A study on the *in vitro* antioxidative property of some plant extracts and the *in vivo* effect of *Stevia rebudiana* extract against lindane induced liver toxicity.

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BY

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(08Mbc015, 08MBc011, 08MBc013).

UNDER THE GUIDANCE

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CERTIFICATE

This is to certify that the thesis entitled **"A study on the in vitro antioxidative property of some plant extracts and the in vivo effect of Stevia rebudiana extract against lindane induced liver toxicity"** submitted to the Department of Biochemistry and Biotechnology, Institute of Science, in partial fulfillment of the requirement for the award of the Degree of M.Sc. in Biochemistry, is a faithful record of bonafide research work carried out by **Ms. VAIBHAVI RAVAL** under the guidance of **Dr. sarika sinha** .No part of the thesis has been submitted for any degree or diploma. The candidate possesses minimum 75% attendance in the current academic session. The animal studies done for this thesis were approved by the CPCSEA (certificate attached).

I further certify that any help or information received during the work on this thesis has been duly acknowledged.

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ABSTRACT:

Antioxidants are the substances able to prevent or inhibit oxidation processes in human body as well as in food products. The important roles of reactive oxygen species in diseases related to aging and the necessity and benefits of antioxidative nutraceuticals in the prevention of diseases and promotion of healthy aging have been extensively reported in recent years. The natural antioxidants are a stable part of nutrition as they occur in almost all edible plant products. In current project, plants (*Prosopis cineraria, Rhus myserensis Cordia dichotoma)* from arid zone of INDIA and *Stevia rebaudiana* were investigated for their antioxidative activity in RBC model system using parameter like SOD, MDA, reduced glutathione. The synthetic antioxidant BHT (butylated hydroxyl toluene) was used as standard and oxidative stress induced using H_2O_2. These extract also used in the study for their prevention effect against oxidative damage using pBR322 plasmid DNA treated with H_2O_2 for inducing oxidative stress.

These extract prepared using peels/fruits/seeds/leaves of the plant by cold extraction in solvent like water, methanol, and acetone. Out of all ,extract acetone extract of *Stevia rebaudiana* leaves exhibit best antioxidant activity and it was further investigated *in vivo* in wistar rats for its probable curative effect against lindane induced liver toxicity using parameters like AFP, SGPT, SGOT, GGT, ALP, ALBUMIN. Positive results have been obtained after 15 days of treatment and the treatment will be continued for about 1 month.

Introduction:

Oxidative processes occurring in living organisms result in the formation of highly reactive free radicals that are known to cause accelerated aging of living cells and to increase the risk of health impairment (Finley *et al.,* 1993).It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (Diaz MN *et al.,* 1997, Effat souri *et al.,* 2007*)*

The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders. Currently, there is a growing interest towards natural antioxidants of herbal resources. Epidemiological Antioxidants are chemical species capable of terminating radical chain reactions (Meyskens *et al;* Byers *et al.,* 1995).In relation to these findings an extensive range of antioxidants both exogenous and endogenous whether synthetic or natural have been presented for the treatment or prophylaxis of disorders attributed to free radical oxidative damages (Cesquini *et al.,* 2003). The fact that various antioxidants occur naturally in plants has been proven (Yen *et al.,* 2000).Therefore, identification

and development of safer, natural antioxidants is more beneficial. Restriction on the use of synthetic antioxidants due to their probable side-effects has increased the contribution of natural antioxidants (Velioglu *et al.,* 1998). The antioxidant activity of several plant constituents, beyond the vitamins, in the form of crude extract or isolated compound has been put widely into consideration (Gazzani *et al.,* 1998; Larson *et al.,* 1988). Consumption of the flavonoids and their potential significance as antagonists of oxidative stress has been the interesting subject of many investigations (Cao *et al.,* 1996). Vegetables and fruits are also reported to decrease the risk of degenerative diseases and could have a protective effect against oxidative stress (Vinson *et al.,* 1998). Antioxidants are also important for food protection against deterioration reactions caused by atmospheric oxygen.

There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer (Salah *et al.,* 1995). Considerable effort has been directed in search for safe antioxidants from natural sources. Naturally occurring antioxidants could be found in fruits, vegetables, nuts, seeds, leaves, flowers, roots and barks. Extensive investigation of plants as sources of efficient radical-scavenging compounds has been under way in number of research groups in recent decade.

Detection of antioxidant property of plant extracts can be done by evaluating several blood parameters and check effect on RBC.

The natural antioxidants are a stable part of nutrition as they occur in almost all edible plant products. Polyphenols are the most numerous group of antioxidant components, and they are present in fruits and vegetables, their products, leguminous plants, grains, teas, herbs, spices and wines (Horubała 1999). Consumption of food containing a lot of polyunsaturated fatty acids raised the significance and usage of substances that protect them against oxidation. The antioxidant supplementation is a generally accepted method of prolonging the stability and storage life of food products, in particular the ones including fat.

However, the artificial compounds with antioxidant properties like butylated hydroxytoluene (BHT) have a limited allowance for food due to their potential cancerogenicity (Jayaprakasha *et al.*, 2003). The growing demand for natural antioxidants observed in food and cosmetic industries forces the search for new sources of these compounds. It has been attempted to discuss which plants and their by-products can be considered as a rich source of natural antioxidants and what methods should be used for their efficient extraction.

The competition between natural and synthetic antioxidants, in terms of consumer acceptance, legal needs for market access, toxicity and thermal stability, is a big problem. Great number of medicinal and other plants contains chemical compounds exhibiting antioxidant properties. Various studies could be carried out on such plants which may results in a development of natural antioxidant formulations for food, cosmetic and other application. Use of natural antioxidants, as food additives for inactivating free radicals receives a lot of attention nowadays, not only for their scavenging properties, but also because they are natural, non-synthetic products and their appreciation by consumers are very favourable. The fact that various antioxidants occur naturally in plants has been proven. Therefore, identification and development of safer, natural antioxidants is more beneficial. There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer, and it is generally assumed that these dietary elements, responsible for the protective effects, are antioxidant nutrients.

(http://www.labtestsonline.org/map/aindex.html)

In this study, plants were selected from arid zone of Rajasthan, *(Prosopis cineraria, Rhus myserensis, Cordia dichotoma) and Stevia rebaudiana*. However, scientific information on antioxidant properties of plants of arid zone is scarce.

Many plant-derived products exhibit potent antitumour activity against several rodent and human cancer cell lines [*Lin et al* 1996, *Nair et al* 2004]. They also possess potent anti-invasive and anti-metastatic activities [*Bracke et al* 1994, *Hirano et al* 1995].

During fetal development, AFP maintains high levels in the serum and drops to very low levels throughout the remaining of life. AFP may be slightly elevated or persisted in patients with large hepatic metastases or viral hepatitis.AFP measurement is widely accepted as tumor marker and for monitoring the therapeutic effectiveness of hepatocellular cancer. Slightly increased titres of AFP are pathognomic for an inflammation. If, during follow up investigations,a continous increase is stated a cancerous is probable.(Zhou L *et al* ,2006).

Introduction to plants:

1. ***Prosopis cineraria***

- **Binomial distribution**

Kingdom	Plantae
Division	Magnoliopsida
Class	Magnoliophyta
Order	Fabales
Family	Fabaceae
Genus	*Prosopis*
Species	*P. cineraria*

Table: 1

It is an extremely important tree of arid India. Green and unripe pods, known as 'Sangri', are used as vegetable and in the preparation of curries and pickles. The dry pods, known as 'Khokha' are used as an emergency food and help in preventing protein-calorie malnutrition. The powdered flowers mixed with sugar are used by women to safeguard themselves against miscarriage during pregnancy, and the flowers are used as a tonic for blood purifier and in the skin diseases (Toky).

Prosopis cineraria flower is pounded, mixed with sugar and used during pregnancy as safeguard against miscarriage. Water-soluble extract of the residue from methanol extract of the stem bark exhibits anti-inflammatory properties.

Prosopis cineraria plant produces gum, which is obtained during May and June. The bark of the tree is dry, acrid, and bitter with a sharp taste; cooling anthelmintic; tonic, cures leprosy, dysentery, bronchitis, asthma, leukoderma, hemorrhoids and muscle tremors. The smoke of the leaves is good for eye troubles. The bark is used as a remedy for rheumatism, cough, the common cold, asthma, and scorpion stings.

2.*Stevia rebaudiana*

- **Binomial distribution**

Kingdom	Plantae
Division	Magnoliopsida
Class	Magnoliophyta
Order	Fabales
Family	Fabaceae
Genus	*Prosopis*
Species	*P. cineraria*

Table: 2

Stevia is a genus of about 240 species of herbs and shrubs in the sunflower family (Asteraceae), native to subtropical and tropical regions from western North America to South America. The species *Stevia rebaudiana*, commonly known as sweet leaf, sugarleaf, or simply *Stevia*, is widely grown for its sweet leaves. As a sweetener and sugar substitute, *Stevia's* taste has a slower onset and longer duration than that of sugar, although some of its extracts may have a bitter or licorice-like aftertaste at high concentrations.(Tadesse bekele *et al*, 208)

With its extracts having up to 300 times the sweetness of sugar, *Stevia* has garnered attention with the rise in demand for low-carbohydrate, low-sugar food alternatives. Medical research has also shown possible benefits of *Stevia* in treating obesity and high blood pressure. Because *Stevia* has a negligible effect on blood glucose, it is attractive as a natural sweetener to people on carbohydrate-controlled diets. Health concerns and

political controversies have limited *Stevia's* availability in many countries. More recent medical research has shown promise in treating obesity (M.suttajit et al.,1993) and hypertension *Stevia* has a negligible effect on blood glucose, even enhancing glucose tolerance; therefore, it is attractive as a natural sweetener to diabetics and others on carbohydrate-controlled diets. (http://www.rain-tree.com/stevia.htm)

3. Cordia dichotoma

Used as a vegetable, raw, cooked, or pickled, and are known by many names, including *lasora* in Hindi. The objective of the investigation performed was to determine the antioxidant properties of pods, peels and seeds of different domestic fruits that are commonly available and readily consumed in Rajasthan, and to indicate which of them

can become a new source of natural antioxidants for food, cosmetic and pharmaceutical industries.

