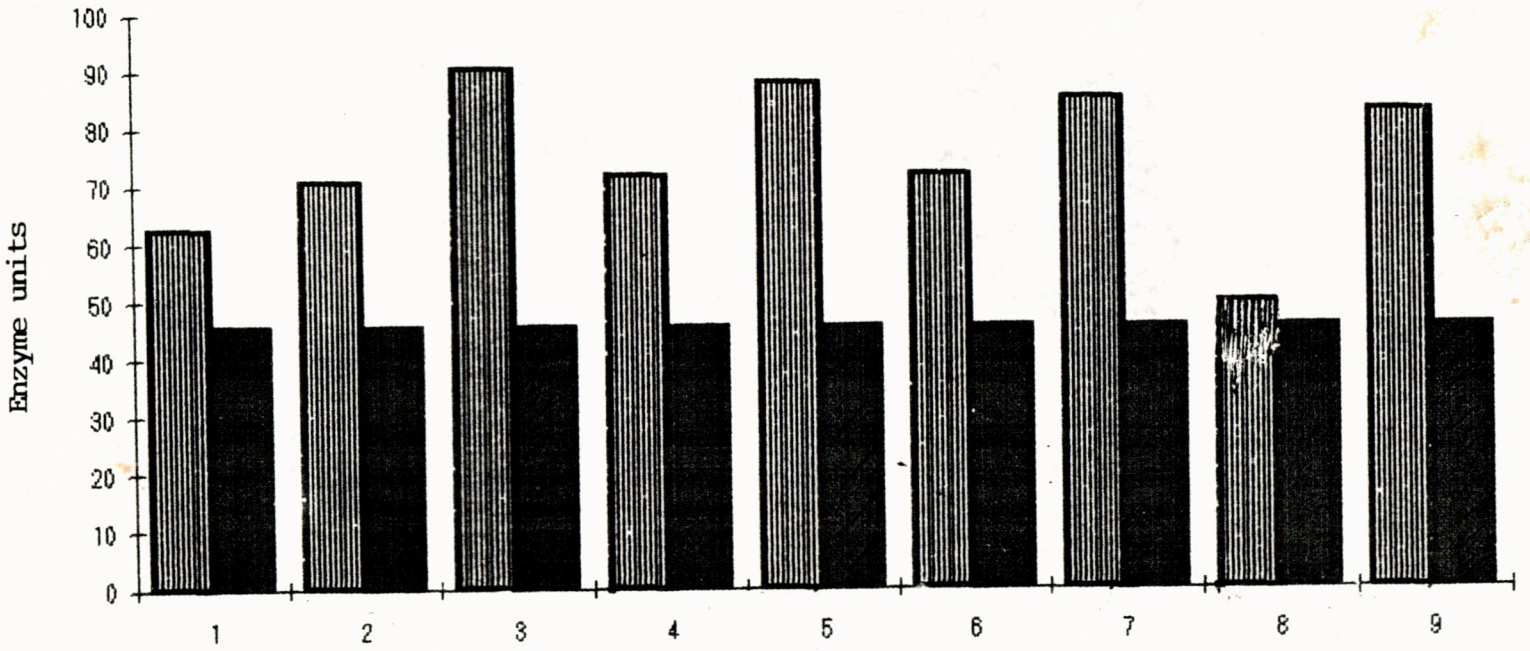
Fig. 8 **Evaluation of antiperoxidative enzyme levels (Superoxide dismutase)**



T_1 - Normal T_2 - CCl_4 + Saline fresh T_3 - Saline fresh T_4 - Water extract + CCl_4 T_5 - Water extract T_6 - CCl_4 Acid extract T_7 - Acid extract T_8 - CCl_4 + Fresh juice T_9 - Fresh juice

T - Treatment

Fig.9.Evaluation of antiperoxidative enzyme levels (catalase)



T_1 - Normal T_2 - CCl_4 + Saline fresh T_3 - Saline fresh T_4 - Water extract + CCl_4 T_5 - Water extract T_6 - CCl_4 Acid extract T_7 - Acid extract T_8 - CCl_4 _ Fresh juice T_9 - Fresh juice

T = Treatment

Intraperitoneal administration of different extracts of CYNODONDACTYLON along with CCl_4 could effectively prevent lipid peroxidation and hepatic injury.

The protective actions of superoxide dismutase and catalase complemented each other in a seqential fashion in CCl_4 induced lipid peroxidation. The activity of superoxide dismutase and catalase in the liver decreased in CCl_4 treated mice.

In the case of extracts administered mice along with CCl, the order of increment in antiperoxidative enzyme levels are as follows.

Fresh Juice < acid extract < water exctract < saline fresh juice as shown in Table XI and in Figures VIII & IX and the values are significant at 5 percent level.

