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PHARMACOLOGICAL EVALUATION OF ANTIOXIDANT AND CARDIOPROTECTIVE ACTIVITIES OF AERIAL PART OF *TAGETES ERECTA* IN EXPERIMENTAL ANIMAL MODEL

[1]Suresh Chandra*, [2]Saurabh Mishra, [3]Ridhima Mehra
[1]Assistant Professor, Department of Pharmacology; Pranveer Singh Institute of Technology, Bhauti road, Kanpur (U.P) 208020. India.
[2,3]Research Scholar, Department of Pharmacology; Pranveer Singh Institute of Technology, Bhauti road, Kanpur (U.P) 208020. India.
Email: sureshcology81@gmail.com

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Abstract

Aim: To evaluate the Antioxidant and Cardioprotective activity of the ethanolic extract of the leaves of *Tagetes erecta* against CCl4 and paracetamol induced hepatotoxicity and against doxorubicin induce cardiotoxicity in experimental rat model.

Methods: The powdered leaves were extracted with pet ether and ethanol the resultant ethanolic extract was subjected for phytochemical analysis to identify different phytoconstituents. Acute toxicity study for extract was carried out up to the dose 2000mg/kg according to OECD guidelines-425.

Antioxidant activity of extract was studied against CCl4 and paracetamol induced hepatotoxicity in rats. Biochemical (ALT, AST, ALP), Antioxidant parameter (SOD) are evaluated. Cardioprotective activity of extract studied against doxorubicin induce cardiotoxicity and Lipid profile parameter (TC, TG, HDL, LDL) and cardiac biomarker (LDH, CK-MB) are evaluated.

Results: The activities of antioxidant enzyme were significantly increased by extract at 200 and 400 mg/kg. The Level of ALT ALP and AST also reduced to normal compared to the induce group. It also prevents the weight loss in animals. Pretreatment with *Tagetes erecta* extract showed reduction in blood lipid profile levels with concomitantly increase in HDL cholesterol was observed. Heart tissue injury by doxorubicin in rats was indicated by elevated level of the marker enzymes such as LDH and CK-MB. Extract was found to inhibit the doxorubicin induced CK-MB and LDH release in the serum of rats.

Conclusion: From the above all findings, it was concluded that Ethanolic extract of *Tagetes erecta* exhibited antioxidant and cardioprotective activity in validated animal model.

Keywords: *Tagetes erecta,* Antioxidant, Carbon tetra Chloride, Paracetamol, Doxorubicin.

Introduction

Oxidative process that is regularly going on in cell is essential for life and death of a cell. Some important points taken into consideration are molecular oxygen has ability to un-pair and leave free radicals which are unstable. This unstable radical is highly reactive and causes formation of reactive oxygen species. Beneficial biological functions such as apoptosis, necrosis, phagocytosis are mediated by reactive oxygen species. These reactive metabolites are selectively neutralized by body's defensive mechanism. Principal defensive agents are antioxidant enzymes and endogenous antioxidants. Balance is created between pro-oxidant and antioxidant in a cell and any impairment in equilibrium cause deleterious effects on cell's life. Increased level of antioxidants may interfere the normal oxidative process while decreased level of antioxidants generates reactive metabolites. It is known that unpaired electron of molecular oxygen react to form highly reactive species, which are known as reactive oxygen species. Reactive oxygen species are generated from enzymatic and non-enzymatic sources (Orient A et.al, 2007; Bedard K et. al, 2007).

Oxidative stress has been linked to the pathogenesis of numerous diseases including asthma (mitochondrial dysfunction) (Vogiatzi g et. al, 2009), atherosclerosis (oxidative modification of LDL) (Li H et al, 2012), endothelial cardiovascular disease, which is more prompt to inactivation of NO and ROS, thus predispose of these reactive molecules.