Free radicals and reactive oxygen species (ROS) damages the DNA by producing oxidized purines and pyrimidines, single strand breaks and alkali labile sites.

PLANT NAME	COMPOUNDS PRESENT
Prosopis cineraria	Sugar,flavonones, tannins
Stevia rebaudiana	Sterols,flavonoids, tannins.
Cordia dichotoma	Tannins,alkaloids, flavanoids.
Rhus myserensis	Tannins, terpenoides.

Table: 3

Oxidative damage of DNA, proteins, and lipids by oxidants has been implicated in a number of pathological conditions such as cancer, aging, and cardiovascular diseases. Hydrogen peroxide (H_2O_2), a ROS may be involved in the formation of hydroxyl radicals, which are highly reactive, destructive, and results in direct DNA damage. Lipid peroxidation is a highly damaging event that results from the interaction of ROS with cellular membrane lipids. It not only drastically alters the structure and function of membranes, but also generates highly toxic by-products (Meneghini R, 1993). Fortunately, various beneficial compounds known as antioxidants controlled free radical formation naturally. Antioxidants are a group of substances when present at low concentrations compared to oxidized substrates significantly inhibit or delay oxidative processes, while being oxidized themselves.

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1. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily. Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion ($\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$) (Halliwell 1997). Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems (Stief 2000, 2003). Hydrogen peroxide can degrade certain heme proteins, such as haemoglobin, to release iron ions.

2. DNA strand breaks and modification and aging

Mitochondria and nuclei have their own DNA. Mitochondrial DNA is susceptible to oxidative damages because of the lack of protective protein, histones, and close locations to the reactive oxygen species-producing systems. Hydroxyl radical oxidizes guanosine or thymine to 8–hydroxy–2 deoxyguanosine and thymine glycol, respectively, which changes DNA and leads to mutagenesis and carcinogenesis (Ames *et al.*, 1993). 8–Hydroxy–2–deoxyguanosine has been used as a biological marker for oxidative stress. Altered DNA can be repaired by DNA glycosylase. A low level of oxidative base damage in DNA is found in the cells of a healthy person. However, concentration of oxidized DNA base increases in humans with chronic inflammatory diseases such as rheumatoid arthritis or under oxidative stresses such as smoking (Halliwell 1997). If oxidative stress is too great, the DNA repair system using glycosylase is not enough, and mutagenesis and/or carcinogenesis can be induced.

3. DNA Damage and Cancer

Damage to DNA by ROS has been widely accepted as a major cause of cancer (Ames BN 1983). In patients with diseases associated with a risk of cancer indicates an increased rate of oxidative DNA damage or in some instances deficient repair system such as Fanconi anaemia, chronic hepatitis, cystic fibrosis and various autoimmune diseases (Takeuchi T *et al.*, 1993, Hagen TM *et al.*, 1994, Shimoda R *et al.*).

Human studies support the experimentally based notion of oxidative DNA damage as an important mutagenic and apparently carcinogenic factor. ROS can damage DNA and the division of cells with unpaired or misrepaired damage leads to mutations. The majority of mutations induced by ROS appear to involve modification of guanine, causing G→T transversions (Du MQ *et al.*, 1994, Denissenko MF et *al.*, 2002, Ames BN *et al.*, 1993). If it relates to critical genes such as oncogenes or tumor suppressor genes, initiation/progression can result (Ames BN *et al.*, 1993). Indeed, these species can act at several steps in multistage carcinogenesis. It is now assumed that ROS are involved both in the initiation and progression of cancer (Moller P *et al.*, 1998). Mutations caused by oxidative DNA damage include a range of specifically oxidized purines and pyrimidines, alkali labile sites, single strand breaks and instability formed directly or by repair processes. Because of the multiplicity of DNA

modifications produced by ROS, it has been difficult to establish the frequency and specificity of mutations by individual oxygen radical induced lesions. Some of these modified bases have been found to possess mutagenic properties. Therefore, if not repaired they can lead to carcinogenesis. In human tumors, G to T transversions are the most frequent mutations in the p53 suppressor gene (Hollstein M *et al.,* 1991, Harris CC *et al.,*1993).Using single stranded DNA template in a sensitive forward mutation system, various mutations, including tandem double CC→TT substitution have been observed in DNA treated with oxygen free radicals (Reid TM *et al.,* 1993). Elevated levels of modified bases in cancerous tissue may be due to the production of large amount of H_2O_2, which has found to be characteristic of human tumor cells (Szatrowski TP *et al.,* 1991). Initiation of cancer in humans by ROS is further supported by the presence of oxidative DNA modifications in cancer tissue. (Ames BN *et al.,* 1993).

4. Antioxidant

4.1 History

The term antioxidant (also “antioxygen”) originally was used to refer specifically to a chemical that prevented the consumption of oxygen. In the late 19th and early 20th century, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines (Matill HA 1947). Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity (German J 1999). Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of Vitamins A, C and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in biochemistry of living organisms. (Jacob R 1996, Knight J 1998).

The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized. Research into how Vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent

oxidative reactions, often by scavenging reactive oxygen species before they can damage cells .(Wolf G 2005).

4.2 Antioxidative enzymes

Antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase/reductase, convert reactive oxygen species into nonreactive oxygen molecules. Proteins showing antioxidant properties are Superoxide dismutase (SOD) which converts superoxide anion into hydrogen peroxide and oxygen. There are 2 types of SOD: a magnesium- containing SOD and a copper-zinc–dependent SOD. Catalase is involved in cellular detoxification and can convert hydrogen peroxide into water and oxygen. Glutathione peroxidase is the most important hydrogen peroxide–removing enzyme existing in the membrane. Glutathione disulfide reductase is a flavoprotein that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADH to NAD^+.

4.2a SUPEROXIDE DISMUTASE

Superoxide dismutase (SOD) was first isolated by Mann and Keilis (1938) and thought to be a copper storage protein. SOD is known to catalyze the dismutation of superoxide to hydrogen peroxide and oxygen:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2$$

Since SOD is present in all aerobic organisms and most subcellular compartments that generate activated oxygen, it has been assumed that SOD has a central role in the defence against oxidative stress. There are three distinct type of SOD classified on the basis of the metal factor:
Copper/Zinc (Cu/Zn – SOD), Manganese (Mn – SOD) and Iron (Fe – SOD)

These isozymes can be separated by native polyacrylamide gel electrophoresis, their activity detected by negative staining and identified on the basis of their sensitivity to KCN and H_2O_2.

SOD activity is increased in cells response to diverse environmental and xenobiotic stresses including high light, water logging and drought. Apparently, each of the SOD isozymes are independently regulated according to the degree of oxidative stress experienced in

the respective sub cellular compartments, but how this is communicated at the molecular level is unknown.(Bowler *et al* 1992) have suggested that this role may be served by unique lipid peroxidation products from each organelle that diffuse from the site of oxidative damage to the nucleus where they would enhance transcription of specific SOD genes.

4.2b REDUCED GLUTATHIONE

Glutathione acts as a disulfide reductant to protect thiol groups on enzymes, regenerate ascorbate and react with singlet oxygen and hydroxyl radicals. It acts as a protein disulfide reductant, which detoxifies herbicides by conjugation and regulates gene expression in response to environmental stress and pathogen attack. It also participates in the regeneration of ascorbate from dehydroascorbate reductase. In such reactions GSH is oxidized to glutathione disulfide (GSSG). GSH is regenerated by glutathione reductase (GR) in a NADPH – dependent reaction.

4.2c LIPID PEROXIDATION:

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between

which tie methylene -CH2- groups that possess especially reactive hydrogens. As with any radical reaction the reaction consists of three major steps: initiation, propagation and termination.

Quantification of the end products of lipid peroxidation, specifically malonaldehyde (MDA) (A.B. Hancock., 1998) the most commonly used test is called a TBARS Assay (thiobarbituric acid reactive substances assay). Thiobarbituric acid reacts with malonaldehyde to yield a fluorescent product. However, there are other sources of malonaldehyde, so this test is not completely specific for lipid peroxidation. (Maurizio Trevisan)

Malonaldehyde is the organic compound with the formula CH2 (CHO) 2. (A.B. Hancock., 1998) The structure of this species is more complex than this formula suggests. This reactive species occurs naturally and is a marker for oxidative stress.

Reactive oxygen species degrade polyunsaturated lipids, forming malonaldehyde. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells.

5. PESTICIDES

A pesticide is a substance or mixture of substances used to kill a pest. A pesticide may be a chemical or biological agent such as a virus or bacteria used against any pest [http://www.epa.gov/pesticides/about/index.htm].

Since before 2500 BCE, humans have utilized pesticides to protect their crops. The first known pesticide was elemental sulfur dusting used in Sumeria about 4,500 years ago. By the 15th century, toxic chemicals such as arsenic, mercury and lead were being applied to crops to kill pests. In the 17th century, nicotine sulphate was extracted from tobacco leaves for use as an insecticide. The 19th century saw the introduction of two more natural pesticides, pyrethrum which is derived from chrysanthemums and rotenone which is derived from the roots of tropical vegetables [*Miller 2002*].

In 1939, Paul Muller discovered that DDT was a very effective insecticide. It quickly became the most widely-used pesticide in the world.