Subramaniyam and Selvam (1992) also reported decreased activity of superoxide dismutase and catalase in CCl induced rats for 7 days.

Induction of antiperoxidative enzymes such as superoxide dismutase and catalase in alcohol induced rats is also reported by Jaya et al., 1993 and Sharma 1993)

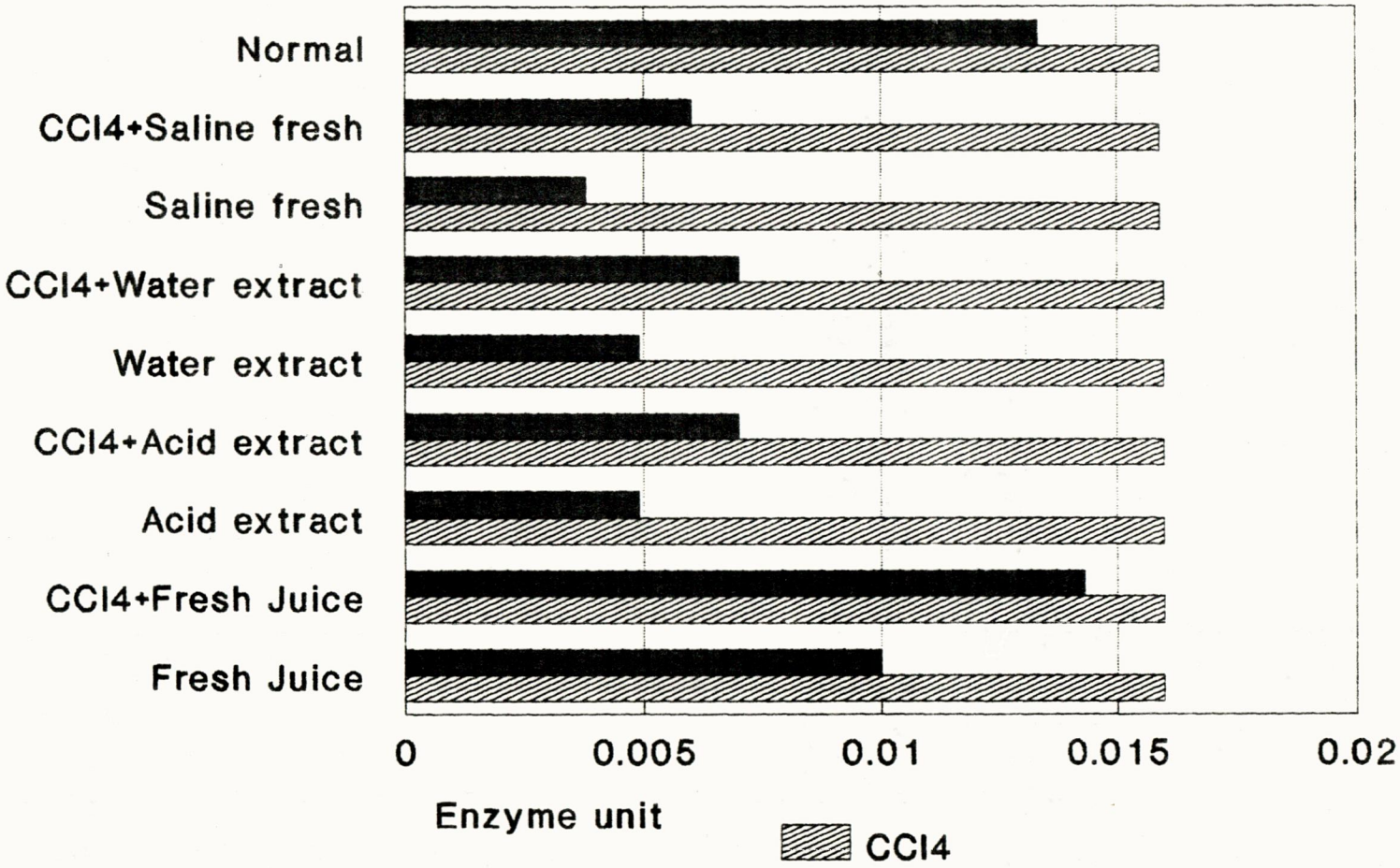
Effect of testosterone on lipid peroxides and antiperoxidative enzymes in normal and orchidectomised animals had been studied by Kumari et al., (1993).

PEROXIDATIVE STATUS DUE TO VARIOUS EXTRACTS OF CYNODON DACTYLON IN THE LIVER OF CCl_4 INDUCED MICE

TABLE XII

Treatment	Peroxidase Activity Enzyme unit
Normal	0.013
CCl_4	0.0158
CCl_4 + Saline Fresh Juice	0.0055
Saline Fresh Juice	0.0040
CCl_4 + Water Extract	0.0070
Water extract	0.0050
CCl_4 + acid extract	0.0060
acid extract	0.0050
CCl_4 + fresh juice	0.0120
fresh juice	0.0100

CD = 0.0016 SED = 0.0006



EVALUATION OF PEROXIDASE

Figure 10

4.0 **ASSAY OF PEROXIDASE :-**

Peroxidase initiate lipid peroxidation indirectly through Co - oxidation of organic molecules to radical intermediates (Marnett 1990).