CVD remains the principal cause of death in both developed and developing countries, accounting for roughly 20% of all worldwide deaths per year. In addition, hypertension, arrhythmia, coronary artery disease, MI and cardiac failure represent the leading killer of males over the age of 45 and females over the age of 65 in the United State and accounts for 750,000 deaths annually (Kubler W, Haass M, 1996).

Cardioprotection includes “All mechanisms and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage” (Murray CJ et al., 1996).

Although some of the major risk factors for CVD are not modifiable- age, sex, genetic predisposition-diet and lifestyle issue are recognized as the major modifiable risk factors

(Luthra A, 1998).

Accordingly, current knowledge favors the notion that the risk factors other than raised plasma cholesterol play an important role in the development of CVD (Abeywardena MY, 2003). Indeed, recent findings have highlighted the importance of oxidative stress, vascular inflammation, and endothelial dysfunction (as central) to the development of CVD (Zalba G et. al., 2007). Such advancements in the knowledge of the disease process have also provided new avenues to develop novel pharmaceutical and/or dietary strategies to cure the development of vascular diseases (Renaud S et. al, 1992).

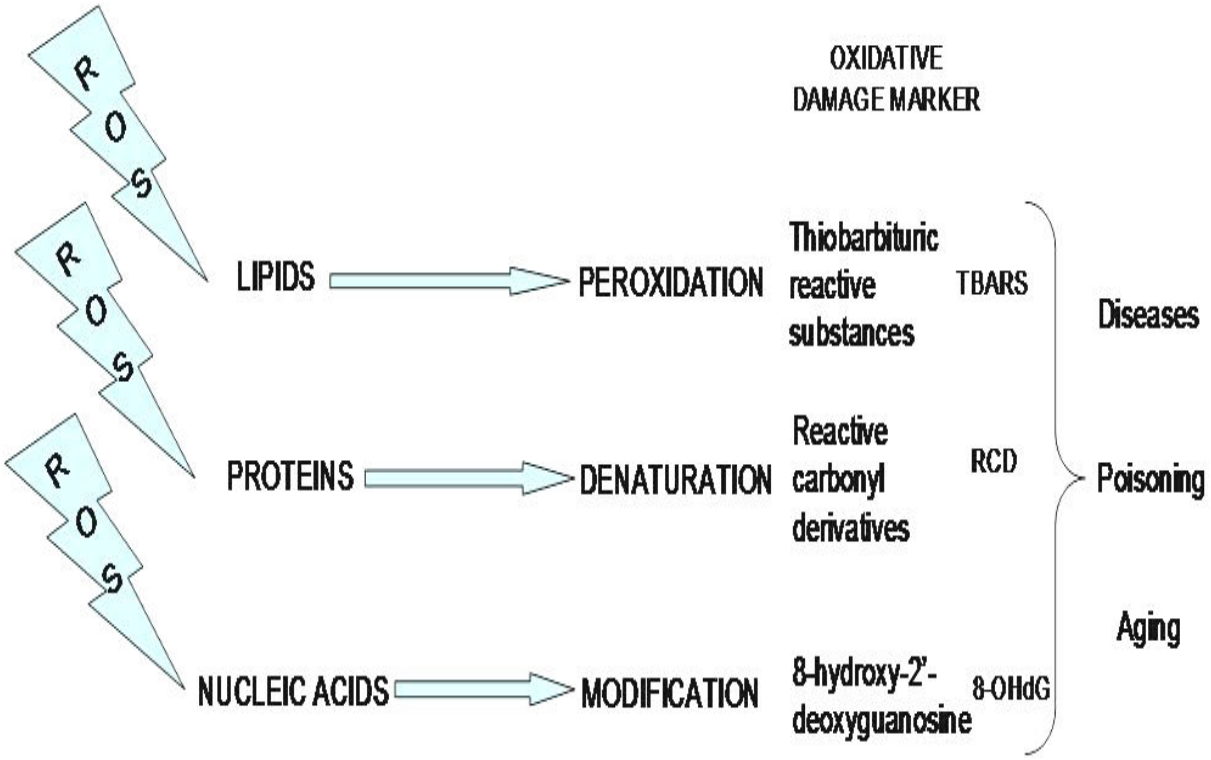

Figure.1: The figure shows the effect of excess ROS production on cell's lipids, proteins and nucleic acids and the general effect on the organism. In addition, it is shown which damage marker is investigated to determine oxidative damage for each macromolecule.