5.1LINDANE

Lindane is an organochlorine insecticide and fumigant which has been used on a wide range of soil-dwelling and plant-eating (phytophagous) insects. It is commonly used on a wide variety of crops, in warehouses, in public health to control insect-borne diseases, and (with fungicides) as a seed treatment. Lindane is also presently used in lotions, creams, and shampoos for the control of lice and mites (scabies) in humans. Lindane is a moderately toxic compound in EPA toxicity class II. Labels for products containing it must bear the Signal Word WARNING. Some formulations of lindane are classified as Restricted Use Pesticides (RUP), and as such may only be purchased and used by certified pesticide applicators. Lindane is no longer manufactured in the U.S., and most agricultural and dairy uses have been cancelled by the EPA because of concerns about the compound's potential to cause cancer.

Technically lindane is comprised of the gamma-isomer of hexachlorocyclohexane, HCH. Five other isomers (molecules with a unique structural arrangement, but identical chemical formulas) of HCH are commonly found in technical lindane, but the gamma-isomer is the predominant one, comprising at least 99% of the mixture of isomers. Data presented in this profile are for the technical product unless otherwise stated; lindane, HCH, or BHC refer to technical lindane, i.e., Gammahexachlorocyclohexane.

Gamma-HCH has been shown to be the insecticidally effective isomer. [*Ebadi 2006*]

Lindane may also be found in formulations with a host of fungicides and insecticides. It is available as a suspension, emulsifiable concentrate, fumigant, seed treatment, wettable and dustable powder, and ultra low volume (ULV) liquid.

5.2 TOXICOLOGICAL STUDIES

Lindane is a moderately toxic compound via oral exposure, with a reported oral LD50 of 88 to 190 mg/kg in rats. Other reported oral LD50 values are 59 to 562 mg/kg in mice, 100 to 127 mg/kg in guinea pigs, and 200 mg/kg in rabbits (*Smith 1991)*. Gamma-HCH is generally considered to be the most acutely toxic of the isomers following single administration .It is moderately toxic via the dermal route as well, with reported dermal LD50 values of 300 mg/kg in mice, 500 to 1000 mg/kg in rats, 400 mg/kg in guinea pigs, and 300 mg/kg in rabbits [*Kidd and James 1991, Ebadi 2006*]. Notably, a 1% solution of lindane in vanishing cream resulted in a six-fold increase in acute toxicity via the dermal route in rabbits, with a reported dermal LD50 of 50 mg/kg (*Ebadi 2006*). It is reported to be a skin and eye irritant [*Kidd and James 1991*]. Calves are especially susceptible to dermal application [*Ebadi 2006*].

5.3 METABOLISM OF LINDANE

HCH (lindane) breaks down into many other substances. One of these substances is pentachlorophenal which is known to be harmful. Another substance, called chlorinated hydrocarbon epoxide, may cause cancer in animals. Angerer *et al* (1983) determined that chlorophenals were the primary urinary metabolites of γ-HCH excreted by workers involved in γ-HCH production. The metabolites 2,3,5-, 2,4,5- and 2,4,6-trichlorophenal accounted for almost 57.7% of the γ-HCH metabolites identified in the urine collected during the last 2 hours of the workers' shifts. Other urinary metabolites included other trichlorophenols, dichlorophenols, tetrachlorophenols, and dihydroxychlorobenzenes. Pentachlorophenol has also been identified as a urinary metabolite in humans following occupational exposure [*Engst et al 1979*]. In vitro investigations indicate that human liver microsomes convert γ-HCH by dechlorination, dehydrogenation, dehydrochlorination, and hydroxylation to five primary metabolites: 3, 6/4,5- hexachlorocyclohexane, pentachlorocyclohexane, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorobenzene [*Fitzloff et al 1982*]. Similar in vitro studies have demonstrated that an epoxide forms during the metabolism of pentachlorocyclohexane. This stable halogenated hydrocarbon epoxide metabolite may be responsible for the mutagenic and carcinogenic effects of γ-HCH [*Fitzloff and Pan 1984*].

In animals, γ-HCH appears to be transformed by hepatic enzymes to form chlorophenols, chlorobenzene, chlorocyclohexanes, chlorocyclohexanals, and conjugates of mercapturic acid glucuronide, and sulfate [*Chadwick and Freal 1972,* 14 *Kujawa et al 1977, Chadwick et al 1978, Engst et al 1979*]. These metabolites have been identified in various tissues and in the urine of laboratory animals. Metabolites found in the liver of rats following intermediate exposure of γ-HCH via gavage or diet include di-, tri-, tetra-, and pentachlorobenzenes; pentachlorocyclohexanes; and pentachloro-2-cyclohexen-1-ol [*Chadwick and Freal 1972, Kujawa et al 1977*]. Metabolites identified in the blood of these rats include di-, tri-, tetra-, and pentachlorophenols and pentachloro-2-cyclohexen-1-ol [*Kujawa etal 1977*] Di-,tri-,and tetrachlorophenols; pentachlorocyclohexenes; and pentachloro-2 cyclohexen-1-ol have been identified in samples of kidney, spleen, heart, and brain tissue from rats fed γ-HCH [*Kujawa et al 1977*]. Metabolites found in the urine include tri-, tetra-, and pentachlorophenol; pentachloro-2-cyclohexen-1-ol; and isomers of tetrachloro-2- cyclohexen-1-ol [*Chadwick and Freal 1972, Kujawa et al 1977, Chadwick et al 1978*]. The metabolism of γ-HCH in the intestine was reported to be very minor, or the metabolites were completely absorbed. No metabolites were detected in the faeces or in the adrenal gland [*Kujawa et al 1977*]. *In vitro* preparations using rat liver slices have also found that γ-HCH is converted to hexachlorobenzene [*Gopalaswamy and Aiyar 1984*].

OBJECTIVES:

1) To study antioxidant potential of *Prosopis cineraria, Stevia rebaudiana, Rhus myserensis* and *Cordia dichotoma* extracts against H_2O_2 induced oxidative stress in RBC model system and pBR322 Plasmid DNA.

2) To study the effect of acetone extract of *Stevia rebaudiana* on lindane induced liver toxicity in wistar rats using parameters like AFP, SGPT, SGOT, ALP, ALBUMIN, and GGT.

MATERIALS AND METHODS:

1) *IN VITRO*

Blood (Prathma diagnostic lab) Trichloroacetic acid, Dextrose, Phenol, Isoamyl alcohol, Agarose (SRL Pvt Ltd.) , Hydogen peroxide,$Na_2HPO_4.2H_2O$, Sodium Chloride, Boric acid, Glaciel acetic acid, Chloroform, Bromo phenol blue (MERCK), Sodium Citrate, EDTA, Potassium acetate , Sodium hydroxide, KH_2PO_4 and K_2HPO_4 (sd fine chemical pvt. Limited), DTNB, Riboflavin, NBT (HIMedia), Thiobarbituric acid (Chemport pvt Ltd), Ampicillin (CDH Lab system), Ethanol (Ureca consumer).

2) *IN VIVO*

Wistar rats (Female) (zydus pharmaceuticals), NaCl (sd fine chemical pvt limited) , Fomaldehyde, Methanol, Acetone. (SRL Pvt Ltd), AFP (OMEGA diagnosis Ltd. UK), SGPT, SGOT, ALP, GGT Kit (Accucare), Surgical instruments, Lindane (65%), pesticide (Indian pesticide Ltd.lucknow, INDIA).

3) PLANT MATERIALS

Fruits of *Rhus myserensis* and *Cordia dichotoma*, leaves of *Stevia rebaudiana* while pods of *Prosopis cineraria* were collected from arid regions of Udaipur, Rajasthan (India). The fruits and pods were gathered directly from trees and shrubs and were stored in cold conditions until the moment of analysis. For examination only healthy looking fruits and pods were chosen (without mechanical damages and bacterial infection).

Plant	**Part used**
Prosopis cineraria	Pods
Stevia rebudiana	Leaves
Rhus myserensis	Peel
Cordia dichotoma	Seeds

Table: 4

Preparation of plant extract

Peels were cut off with a stainless steel knife. Peels and seeds were separated and dried naturally for five days. Dried samples were crushed with mortar and pestle and grinded into powdered form and

stored in a dry place, protected from light until used. Powdered samples were extracted with Methanol (80%), Water and Acetone.

1) Stevia rebaudiana

25 g of air-dried powder of *Stevia rebaudiana* leaves was immersed in 100 mL of organic solvent (methanol, acetone, chloroform) and water separately in a conical flask. It was incubated at room temperature for 48 hour at 150 rpm in an orbital shaker. The suspension was filtered and concentrated to dryness at 40^{o}C. The extract was dissolved in 0.25% Dimethyl Sulphoxide (DMSO, Merck) to a concentration of 100 mg/mL. (Satishkumaar *et al* , 2007)

2) *Prosiopis cinereria, Rhus myserensis, Cordia dichotoma.*

Extraction was applied by flasks with 10 g of sample and 200 ml of solvent ($CH_3OH/H_2O/CH_3COCH_)$ flasks are then incubated in shaker at 1200 rpm for 2 days. The obtained extracts were filtered and concentrated to dryness at 64ºC for methanol and 100ºC for water extracts and dissolved in minimum respective solvents.

4) BLOOD

Human blood samples were obtained from PRATHMA BLOOD CENTRE - ADVANCED TRANSFUSION MEDICINE RESEARCH FOUNDATION. Blood samples were provided in vacutainers coated with anticoagulant K_3EDTA.

ISOLATION OF ERYTHROCYTES FROM WHOLE BLOOD.

MATERIALS:

150 mM NaCl (freshly prepared), 100 mM isotonic sodium phosphate buffer (pH 7.4) and refrigerated centrifuge.

METHOD:

Human blood (5 mL) obtained in vacutainer was centrifuged at 3000 rpm for 15 minutes in a refrigerated centrifuge. Plasma and buffy coat were removed by aspiration. Cells were washed three times with freshly prepared 150 mM NaCl. After the last wash the cells were suspended in 100 mM sodium phosphate buffer (pH 7.4) such that the total volume obtained was 5 mL. The cells were then diluted with isotonic sodium phosphate buffer so as to obtain 50×10^6 cells per mL. (Mohan singh *et al*, 2004).