Table XII and figure X reveal the induced levels of peroxidase in mice treated with CCl_4 and decreased levels of peroxidase in mice treated with CCl_4 + different extracts of CYNODONDACTYLON and mice given with different extracts of CYNODONDACTYLON alone. The se quential increase in the inhibition of peroxidase is ordered as below.

Fresh Juice < acid extract < Water extract < Saline Fresh Juice.

5.0 **HISTOPATHOLOGICAL RESULT:**

To bring out evidences of inhibition by CYNODONDACTYLON histopathological studies were carried out. Longitudinal section of liver that has been in duced with CCl followed by subsequent treatment with various extracts of CYNODONDACTYLON was taken and cb served for cellular changes.

TABLE XIII

Histopathological results obtained from different treatments

Serial No.	Microscopic Features	CCl_4	CCl_4 + Saline Fresh Juice	CCl_4 + Fresh Juice	CCl_4 + Acid Extract	CCl_4 + Water extract
1.	Congestions	-	+++	+	++	+++
2.	Lymphocytic collections around portal triad	+	++	+++	+++	++
3.	Vacuolar degeneration	+	+	+	+	+
4.	Necrosis	+	+	+	+	+
5.	Bilestasis	+	-	+	++	-
6.	Nuclear changes	+++	++	+	+	+
				Minimal +		
				Moderate ++		
				Severe +++		

TABLE XIV

NET OUTCOME OF THE HISTOPATHOLOGICAL STUDIES

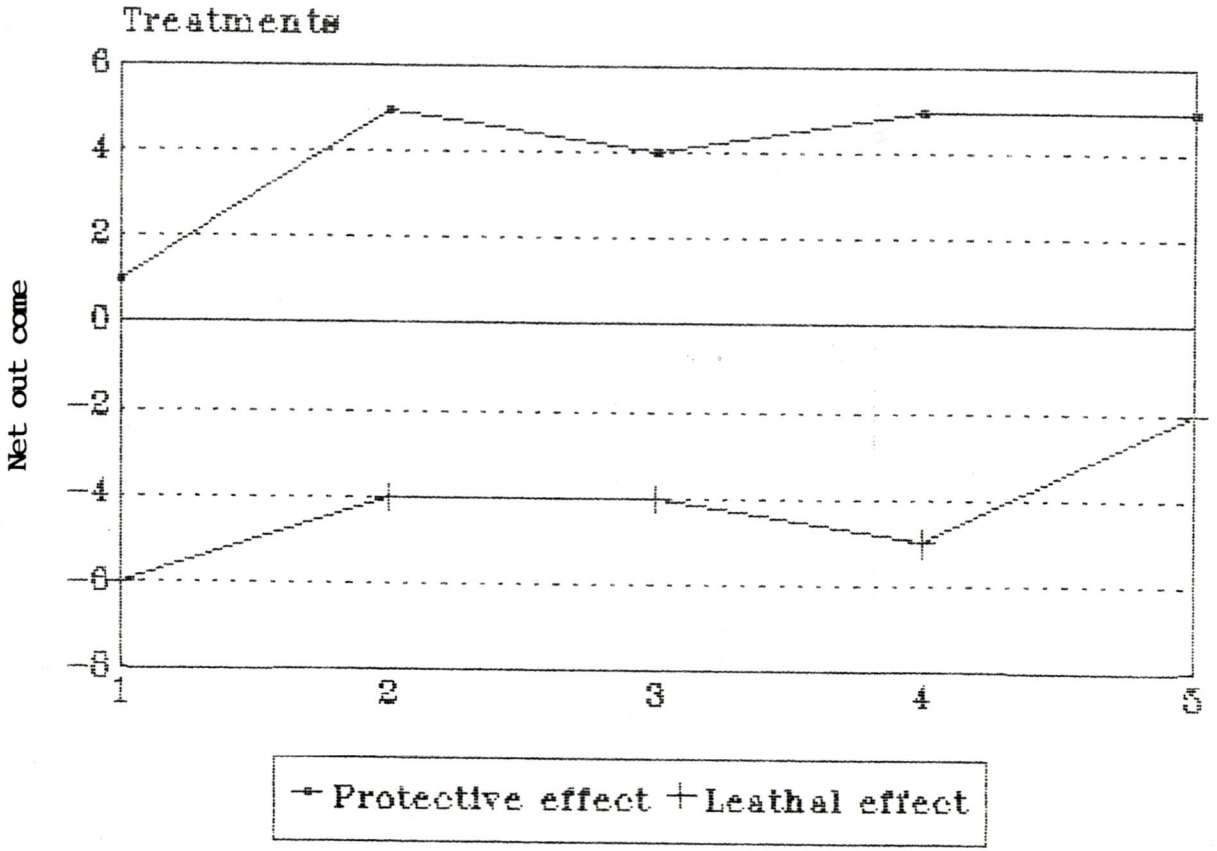
Type of treatment	Protective effect	Lethal effect	Net outcome
CCl_4	+	++++++	(5) lethal
CCl_4 +saline			
Juice	+++++	++++	(1) PROTECTIVE
CCl_4 + fresh juice	++++	++++	-(Neutralization)
CCl_4 + Acid Extract	+++++	+++++	-(Neutralization)
CCl_4 +H_2O Extract	+++++	++	(3) PROTECTIVE