Several medicinal plants have been found to possess antioxidant properties and have beneficial effects in pathological conditions like cancer, liver diseases, cataract and myocardial ischemia (Hertog MGL et al., 1993, Shalini VK et al., 1987). The use of herbal medicines has been steadily increasing over the past decade. A considerable number of these plants/plant based products have been widely used (Das DK et Al., 1993). Therefore, interest in the examination of plants as potential sources of new drugs is increasing. The prophylactic and therapeutic effect of many plant foods and extracts in reducing cardiovascular disease has been reviewed as they are inexpensive, efficacious and safe (Fugh Berman A; 2003).

The present study evaluates the Antioxidant and Cardioprotective activity of the ethanolic extract of the leaves of *Tagetes erecta* against CCl4 and paracetamol induced hepatotoxicity and against doxorubicin induce cardiotoxicity in experimental rat model.

Materials and Methods:

Plant material: *Tagetes erecta L.* (Compositae) is commonly known as "Marigold". The plant is widely distributed in India, asia, central Europe, USA and Africa. Marigold is a common garden plant which is rather coarse, erect, branched and grows to about 1 meter high.The leaves are very deeply incised and sharply toothed. Flower heads are solitary, long stalked and thickening upward. The flowers are bright yellow, brownish-yellow or orange. Phytochemical studies of different parts have resulted in the isolation of various chemical constituents such as thiophene, flavanoids, carotenoids and triterpenoids. Plant *T. erecta* has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, synergic acids, methyl-3,5-dihydroxy -4-methoxy benzoate, quercetin, thienyl and ethyl gallate(Kirmani M, Ibrahim M, 2012).

Scientific Classification:

Kingdom: Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Asterales
Family : Asteraceae
Genus : *Tagetes*
Species : *erecta*

Tagetes erectus L. is rich in the xanthophylls, lutein, which occurs acylated with fatty acids.

Carotenoids, which are present, have excellent antioxidant properties while alpha and beta-carotene, xanthophylls and retinoids have been reported to inhibit some types of cancers. Lutein also shows greater antioxidant activity than the other two common carotenoids, beta-carotene and lycopene (Shinde V et al., 2009). The leaves are reported to be effective against piles, kidney troubles, muscular pain, ulcers, wounds and earache. The pounded leaves are used as an external application to boils and carbuncles. It is reported to have antioxidant, antimycotic, analgesic activity and 18 active compounds are identified by GC-MS, many of them are terpenoids. The flower is useful in fevers; epileptic fits,

astringent, carminative, stomachic, scabies, liver complaints, and also employed in diseases of the eyes. They are said to purify blood and flower juice is given as a remedy for bleeding piles and also used in rheumatism, cold and bronchitis.

Aerial part of *Tagetes erecta* were collected (in the month of February) and identified by Dr. P.K. Singh, HOD, Department of botany, Pt J.N.P.G. College Banda.

Extraction procedure:

Leaves of *Tagetes erecta L.* (150 g) were dried at 40 °C for 1 week and pulverised. The powder was packed into the thimble of a Soxhlet extractor and refluxed continuously for 6 h. The solvent - either petroleum ether (PEE), ethanol (EE)) - was changed at the end of every 6 h. The solvent was removed by distillation on a Boiling water-bath at atmospheric pressure and then under reduced pressure in a rotary evaporator. The alcoholic extract was filtered, concentrated under reduced pressure to a semisolid mass and was made free from solvent. The final obtained extract was weighed; percentage yield was calculated and stored in a cool place. Before administration, extract was reconstituted by dissolving in water.