5) **Determination of Antioxidative enzymes**

a) REDUCED GLUTATHIONE

Principle

Glutathione is present inside cells mainly in the reduced form (90-95% of the total glutathione).Oxidation of glutathione leads to the formation of glutathione disulfide (GSSG).SH group of reduced glutathione reduce 5,5-dithiobis,2-nitrobenzoic acid (DTNB) and form a yellow coloured product. It can be determined spectrophotometrically at 412nm. (Ferreira *et al,* 1999).

Reagents

1)10% TCA

2)0.3 M $Na_2HPO_4.2H_20$

3)1% Sodium Citrate

4) DTNB (5, 5-dithiobis,2-nitrobenzoic acid)

Procedure

1 ml RBC mixed with 1 ml 10% chilled TCA, mixture kept in ice for 30 min and centrifuge at 3000 rpm for 10 min at 4°C .After centrifugation at 3000 rpm for 10 min, 0.5 ml supernatant was added to 2 ml of 0.3 M Na_2HPO_4 solution. Then 0.2 ml DTNB (0.4 mg/ml in

1% sodium citrate) was added and absorbance was measured immediately at 412 nm.

b) Malonaldehyde

Principle

Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid reactive substances (TBRAS) as described by Health and Packer (1968). MDA forms pink color complex with TBA which is determined spectrophotometrically at 532nm. The intensity of pink color increases with concentration of MDA. (Ferreira *et al*, 1999).

Reagents

- 5% TCA (Trichloro acetic acid)

2.5% g of TCA was dissolved in DDW and final volume was made to 50 ml.

- 20% g TCA

20 g of TCA was dissolved in DDW and final volume was adjusted to 100ml.

- 0.5% TBA (Hi media) in 20% TCA

0.25 g TBA was dissolved in 20% TCA and final volume was made to 50ml.

Method

1.5 ml of 5% TCA and 4ml of TBA reagent (0.5% in 20% TCA) was mixed and used as a blank. For correction blank,1 ml of homogenate and 4ml TBA reagent were mixed .After heating for 30 min at 95°C in a water bath the mixture was cooled and centrifuged for 10 min at 4000g. The absorbance was measured at 532nm and corrected for non specific absorbance at 600nm and for the absorbance at 532nm of the correction blank. The correction of MDA was calculated by using an extinction coefficient at $155mM^{-1}cm^{-1}$.

c) Superoxide dismutase

Principle

SOD catalyzes the dismutation of the peroxide radical (O_{2}^{-}) into H_2O_2 and elemental oxygen. The most typical SOD detection method is the one based on spectrophotometric detection. This method uses nitroblue terazolium (NBT). In the assay, superoxide ions, generated by riboflavin converts NBT to NBT – diformazan, which absorbs light at 560nm. SOD reduces the superoxide ion concentration and thereby lowers the rate of NBT-diformazan formation. The extent of reduction in an appearance of NBT-diformazan is a measure of SOD

activity present in an experimental sample. (Giannopolitis *et al*, 1977).

Reagents

a) 1M Tris buffer (pH8.9)(Hi media)

6.0565 g of tris buffer was dissolved in DDW and volume was made to 40 ml. The pH was adjusted to 8.9 with 10% HCL and final volume was adjusted to 50 m.

b) NBT (6mM) (Hi media)

0.0245 g of NBT was dissolved in DDW and final volume was made to 5 ml.

c) Riboflavin (600 µM in 5 mM KOH)(Hi media)

0.00225 b of riboflavin was dissolved in 5 mM KOH and final volume was adjusted to 10 ml.

d) BSA (3.3 multiplied by 0.003%w/v) (sigma)

0.165 BSA was dissolved in DDW and final volume was made to 50 ml.

Method (Giannopolitis and ries 1977):

3.0 ml reaction mixture consisted of 2.5 ml Tris buffer (pH 8.9), 0.1 ml bovine serum albumin, 0.1 ml riboflavin and 0.2 ml of the enzyme extract. Identical unilluminated assay mixtures used as a reference. For blank enzyme extract was replaced with Tris buffer.

Both sample and blank tubes were illuminated under fluorescent light for 1 minute at an interval of 5 seconds.(OD at 560 nm.)

Formula used

$$\frac{\text{O.D OF COLOUR CONTROL (positive control) - OD OF SAMPLE}}{\text{O.D OF CONTROL}} \times 100$$

6) ISOLATION OF PLASMID

Preparation of Plasmid DNA by Alkaline Lysis with SDS: Mini preparation

Plasmid DNA is isolated from small-scale (1-2 ml) bacterial cultures by treatment with alkali and SDS. (Sambrook and russel).

MATERIALS

Buffers and Solutions

Alkaline lysis solution I

Alkaline lysis solution II

Alkaline lysis solution III

Antibiotic for plasmid selection

Ethanol

Phenol:chloroform (1:1, v/v)

TE (pH 8.0) containing 20 µg/ml RNase A

Alkaline Lysis Solution I

50 mM glucose

25 mM Tris-Cl (pH 8.0)

10 mM EDTA (pH 8.0)

Prepare Solution I from standard stocks in batches of approx. 100 ml, autoclave for 15 minutes at 15 psi (1.05 kg/cm2) on liquid cycle, and store at 4°C.

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Alkaline Lysis Solution II

0.2 N NaOH (freshly diluted from a 10 N stock)

1% (w/v) SDS

Prepare Solution II fresh and use at room temperature.

Alkaline Lysis Solution III

5 M potassium acetate, 60.0 ml

glacial acetic acid, 11.5 ml

H_2O, 28.5 ml

METHOD

1. Inoculate 2 ml of rich medium (LB) containing the appropriate antibiotic with a single colony of transformed bacteria. Incubate the culture overnight at 37°C with vigorous shaking.

2. Pour 1.5 ml of the culture into a microfuge tube. Centrifuge at maximum speed for 30 seconds at 4°C in a microfuge.

3. Remove the medium by aspiration, leaving the bacterial pellet as dry as possible.

4. Resuspend the bacterial pellet in 100 µl of ice-cold Alkaline lysis solution I by vigorous vortexing.

5. Add 200 µl of freshly prepared Alkaline lysis solution II to each bacterial suspension. Close the tube tightly, and mix the contents by inverting the tube rapidly five times. *Do not vortex!* Store the tube on ice (5 min).

6. Add 150 µl of ice-cold Alkaline lysis solution III. Close the tube and disperse Alkaline lysis solution III through the viscous bacterial lysate by inverting the tube several times. Store the tube on ice for 15 minutes.

7. Centrifuge the bacterial lysate at maximum speed for 5 minutes at 4°C in a microfuge. Transfer the supernatant to a fresh tube.

8. Add an equal volume of phenol:chloroform. Mix the organic and aqueous phases by vortexing and then centrifuge the emulsion at

maximum speed for 2 minutes at 4°C in a microfuge. Transfer the aqueous upper layer to a fresh tube.

9. Precipitate nucleic acids from the supernatant by adding 2 volumes of ethanol at room temperature. Mix the solution by vortexing and then allow the mixture to stand for 2 minutes at room temperature.

10. Collect the precipitated nucleic acids by centrifugation at maximum speed for 5 minutes at 4°C in a microfuge.

11. Remove the supernatant by gentle aspiration as described in Step 3 above. Stand the tube in an inverted position on a paper towel to allow all of the fluid to drain away.

12. Add 1 ml of 70% ethanol to the pellet and invert the closed tube several times. Recover the DNA by centrifugation at maximum speed for 2 minutes at 4°C in a microfuge.

13. Remove all of the supernatant by gentle aspiration as described in Step 3.Take care with this step, as the pellet sometimes does not adhere tightly to the tube.

14. Remove any beads of ethanol that form on the sides of the tube. Store the open tube at room temperature until the ethanol has evaporated and no fluid is visible in the tube (5-10 minutes).

15. Dissolve the nucleic acids in 50 µl of TE (pH 8.0) containing 20 µg/ml DNase-free RNase A.Vortex the solution gently for a few seconds. Store the DNA solution at -20°C.

Effect of varying concentration of hydrogen peroxide at various time intervals

a) RED BLOOD CELLS

To study the effect of hydrogen peroxide on red blood cells, the erythrocytes were incubated with hydrogen peroxide at concentrations of 0.4% hydrogen peroxide (H_2O_2). To study the effect of hydrogen peroxide on lipid peroxidation, glutathione, sod levels, the erythrocytes were incubated with hydrogen peroxide for 20 minutes.

b) PLASMID

pBR322 plasmid was obtained from already transformed cultures of DH5α (M.S.U BARODA) incubated with 30% hydrogen peroxide for one hour.

7) EXPERIMENTS ON SERUM

Rat's blood (5 mL) was centrifuged at 3000 rpm for 15 minutes in a refrigerated centrifuge. The supernatant i.e. the serum was removed with the help of micropipette. The serum was then used for enzyme assays.

DETAILED STUDY PLAN

Group	No.of animals (female wistar rats)
Uninduced &untreated[control]	4
Induced& Untreated	6
Induced &treated [200mg/kg per body weight]	6
Induced &treated [300mg/kg per body weight]	6
Induced &treated [400mg/kg per body weight]	6
Total	**28**

]Table: 5

Lindane was orally fed at a dose regime of 300ppm/kg body wt /day for 30 days (Diluted in coconut oil).

7.1 DETERMINATION OF SGOT [ACCUCARE, lab care diagnosis (INDIA) PVT. Ltd.]

MATERIALS

SGOT (AST) Reagent kit obtained from accucare.