TABLE XV

CHARACTERISTICS OF PROTECTIVE AND LETHAL EFFECT

Protective effect	Lethal effect
Congestion	Vacuolar degeneration
Lymphocytic collection	Necrosis
	Bilastasis
	Nuclear changes

Fig.11 Histopathology



T_1 - CCl_4 T_2 - CCl_4 + Saline fresh juice T_3 - CCl_4 + Fresh juice
T_4 - CCl_4 + Acid extract T_5 - CCl_4 + Water extract

T - Treatment

Cellular changes found in liver were grouped into two broad headings.

(A) Protective or Beneficial Effects/which include

a) Congestion

b) ymphocytic Collections

(B) lethal effect (or) Adverse effect/which include

a) Vacuolar degeneration

b) Necrosis

c) Bilestasis

(5) The changes noted were graded into mild (+), moderate)++) and Severe (+++)

All these changes were clearly noted in Table XIII and XIV

Table XIII illustrates the effects produced in 5 sets of albinomice. Table XIV and fig II shows net outcome whether beneficial effect dominates or lethal effect dominates.

Conclusion:

Of the sets of mice, the mice treated with CCl_4 + Saline Fresh Juice and CCl_4 + Water extract had very

Plate - 1

Normal Cords of liver cells (with few lymphocytic Collections) - Higher magnification 450X.

Plate - 2

Liver lobule with plenty of lymphocytes, congestion and mild vacuolar degeneration - Higher magnification (450X).