Chemical and apparatus: The list of various chemicals and drugs used is being provided in the Table 1 and Table 2 respectively.

Table-1: List of chemical and Drugs used.

S.no	Chemical/drugs	Manufacturer/Supplier
1	Liquid paraffin	Merck Specialities Pvt Ltd.
2	Petroleum ether	Merck Specialities Pvt Ltd
3	Diethyl ether	Merck Specialities Pvt Ltd
4	Silymarin	Day‘s Pharmaceutical
5	Ethanol	SD fine chem. Ltd.
6	Doxorubicn	Dauber chem. Ltd

Table-2: Enzymatic Kits.

S.no	**Kits**	
1	Total cholesterol	Autospan diagnostic Ltd.
2	ALP , ALT , AST	Autospan diagnostic Ltd.
3	Triglycerides	Autospan diagnostic Ltd.
4	HDL-cholesterol	Autospan diagnostic Ltd.

5	LDL-cholesterol	Autospan diagnostic Ltd.
6	LDH	Autospan diagnostic Ltd.
7	Creatine-Kinase	Autospan diagnostic Ltd.

Experimental Methods:

Acute oral toxicity:

The dose limit was selected on the basis of previously performed oral acute toxicity studies in albino mice in accordance with the OECD guidelines. Acute toxicity studies on *Tagetes erecta* aerial parts extract were performed in mice containing 6 animals in each group, the graded doses of the ethanolic extracts of *Tagetes erecta* aerial parts extract doses selected for the study were 100 mg/kg, 200 mg/kg, 400 mg/kg, 800 mg/kg, 1600 mg/kg, 2000 mg/kg were administered orally and the animals were observed for 2 weeks following administration, change in body weight gain, food consumption, any kind of behaviorally changes and mortality were noted. It was found that the ethanolic extract has produced significant toxicity at the dose of 2000 mg/kg as 2 animal of this group was died. Thus the extract was highly tolerable up to 1500 mg/kg for ethanolic extract.

Cl4 Hepatotoxicity (Girish A Chliya et al., 2004; Chidambra Murthy A et al., 2005; Baheti JR et al., 2006)

Male albino rats weighed between 120-150g was divided into 5 groups and each group contains 4 rats. Animals were grouped into following groups: 14 days treatment [everyday]

Group I: Control Group (normal saline- Dose- 3ml/kg)
Group II: CCl4 (1ml/kg) sc
Group III: CCl4+ extract p.o. (200mg/kg)
GroupIV: CCl4+ extract p.o. (400mg/kg)
GroupV: CCl4+Silymarin (100mg/kg) Standard drug

The animals were kept starved overnight on 14th day of experiment. On the next day the animals were sacrifice by decapitation, and the blood was collected by cutting the jugular vein. The liver and kidney in each case was dissect out, blotted of blood, washed in saline and stored in a freezer. Liver, kidney and serum were used for various biochemical estimations.

The marker enzymes ALT, AST and ALP were assayed in serum using standard kits supplied from Autospan diagnostic Ltd.

Doxorubicin induced myocardial toxicity in rats: (BC Koti et al., 2009)

Albino rats of either sex weighing between 150-200g were divided into 4 groups in which each group contained 4 rats.

Animals are grouped into following group:

Group I: Animals were administered with normal saline 5ml/kg body weight (ip)
Group II: Animals were administered with doxorubicin (2.5 mg/kg body weight ip) 6 equal injections alternatively for 2 weeks to make a total cumulative dose of 15 mg/kg body weight.
Group III: Animals were administered with extract (200 mg/kg body weight po) for 2 weeks and then alternatively with vehicle for next 2 weeks.
Group IV: Animals received extract (400 mg/kg body weight po, for 2 weeks) as a pretreatment followed by doxorubicin administration as in group 2.
After 36 hr of the last treatment, orbital blood samples were obtained under light ether anesthesia using heparinized micro capillaries for the estimation of Cardiac biomarkers CPK and LDH and Total cholesterol, triglycerides, LDL.