PRINCIPLE

Aspartate transaminase (GOT-AST) catalyses the reaction between alpha-ketoglutaric acid and L-aspartate giving glutamate and oxaloacetate. Oxaloacetate, in the presence of malate dehydrogenase (MDH) reacts with NADH giving malate and NAD. The rate of NADH decrease is determined spectrophotometrically at 340nm and is directly proportional to the GOT activity in the sample. (Nyblom H *et al, 2004*).

METHOD:

Take 100µl serum sample and 1000µl reagent. Mix well and let stand for 1 min at 37°C. Measure absorbance decrease per minutes and determine the ΔA/min.

7.2 DETERMINATION OF ALBUMIN [ACCUCARE,lab care diagnosis (INDIA) PVT. Ltd.]

MATERIALS

ALBUMIN reagent kit obtained from accucare.

PRINCIPLE

The method of serum albumin is based on its quantitative binding to the indicator 3, 3'5, 5'-tetrabromo-m-creasolo sulphophthalein (bromo cresol green, BCG). The albumin-BCG complex absorbs maximally at 578nm.

METHOD

Take 5µl sample and 1000µl reagent. Mix well and wait for 5 minutes at room temperature. Measure the absorbance of the sample (Ac) and Standard (As) against the reagent blank.

7.3 DETERMINATION OF GGT [ACCUCARE,lab care diagnosis (INDIA) PVT. Ltd.]

MATERIALS

GGT reagent kit obtained from accucare.

PRINCIPLE

Gamm-glutamyl is transferred from Gamma-glutamyl-p-nitroanilide to glycylglycine by Gamma-GT (Gamma-Glutamyl-transferase).The p-nitroaniline formed absorbs at 405nm. The amount of p-nitroaniline formed is directly proportional to gamma-GT activity.

METHOD

Take 100µl sample and 1000µl reagent. Mix well and incubate for one min at 37°C.Read initial absorbance and star timer simultaneously, read again after 1, 2 and 3 min. Calculate (ΔA /min).

7.4 DETERMINATION OF SGPT [ACCUCARE,lab care diagnosis (INDIA) PVT. Ltd.]

MATERIALS

SGPT reagent kit obtained from accucare.

PRINCIPLE

Glutamic-pyruvic transaminase (GPT-ALT) catalyses the reaction between alpha-ketoglutamic acid and alanine giving L—glutamic acid and pyruvic acid. Pyruvic acid, in the presences of lactate dehydrogenenase (LDH) reacts with NADH giving lactate acid and NAD. The rate of NADH consumption is determined spectrophotometrically at 340nm and is directly proportional to the GPT activity in the sample (Nyblom H *et al, 2004*)

METHOD

Take 100µl serum sample and 1000µl reagent. Mix well and let stand for 1 min at 37°C. Measure absorbance decrease per minutes and determine the ΔA/min.

7.5 AFP [omega diagnostics, pathozyme alpha fetoprotein-enzyme-Immunoassay (EIA)]

MATERIALS

AFP reagent kit obtained from OMEGA diagnosis Ltd. UK and Elisa reader [Biorad].

PRINCIPLE

Specific rabbit anti-AFP antibodies are prepared, purified, and coated onto microtitration wells. Test sera are applied and incubated with Zero Buffer. If human AFP is present in the specimen, it will bind to the antibodies in the well. Unbound material is wasted away and mouse monoclonal anti –AFP antibody, labelled with horseradish peroxidise enzyme (conjugate) is added. The conjugate binds to the AFP which is bound to the anti bodies. Unbound material is again washed away.

On addition of the substrate(TMB) ,a color will develop only in those well in which enzyme is present ,indicating the presences of AFP .The enzyme reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of AFP is directly proportional to the color intensity of the test sample. (http://www.webmd.com/baby/alpha-fetoprotein-afp-in-blood)

METHOD

1. Dispense 20µl of test serum or standards and 100µl of zero buffer into each well and mix gently for 30 seconds.

2. Incubate for 30 minutes at room temperature (20°C to 25°C).

3. Discard well contents and wash five times with distilled water.

4. Dispense 150µl of Anti-AFP HRP.Conjugate into each well. Gently mix for 5 seconds.

5. Incubate it for 30 mintutes at room temperature (20°c-25°c).

6. Discard well contents and wash five times.

7. Add 100µl of substrate solution to each well and gently shake for 5 seconds.

8. Incubate in the dark for 20 minutes at room temperature (20°C - 25°C).

9. Add 100µl of stop solution to each well and gently shake for 30 seconds.

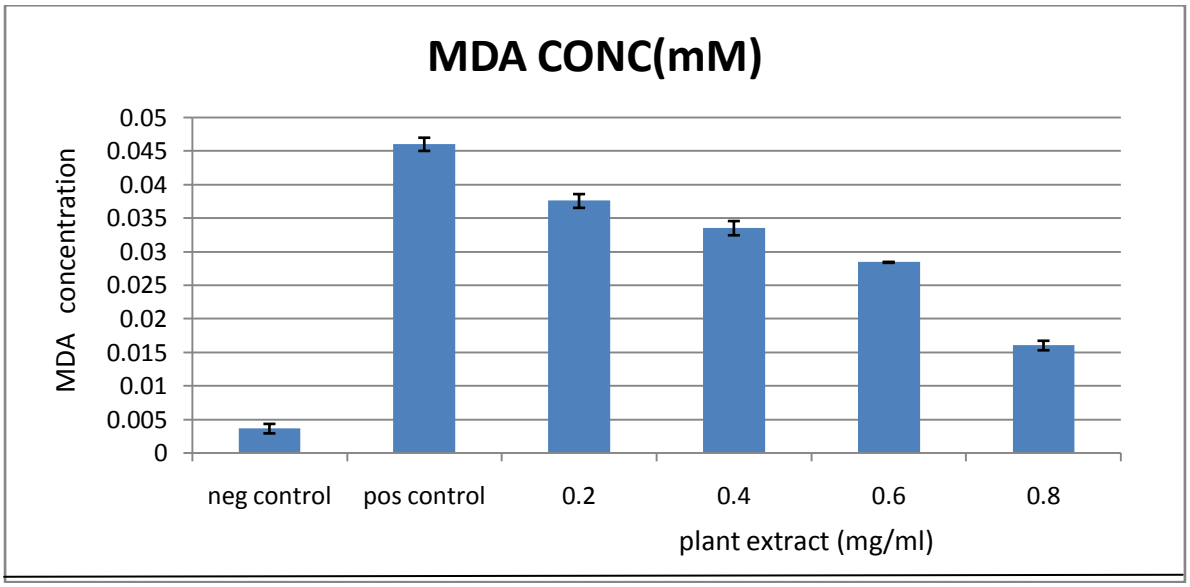
10. Read the optical densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

OBSERVATION AND RESULTS:

1. MALONALDEHYDE

- **Evaluation of antioxidant activity of BHT (standard antioxidant) on MDA levels in erythrocyte membrane.**

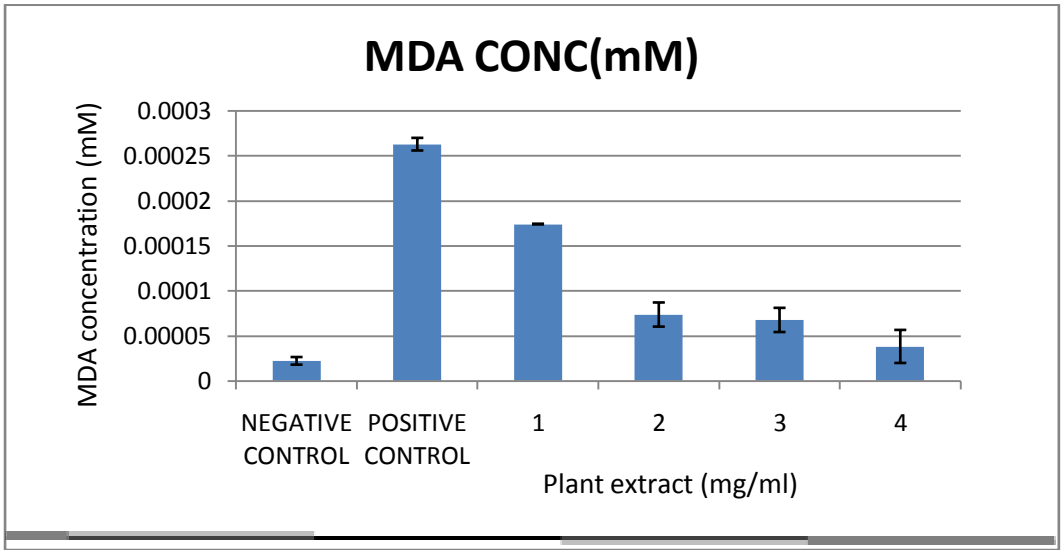
Conc. Of BHT(mg/ml)	Conc. Of MDA (mM)
Positive control	0.0610
Negative control	0.00361
0.2	0.03755
0.4	0.03350
0.6	0.02840
0.8	0.01600



Graph *In vitro* effect of varying conc of BHT (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc of plant extract decrease in MDA levels were obsereved

1a. EFFECTS OF AQUEOUS EXTRACT OF *Stevia rebaudiana* ON MDA LEVELS IN ERYTHROCYTE MEMBRANE.