Plate 1

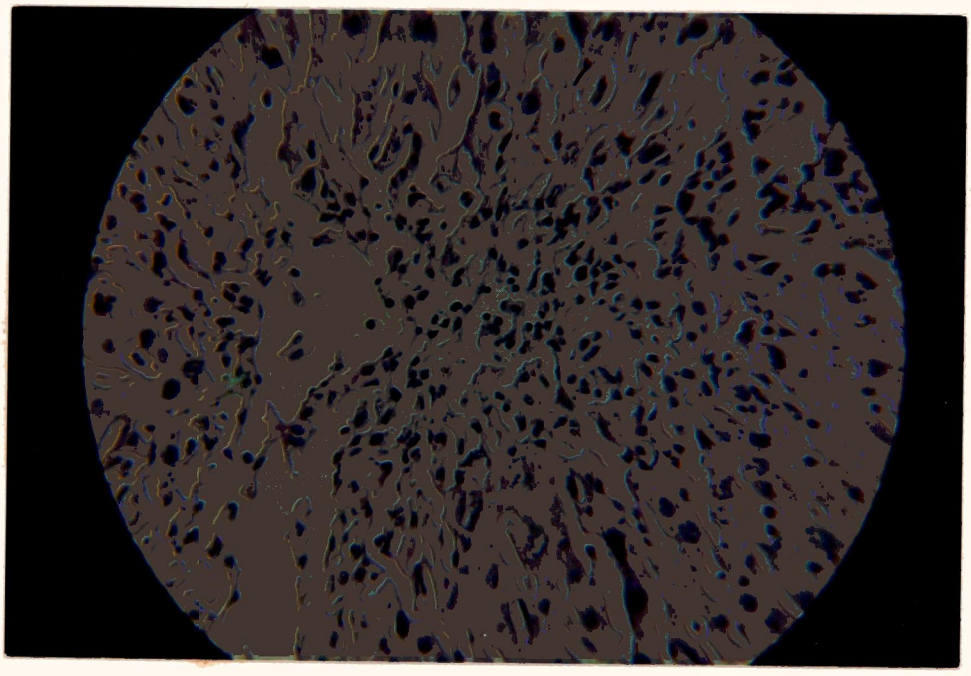


Plate 2

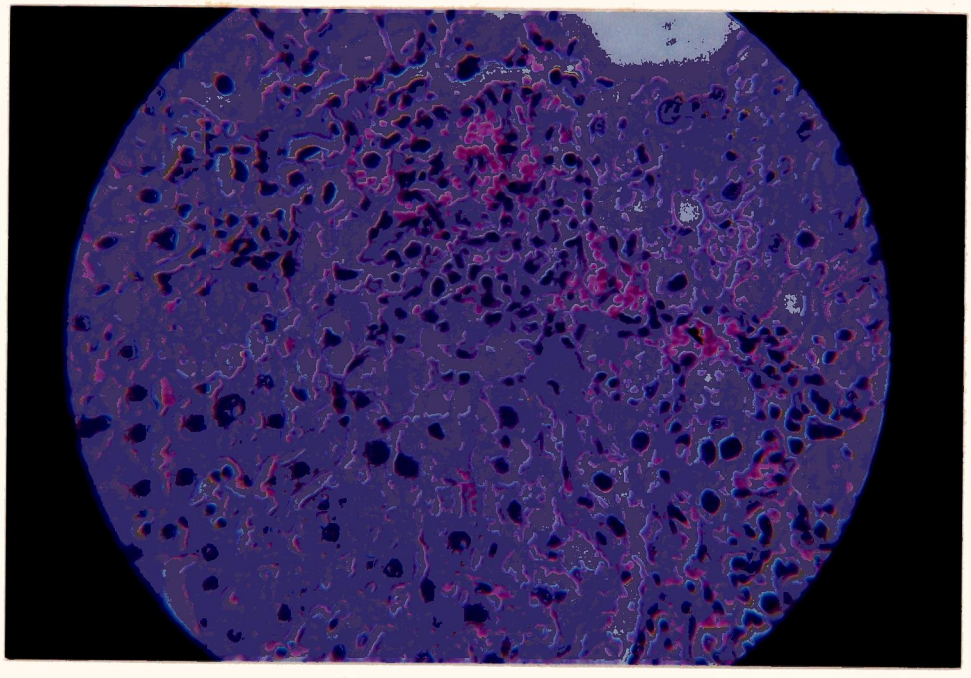


Plate - 3

Liver lobules - congestion and pale vacuolar areas

Low power (100X)

Plate - 4

Liver lobule with vacuolar degeneration and

necrosis. Higher magnification (450X)