The cardiac biomarkers CPK, LDH, Total cholesterol, triglycerides and LDL were assayed in serum using standard diagnostic kits.

Paracetamol-induced hepatotoxicity in rats: (Dash K Deepak et al., 2007)

Albino rats of either sex weighing between 150-200g were divided into 5 groups and each group contains 4 rats.

Group I: Control administered with normal saline 5ml/kg body weight.
Group II: Similarly as group I
Group III: administered with 250mg/kg extract orally.
Group IV: administered with 500mg/kg extract orally.
Group V: administered with 25mg/kg standard drug silymarin orally.
On the seventh day, paracetamol suspension was given by oral route; in a dose of 750mg/kg body weight to all rats except the rats in group I .The biochemical parameters were estimated after an 18h fast following the last dose. The parameters estimated ALT, ALP, AST SOD were analysed in serum using standard diagnostic kits.

Results:

CCl4 Hepatotoxicity

Rats were treated with carbon tetra chloride (CCl_4) developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like ALT, AST and ALP when compared to normal control. Pretreatment with ethanolic extract 200mg/kg showed better protection. Silymarin (100 mg/kg) and extract (200mg/kg and 400mg/kg) showed good

protection against CCl4 induced toxicity to liver. dunnet's test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals

Table-3: Effect of ethanolic extract of *Tagetes erecta* on ALP, ALT, AST and SOD in Serum of Control and Experimental rats.

S.no	TREATMENT	ALP	ALT	AST	SOD
1	Normal	92.98±2.19	35.82±2.55	41.08±1.55	6.08±0.57
2	CCl_4 induce (1ml/kg)	194.16±2.59***	176.99±7.60***	138.54±0.69***	4.06±0.08**
3	Silymarin (100mg/kg)	89.59±1.16^{ns}	58.65±1.82**	40.48±1.11^{ns}	6.70±0.21^{ns}
4	Low Dose (200 mg/kg)	114.36±2.42***	80.81±1.25***	56.35±1.38***	4.93±0.41^{ns}
5	High Dose (400 mg/Kg)	100.54±2.65^{ns}	69.86±1.66***	47.84±0.68**	5.50±0.31^{ns}

Values are in mean±SE; Number of animals in each group = 4, ***p< 0.05 Group l Vs Group ll; *** ** *p<0/05 Vs Group ll. As compared with normal group (one way ANOVA followed by dunnet test)