Concentration Of Extract (mg/ml)	Concentartion Of MDA (mM)
Positive control	2.63×10^{-5}
Negative control	2.20×10^{-4}
1	17.42×10^{-5}
2	73.50×10^{-5}
3	67.50×10^{-5}
4	38×10^{-4}

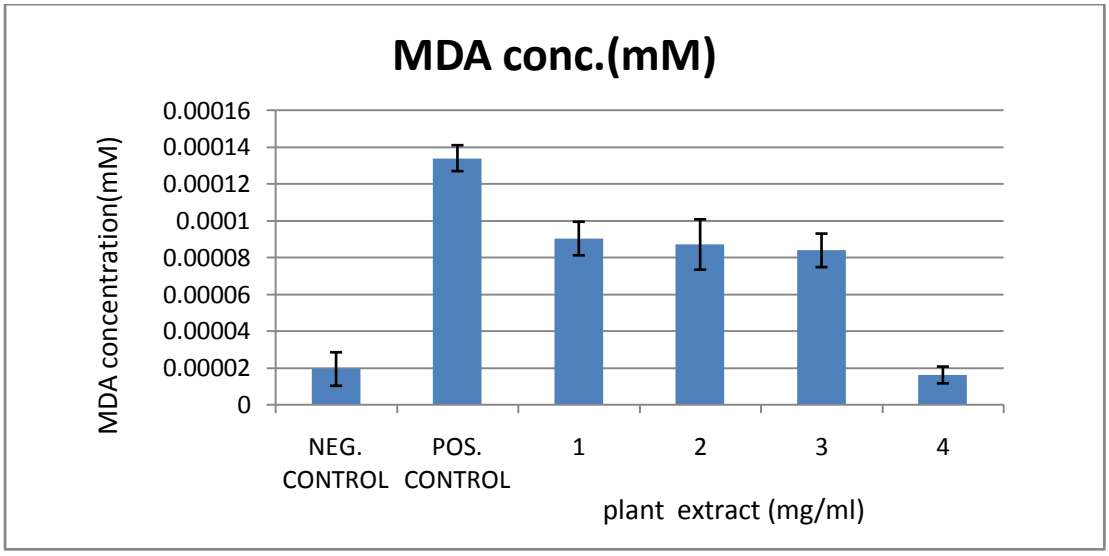


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 1.*In vitro* effect of varying conc of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc of plant extract increase in MDA levels were obsereved.

1b. EFFECTS OF METHANOLIC EXTRACT OF *Stevia rebaudiana* ON MDA LEVELS IN ERYTHROCYTE MEMBRANE.

Concentartion Of Extract(mg/ml)	Concentration Of MDA (mM)
Positive control	1.90×10^{-5}
Negative control	13.40×10^{-4}
1	9.03×10^{-5}
2	8.70×10^{-5}
3	8.30×10^{-5}
4	1.61×10^{-5}

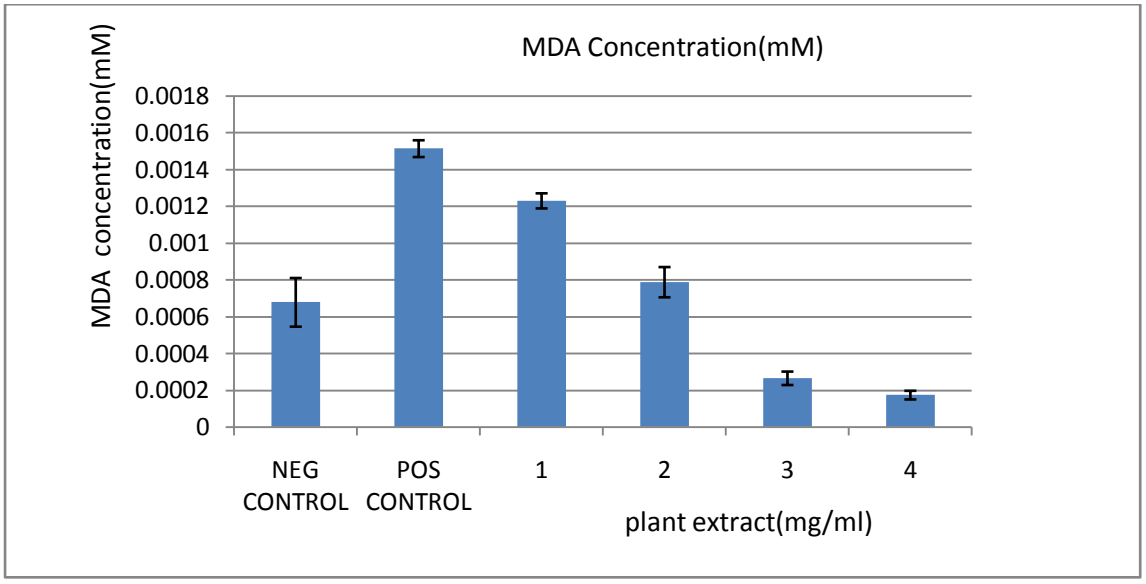


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 2.*In vitro* effect of varying conc of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc of plant extract decrease in MDA levels were obsereved.

1c. EFFECTS OF ACETONE EXTRACT OF *Prosopis cineraria* ON MDA LEVELS IN ERYTHROCYTE MEMBRANE.

Concentartion Of Extract(mg/ml)	Concentration Of MDA (mM)
Positive control	.00151
Negative control	.000677
1	.00122
2	.00078
3	.00026
4	.00017

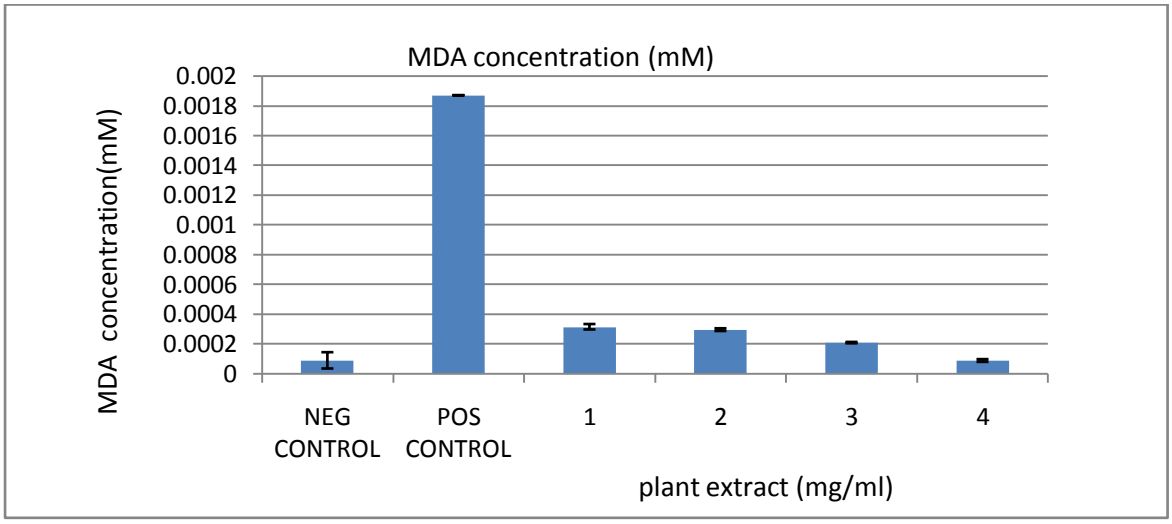


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 3..*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract decrease in MDA levels were observed.

1d. EFFECTS OF METHANOLIC EXTRACT OF *Prosopis cineraria* ON MDA LEVELS IN ERYTHROCYTE MEMBRANE.

Concentartion Of Extract(mg/ml)	Concentration Of MDA (mM)
Positive control	90.5×10^{-5}
Negative control	22.6×10^{-4}
1	31.61×10^{-4}
2	29.68×10^{-4}
3	20.97×10^{-4}
4	89.5×10^{-5}

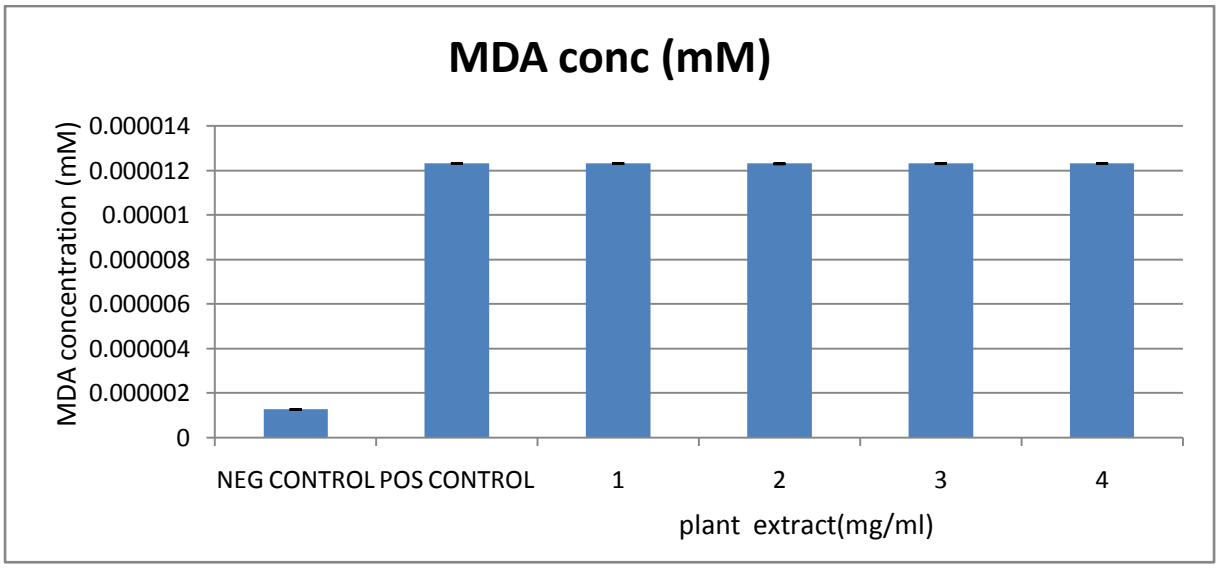


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 4..*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract decrease in MDA levels were observed.

1e. EFFECTS OF AQUEOUS EXTRACT OF *Prosopis cineraria* ON MDA LEVELS IN ERYTHROCYTE MEMBRANE.