Plate 3

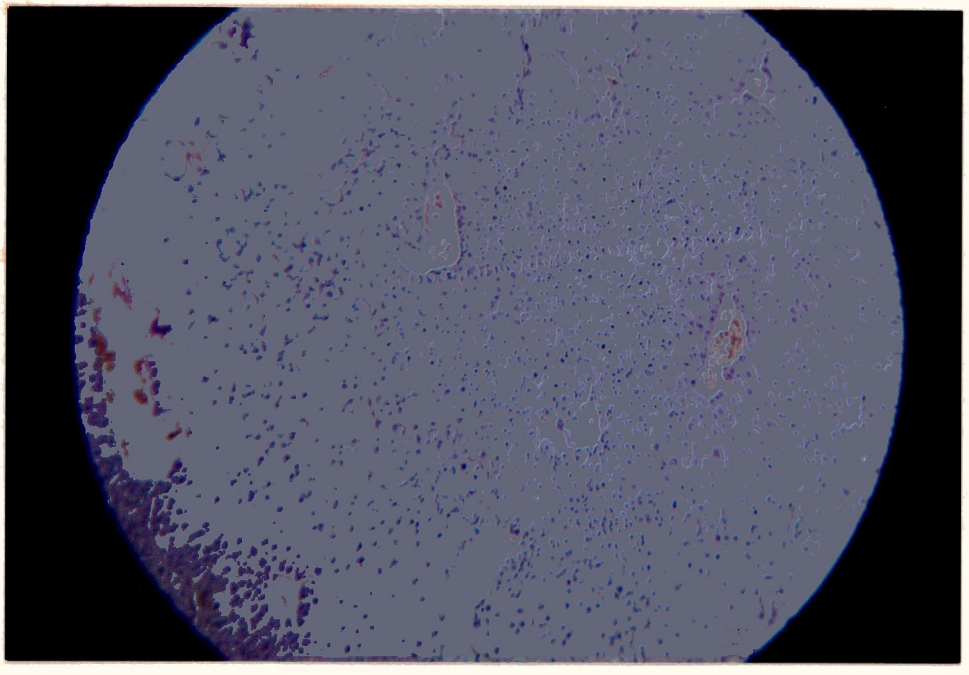


Plate 4

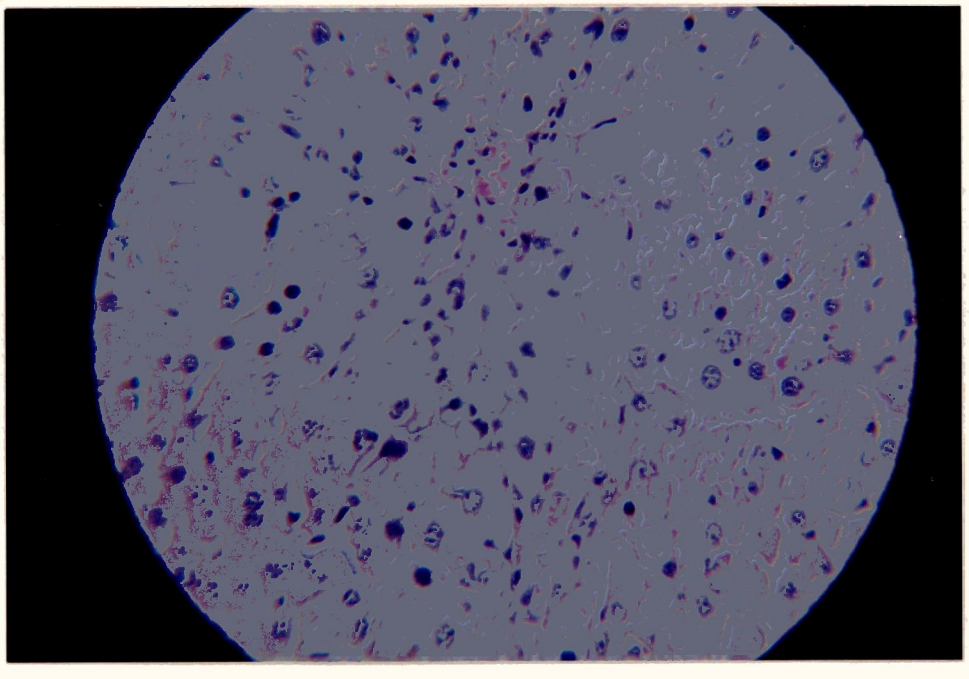
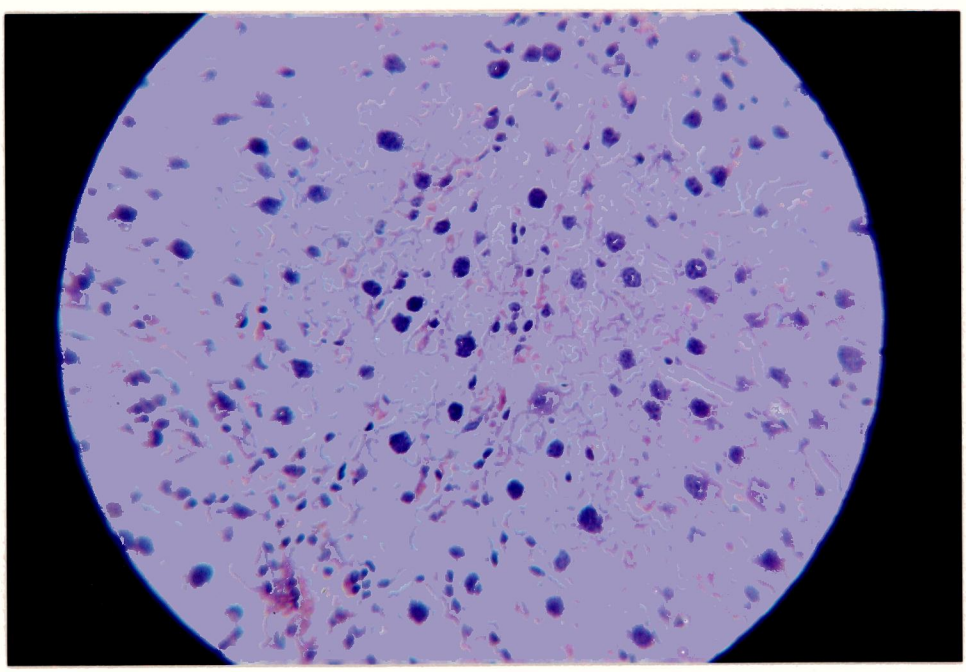


Plate - 5

Liver lobule with nuclear changes like hyperchromatism, Pleomorphism, and mitoric activity in Central field liver cells compared to peripheral normal liver cells.

Plate 5



minimal lethal effect and maximum beneficial efect and mice treated with plain CCl_4 had maximal lethal effect. Fresh Juice treated (CCl_4 injected) mice liver and Acid extract of Arugampul with CCl_4 injected mice had equal beneficial and lethal effect.

Both adverse and protective effects in liver of CCl_4 induced mice and treated with various extracts of CYNODONDACTYLON by histological analysis were photographed and shown in plates I - V.

The conclusion which can be arrived at based on our observations are summarized under the following chapter.

Summary and Conclusion

SUMMARY AND CONCLUSION

Revolutionised modern medical practices are almost all from plants which for one purpose or another have been employed by traditional societies.