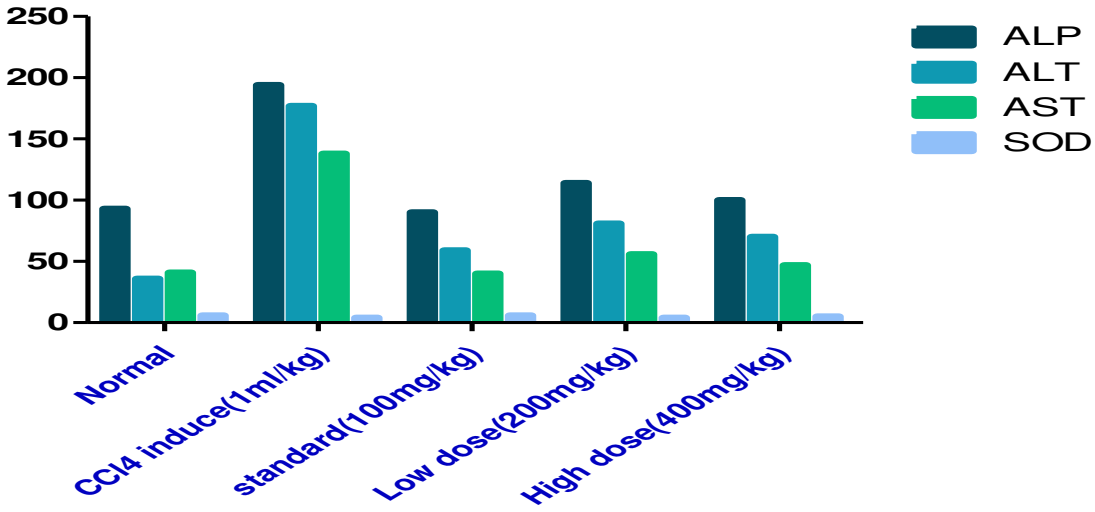

Figure.2: Showing different Enzyme level between Normal, Induced and Treated.

Doxorubicin induced myocardial infraction

Chronic administration of doxorubicin induced cardiac toxicity and effect of *Tagetes erecta* was established by significant increase in cardiac biomarker enzymes. Animals treated with doxorubicin produced significant increase in the levels of cholesterol, triglycerides compared to group 1 and there was very slight difference in HDL levels compared to group 1. Group 3 and 4 produced significant decrease in the level of cholesterol, triglycerides and increase in level of HDL as compared to group 2.

Animals treated with doxorubicin produced significant increase in the levels of CPK and LDH compared to group 1, Group 3 and 4.

Table-4: Effect of ethanolic extract of *Tagetes erecta* on TC, TG, HDL, LDH, LDL and CK-MB in Serum of Control and Experimental rats.

S.no	TREATMENT	TC	TG	HDL	CK-MB	LDH	LDL
1	Control	60.99±1.51***	106.01±1.01***	30.38±1.79*	19.99±0.67***	149.14±2.91***	28.92***
2	Doxorubicin induced (2.5mg/kg)	107.06±2.21	297.81±1.53	22.15±1.03	34.61±1.33	205.04±2.31	72.47
3	Low Dose (200 mg/Kg)	79.83±2.61***	183.30±1.67***	36.38±1.23^{ns}	26.83±1.16***	177.59±1.24***	46.93***
4	High Dose (400mg/kg)	72.44±1.40**	159.69±1.11***	35.9±3.41^{ns}	21.97±0.54^{ns}	165.34±1.82***	38.42***

Values are in mean±SE; Number of animals in each group = 4, ***p< 0.05 Group l Vs Group ll; *** ** *p<0/05 Vs Group ll. As compared with control group (one way ANOVA followed by dunnet test)

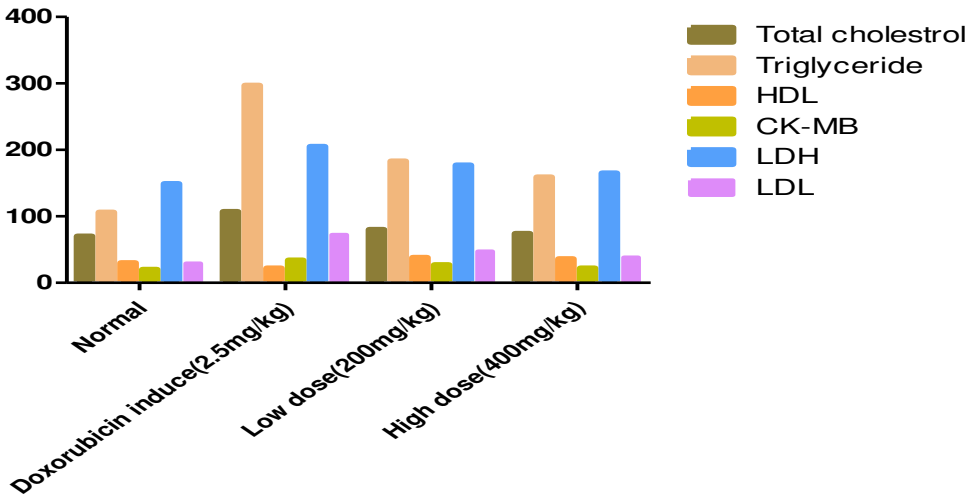

Figure-3: Showing different lipid serum level, cardiac biomarker between Normal, Induced and Treated.