Concentration Of Extarct(mg/ml)	Concentration Of MDA (mM)
Positive control	1.26×10^{-6}
Negative control	1.23×10^{-5}
1	1.2315×10^{-5}
2	1.23005×10^{-5}
3	1.23067×10^{-5}
4	1.23151×10^{-5}

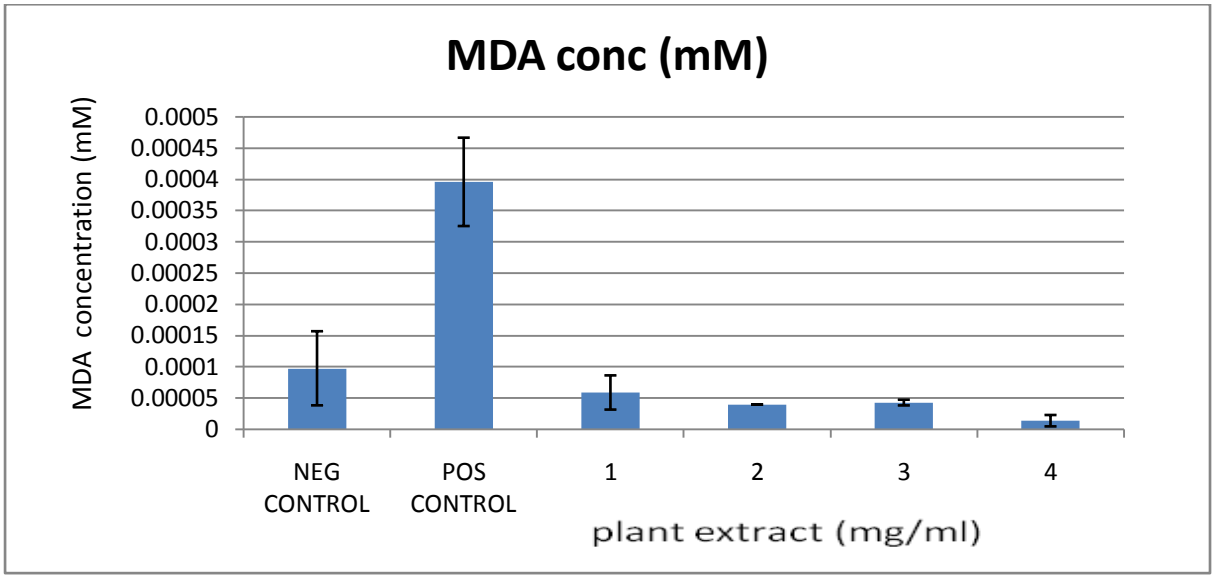


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 5.*In vitro* effect of varying conc of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc of plant extract decrease in MDA levels were obsereved.

1f. EFFECTS OF METHANOLIC EXTRACT OF *Cordia dichotoma* ON MDA LEVELS IN ERYTHROCYTE MEMBRANE.

Concentartion Of Extract(mg/ml)	Concentartion Of MDA (mM)
Positive control	0.000395
Negative control	9.6×10^{-5}
1	5.8×10^{-5}
2	3.8×10^{-5}
3	4.19×10^{-5}
4	1.2×10^{-5}

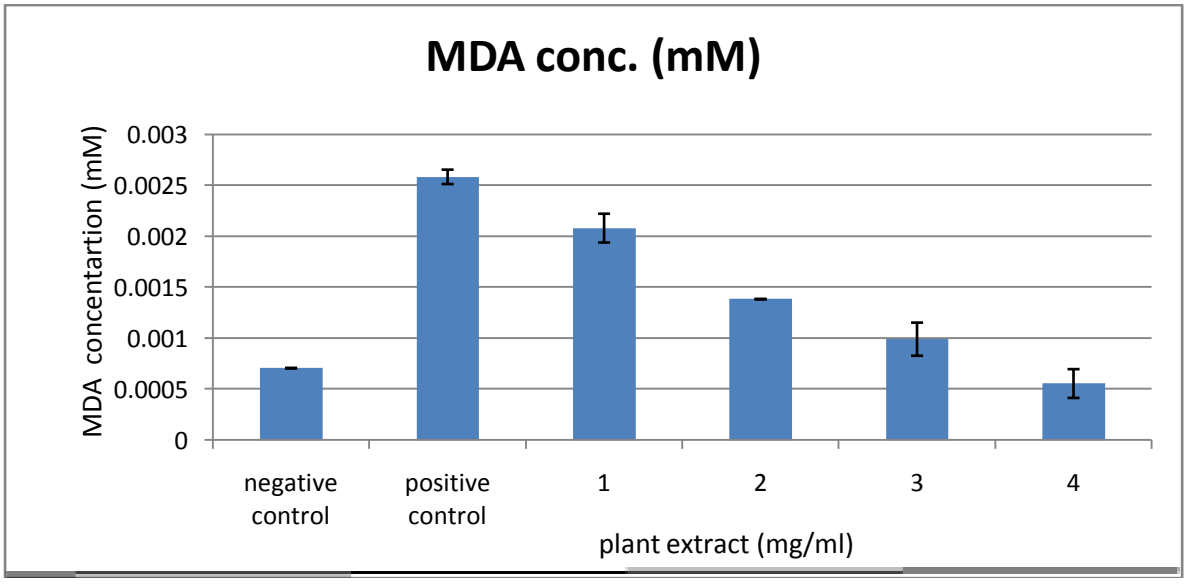


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 6. *In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc.of plant extract decrease in MDA levels were observed.

1g. EFFECTS OF ACETONE EXTRACT OF *Stevia rebaudiana* ON MDA LEVELS IN ERYTHROCYTE MEMBRANE.

Concentration Of Extract(mg/ml)	Concentartion Of MDA (mM)
Positive control	25.87×10^{-4}
Negative control	7.1×10^{-4}
1	20.84×10^{-4}
2	13.87×10^{-4}
3	99.4×10^{-4}
4	55.8×10^{-4}

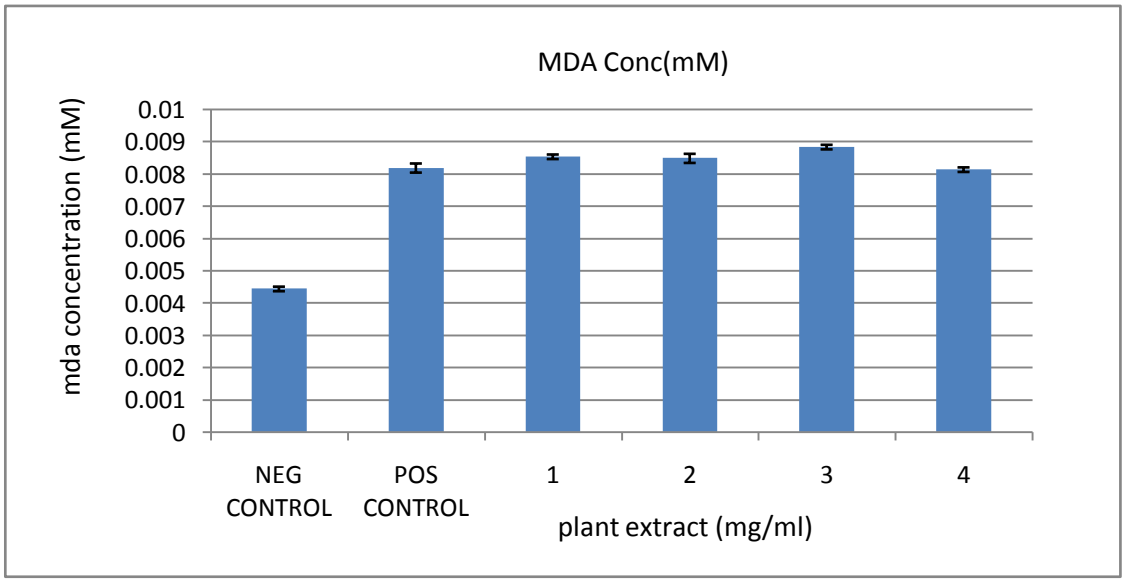


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph7. .*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations; with increase in conc. of plant extract decrease in MDA levels were observed.

1g. EFFECTS OF AQUEOUS EXTRACT OF *Rhus myserensis* ON MDA LEVELS IN ERYTHROCYTE MEMBRANE.

Concentaration Of Extract(mg/ml)	Concentration Of MDA (mM)
Positive control	0.0082
Negative control	0.00445
1	0.00885
2	0.0085
3	0.00885
4	0.00815



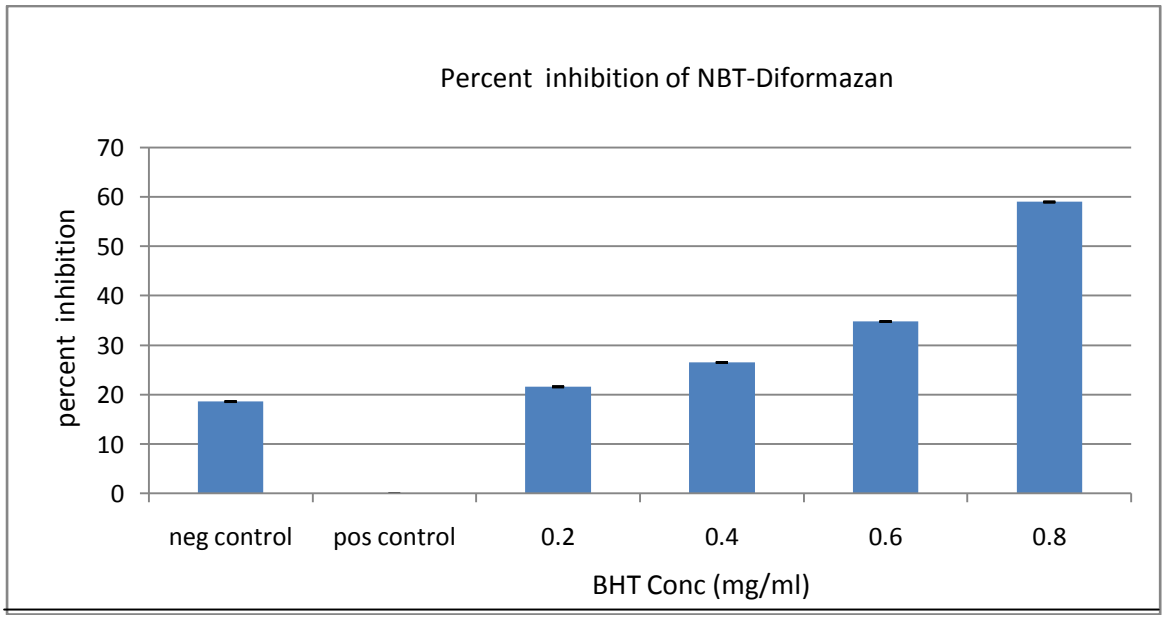
NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 8..*In vitro* effect of varying conc of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc of plant extract no change in MDA levels were obsereved.