Scientific investigations with medicinal plants having folk/ethnomedical uses represent leads that may short cut the discovery of modern therapeutic drugs either directly from the plants or from their synthetic analogs.

In the present investigation the CYNODONDACTYLON traditionally called Arugampal was used as a drug in counteractig Lipid peroxidation and induction of superoxide scavenging activity thereby elevating the levels of antiperxidative enzymes and depressing peroxidases.

Lipid peroxidation a carcinogenic reaction initiated by CCl_4, a fre radical generator is thought to play a key role in many pathological process like carcinogenesis.

The work done by us revealed evaluated changes in organ systems after exposure of CCl_4 to 6-7 weeks old albino male mice weighing 26-31g and in combination with various extracts of CYNODONDACETYLON, there-

by inhibiting Lipid peroxidation and inducing superoxide scavenging activity and antiperoxidative enzymes.

The animals were delineated into two phases. Administration of CCl_4, I.P (0.5ml/Kg body weight) for one day and consequently for 7 days and sacrificing the animal after one day and 7 days in phase I and II respectively.

The lipid peroxide expressed in nanomoles of malonaldehyde per gram weight of tissue was more in brain, followed by liver, kidney, lungs and spleen both in acute and chronic doses.

The extent of inhibition of lipid peroxide formation in different organs of mice varied according to the administration of various extracts of CYNODONDACTYLON.

It can be said from our result that the level of lipid peroxidation increases significantly in all organs of CCl_4 treated mice. Treatment with extract alone significantly reduced the lipid peroxidation. This was significant in both acute (1) dose and chronic (7) dose

Among the four extracts Saline fresh juice counteracted LPO more than acid and water extract which were more when compared to fresh juice.

To support the above findings superoxide scavanging activity, antiperoxidative enzymatic status were also evaluated.

The highest degree of induction of SOSA was by Saline fresh juice followed by acid and water extracts and finally fresh juice extracts. This mainly due to antioxidant properties exhibited by CYNODON DACTYLON.

The extracts of CYNODON DACTYLON were found to increase the tissue levels of antiperoxidative enzymes such as catalase and superoxide dismutase and thus the above extracts be sufficiently considered as protective agents against Lipidperoxidation.

The activities of SOD & CAT were increased in CCl_4 induced mice administrated with the various extracts. On the other hand peroxidase activity was decreased. These changes were observed only in mice given chronic doses of CCl_4.

Histopathological evaluation of liver showed that CCl_4 + Saline Fresh Juice and CCl_4 + Water extracts had maximum beneficial effect, CCl_4 + Fresh Juice and acid extract of CYNODONDACTYLON treated mice had equal beneficial and adverse effect whereas the CCl alone treated mice had maximal adverse effect.

All these investigations conclusively show that extracts of CYNODONDACTYLON are excellent protective agents against CCl_4.

From the studies carried out on "Inhibition of lipidperoxidation and induction of superoxide scavenging activity by CYNODONDACTYLON in CCl_4 induced mice it can be hypothesised that the malfunctioning of the system due to Lipidperoxidation can be depressed by leaf extracts of CYNODONDACTYLON preferably saline fresh juice, water and acid extracts and to a certain extent by fresh juice.

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Appendices

APPENDIX I

ESTIMATION OF TISSUE LIPID PEROXIDE

(Bisharyee et al, 1971)

The CCl_4 induced lipid peroxidation using different tissue homogenates of mice was measured by thiobarbituric acid.

The homogenate (20 percent in 0.2M tris-HCl buffer of pH 7.0-0.1ml) was incubated in a medium (total volume 0.5ml) containing 150mM KCl (0.1ml) tris HCl buffer (0.2M pH 7.0) at 37°C for 1 hour. To the reaction mixture in a total volume of 0.5ml was added 1ml of trichloro acetic acid (20% W/v)at the end of the incubation period. After thorough mixing 2ml of thio-barbituric acid (0.67% W/v) solution was added and the mixture was heated in a boiling water both for 15 minutes. After cooling the tubes to room temperature and centrifugation (5000 rpm for 5 minutes) to remove suspended materials the colour of the supernatant was read at 540nm using uv visible spectrophotometer. Appropriate blanks and controls were run along with the test samples. The amount of lipid peroxidation was expressed as nMoles of malonaldehyde formed in each tube calculated from a standard curve of malonaldehyde.

APPENDIX II

DETERMINATION OF SUPEROXIDE SCAVENGING ACTIVITY

(Wintor bourn et al., 1975)

The ability of various extracts to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide generated by the reaction of photoreduced riboflavin and oxygen was taken as a measure of superoxide scavenging action.