Paracetamol-induced hepatotoxicity in rats

The effects of ethanolic extract of *Tagetes erecta* on serum transaminase, alkaline phosphatase and superoxide dismutase levels in paracetamol-induced liver damage in rats are summarized in Table. Administration of paracetamol (750mg/kg; body weight), after 18 hours of intoxication resulted a significant (P<0.05) elevation of hepatospecific serum markers ALT, AST, ALP, and decrease in SOD level in paracetamol-treated group, in comparison with the normal control group. On administration of Extract and Silymarin at the dose of 25mg/kg the level of these enzymes were found retrieving towards normalcy.

Table-5: Effect of ethanolic extract of *Tagetes erecta* on ALP, ALT, AST and SOD in Serum of Control and Experimental rats.

S.no	TREATMENT	ALP	ALT	AST	SOD
1	Normal	92.69±1.55	37.37±1.96	40.76±1.26	5.80±0.39
2	Paracetamol induce (750mg/kg)	195.36±2.12***	184.98±5.68***	138.83±0.56***	3.66±0.28**
3	Silymarin (100mg/kg)	89.69±0.84^{ns}	58.38±1.05***	40.23±0.92^{ns}	6.04±0.36^{ns}
4	Low Dose (200 mg/kg)	115.36±1.10***	80.23±0.67***	56.11±0.79***	5.23±0.44^{ns}
5	High Dose (400 mg/Kg)	100.26±1.39**	69.59±1.08***	47.84±0.62***	6.42±0.29^{ns}

Values are in mean±SE; Number of animals in each group = 4, ***p< 0.05 Group l Vs Group ll; *** ** *p<0/05 Vs Group ll. As compared with normal group (one way ANOVA followed by dunnet test)

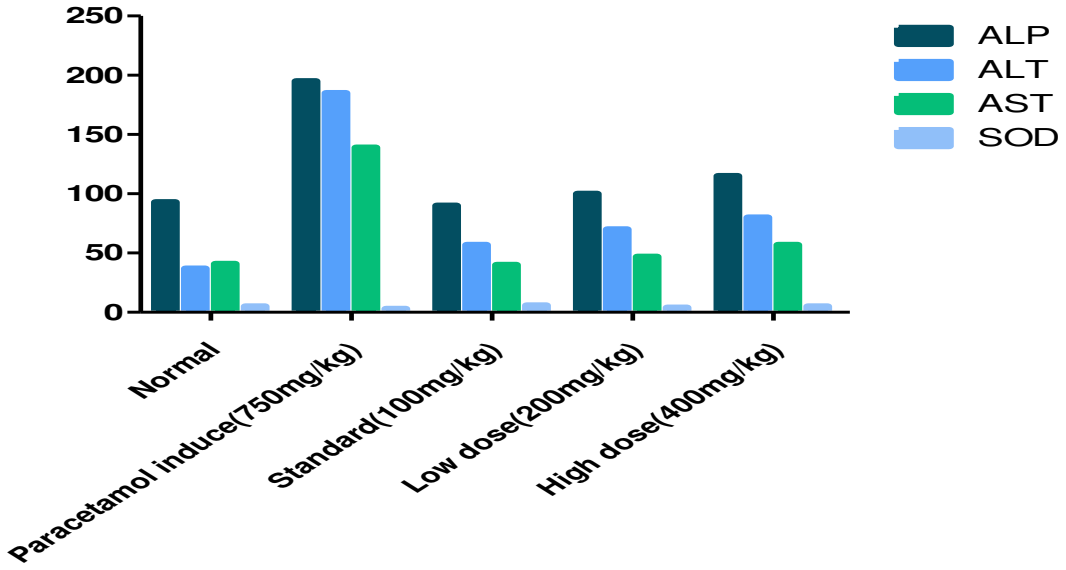

Figure.4: Showing different Enzyme level between Normal, Induced and Treated.

Discussion

Carbon tetrachloride has been extensively studied as a liver toxicant, and its metabolites such as trichloromethyl radical ($CCl_3^{\bullet}$) and trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$) are reported to be involved in the pathogenesis of liver and kidney damage. Liver and kidney are the two important vital organs mostly affected by the drugs.

Liver damage is detected by the measurement of the activities of serum enzymes like AST, ALT and ALP which has been released into the blood from damaged cells. They are also indicators of hepatic cell damage. The normalization of the above enzyme levels in rats treated with the extract clearly establishes the hepatoprotective effect of *Tagetes erecta* which

might be able to induce accelerated regeneration of liver cells, reducing the leakage of the above enzymes into the blood. Superoxide dismutase (SOD), one of the important intracellular antioxidant enzymes, present in all aerobic cells has an antitoxic effect against superoxide anion.

In living systems, liver is considered to be highly sensitive to toxic agents. The study of different

Enzyme activities such as ALT, AST, and ALP have been found to be of great value in the assessment of clinical and experimental liver damage. In the present investigation it was observed that the animals treated with acetaminophen resulted in significant hepatic damage as shown by the elevated levels of serum markers. The pretreatment with extract, significantly attenuated the elevated levels of the serum markers. The normalization of serum markers by extract suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against acetaminophen induced leakage of marker enzymes into the circulation.

Increase in serum level of ALP is due to increased synthesis in presence of increasing billiary pressure. Effective control of alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell.

In the present study, it was observed that the extract significantly increased the hepatic SOD activity in paracetamol induced liver damage in rats. This show extract of *Tagetes erecta* can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

The study entails the cadioprotective effect of *Tagetes erecta* against doxorubicin-induced cardiotoxicity. The present study is aimed to explore the cardioprotective effect of *Tagetes erecta* against doxorubicin induced cardiotoxicity in rats.

The existing experimental evidence suggests that doxorubicin oxidative stress is due to the generation of free radicals in the heart tissue. The generated reactive oxygen species such as superoxide radicals and hydroxyl radicals are potential to cause damage to various intracellular components. The doxorubicin induced mitochondrial injury is critical to the heart because it would presumably have extreme adverse effects on the contractile functioning of the cardiac myocytes by alterations in the energy metabolism. Pretreatment of *Tagetes erecta* was able to reduce the doxorubicin-induced cardiotoxic manifestations in multiple ways. Increase in the level of plasma triglycerides, total cholesterol and HDL in the doxorubicin treated group indicate doxorubicin may be interfering with metabolism or biosynthesis of lipids. Pretreatment with *Tagetes erecta* showed reduction in blood lipid profile levels with concomitantly increase in HDL cholesterol was observed. Decrease in the blood lipid profiles and increase in HDL cholesterol in *Tagetes erecta* treated group. Lipid

lowering effect of *Tagetes erecta* is due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor mediated catabolism of LDL. Heart tissue injury by doxorubicin in rats was indicated by elevated level of the marker enzymes such as LDH and CK-MB. *Tagetes erecta* was found to inhibit the doxorubicin induced CPK and LDH release in the serum of rats.

Conclusion:

Our present study showed that administration of Ethanolic extract of 200 and 400 mg/kg of *Tagetes erecta* was effective to protect against oxidative stress, Hepatoprotective and cardioprotective. The active ingredients present here may recover the disorders in hepatic damage and cardiotoxic state. The present results show that the extracts of *Tagetes erecta* linn produce a significant reduction of ALT AST and ALP and raised the SOD level which is good for oxidative stress and thus it act as drug. Similarly it also inhibit the elevated markers enzyme (CPK, LDH), thus also act as cardioprotective drug and we also concluded that *Tagetes erecta* had lesser side effect than silymarin.

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Corresponding Author:

Suresh Chandra*,

Email: *sureshcology81@gmail.com*