The assay mixture contained different tissue homogenate plus 0.2ml of 0.1M EDTA containing 1.5mg of Sodium Cyanide/100ml, 0.05ml of 0.12mM riboflavin (4.5mg/100ml) and M/15 phosphate buffer of P_H 7.8 to give a total volume of 3ml (winter bourn et al 1975). After the tubes have been brought to a standard temperature of 20 ^{o}C, riboflavin was added and the tubes were placed in a bright light box where they received uniform illumination from a 18W flurescent tube for 15 minutes. Optical densities were then measured at 560nm using a u.v spectro photometer. The difference in optical densities before and after illumination was a measure of the amount of superoxide production. The percentage of inhibition of superoxide by the treated extract was evaluated by comparing the absorbance of controls to that of experimental tubes.

APPENDIX III

ESTIMATION OF PROTEIN

(Lowry *et al.*, 1951)

Total proteins in the samples were determined as follows.

In a two step reaction, Folin's reagent reacts with the aromatic aminoacids in protein after treatment with alkaline copper to give a blue colour. This is the principle of this method.

Protein sample 100ul was diluted to 1.2ml with distilled water and 6ml of solution (A) containing 1ml of $CuSO_4 5H_2O$ (1%) plus 1ml sodium potassium tartarate (2%) and 98ml of 2% Na_2CO_3 in 0.1 N NaOH was added, mixed and left for 10 minutes. 300 ul of solution (B) Folinciocalteau reagent 1:1 in 0.1N NaOH was added with constant mixing and kept for 30 minutes at room temperature. The colour developed was read in a photoelectric colorimeter at 660nm against a reagent blank and with a BSA standard (1mg/ml of saline). The optical dersities were converted to milligram protein from the standard curve.

APPENDIX IV

DETERMINATION OF CATALASE ACTIVITY

(Luck, 1974)

The UV light absorption of hydrogen peroxide solution can be easily measured between 230 and 250nm . On decomposition of hydrogen peroxide by catalase the absorption decreases with time. The enzyme activity can be arrived at from this decrease.

Into the experimental cuvette 3ml of hydrogen peroxide-phosphate buffer (0.16ml of H_2O_2 (10 W/V) in 0.06M phosphate buffer P^{H} 7.0)was taken. Mix 0.01-0.04ml homogenate with a glass or plastic rod flattened at one end. The time change required for a decrease in absorbance from 0.45 to 0.4 is noted. This value is used for calculations.

APPENDIX V

ESTIMATION OF PEROXIDASE

(Addy and Goodman 1972)

The peroxidase can be assayed by adding tissue extract to pyrogallol which in the presence of H_2O_2 is oxidised to a coloured derivative. The amount of purpurogallin formed during the reaction will measure the activity.

3ml of 0.05M pyrogallol phosphate buffer (0.1M) P H 6 and 0.1ml of 20 percent tissue homogenate in to a cuvette and adjust the absorbance to zero at 420nM . 0.5 ml of hydrogen peroxide was added and the cuvette was inverted immediately to mix the content and replaced in the colorimeter. Changes in absorbance at 20 seconds interval for a period of three minutes were measured. To Plot the peroxidase activity the average change in absorbance per 20 seconds between 40 and 160 seconds was used. Suitable controls were maintained.

APPENDIX VI

ASSAY OF Super Oxide Dismutase (SOD)

(By Misra and Fridovich method (1972)

Superoxide dismutase which inhibit superoxide anion, can be assayed by the following procedure.

Assay mixture consisted of 0.2ml of riboflavin, (4mg riboflavin / 10ml citrate phosphate buffer) 0.1 ml of diannisidine (18mg diannisidine/5ml of C_2H_5OH) 0.5ml of tissue homogenate, 5ml of citrate phosphate buffer. This mixture was exposed to a tube light for 15 minutes. The colour was read at 460nm quickly. One unit amount of enzyme required to get double the OD of control.

APPENDIX VII

HISTOPATHOLOGICAL TECHNIQUE

(Culling,1979)

The following steps are followed in histopathological techniques.

(1) Antopsy bits are preserved in 10% formaline solution sahoe for minimum 1 hour.

(2) Dehydration of biopsy bits done by 3 changes of acetone (each 500ml)

(3) Cleaning of bits from acetone done by 3 changes of Xylene (each 500 ml) - total 3 hours.

(4) Incubation of processed tissue bit in paraffin wax - 2 changes for 3-4 hours in an incubator at 58-60 ^{o}c

(5) Embedding of the tissue in paraffin wax after incubated in melted paraffin.

(6) Cutting of sections from autopsy bit embedded in wax (Sections are of 1-3u thicken of autopsy bit)

(7) Sections are taken on this glass slide

(8) Sections on glass slide are cleaned from wax by immersing in Xylene.

(9) Sections were histochemically reacted with haematoxylin and eosin staining to evaluate the morphology and cellular composition.