2. **SUPEROXIDE DISMUTASE**

- Evaluation of antioxidant activity of BHT (s tandard antioxidant) on SOD levels in Haemolysate.

Concentration Of Extract(mg/ml)	PERCENT INHIBITION
Positive control	0
Negative control	18.67
0.2	21.645
0.4	26.57
0.6	34.86
0.8	59.04



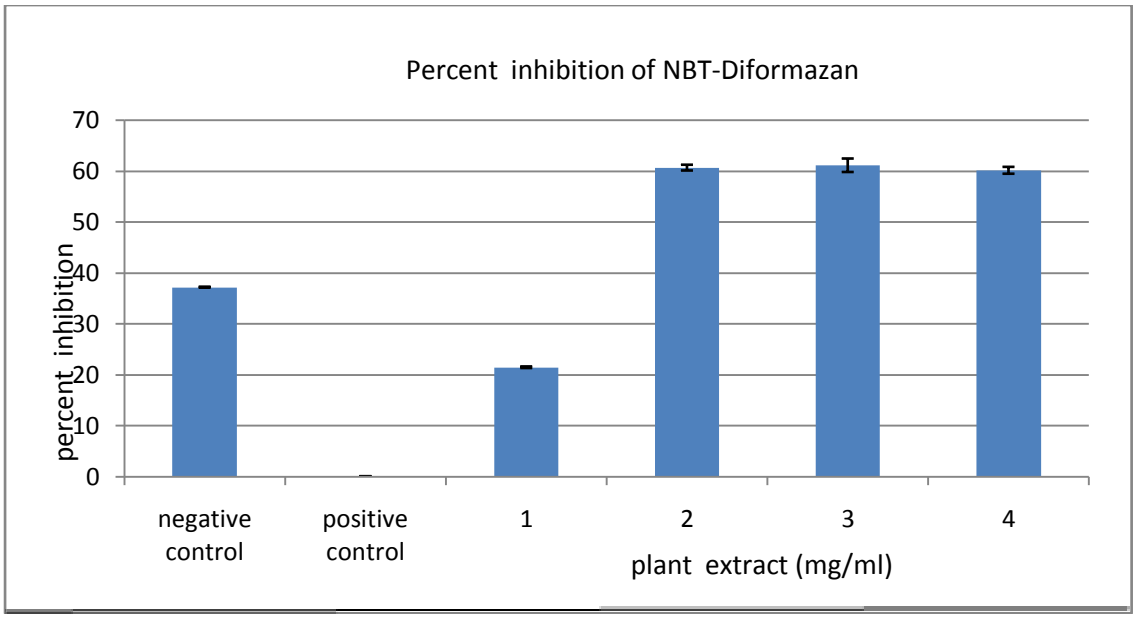
NEGATIVE CONTROL-only RBC + NBT diformazan formation

POSITIVE CONTROL – only NBT diformazan

Graph9 .*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract inhibition of NBT – diformazan formation (in percentage) increases.

2a. EFFECTS OF AQUAEOUS EXTRACT OF *Stevia rebaudiana* ON SOD LEVELS IN HEMOLYSATE.

Concentration Of Extract(mg/ml)	PERCENT INHIBITION
Positive control	0
Negative control	37.29
1	21.41
2	60.11
3	60.15
4	60.575

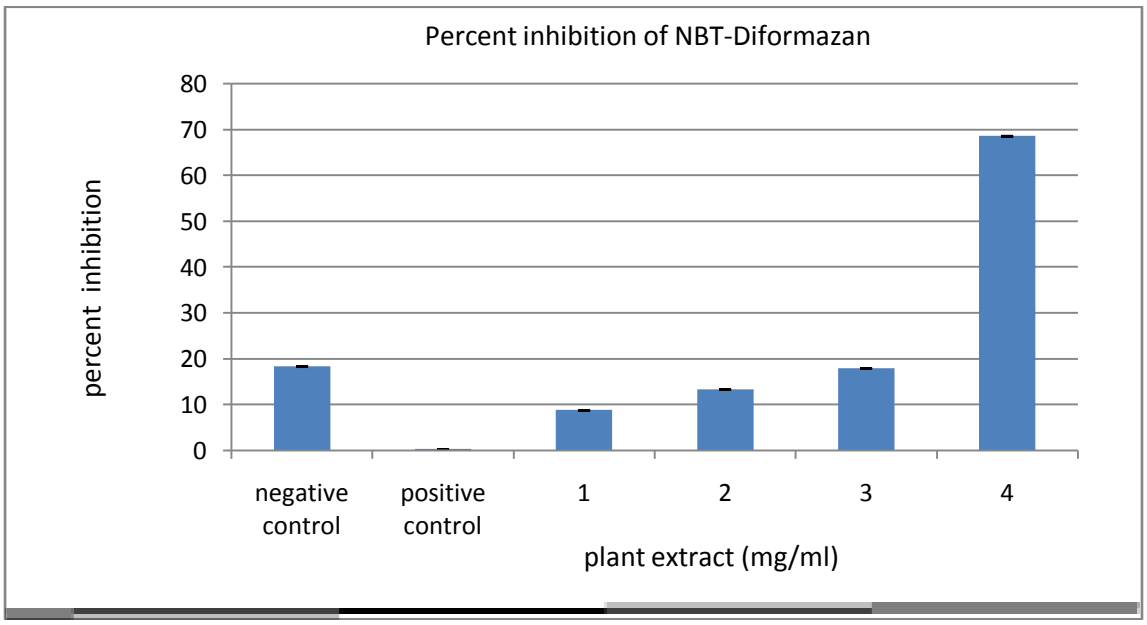


NEGATIVE CONTROL-only RBC + NBT diformazan formation
POSITIVE CONTROL – only NBT diformazan

Graph 10.*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract inhibition of NBT – diformazan formation (in percentage) increases.

2b. EFFECTS OF METHANOLIC EXTRACT OF *Prosopis cineraria* ON SOD LEVELS IN HEMOLYSATE.

Concentration Of Extract(mg/ml)	PERCENT INHIBITION
Positive control	0.26
Negative control	18.35
1	8.79
2	13.34
3	17.97
4	68.64

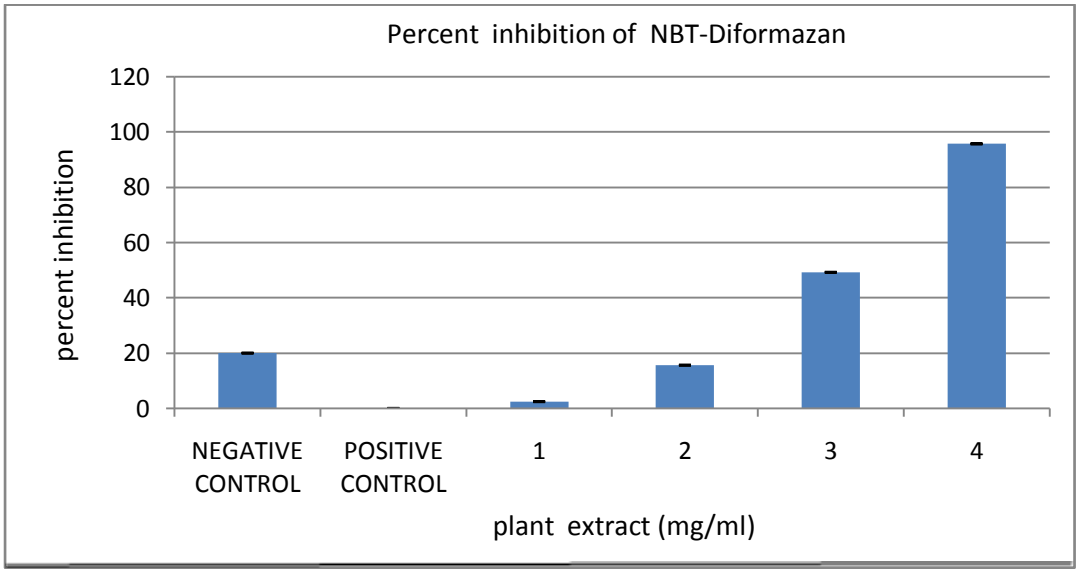


NEGATIVE CONTROL-only RBC + NBT diformazan formation
POSITIVE CONTROL – only NBT diformazan

Graph 11.*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract inhibition of NBT – diformazan formation (in percentage) increases.

2c. EFFECTS OF ACETONE EXTRACT OF *Stevia rebaudiana ON* SOD LEVELS IN HEMOLYSATE.

Concentration Of Extract(mg/ml)	PERCENT INHIBITION
Positive control	0
Negative control	19.96
1	2.37
2	15.61
3	49.2
4	95.65

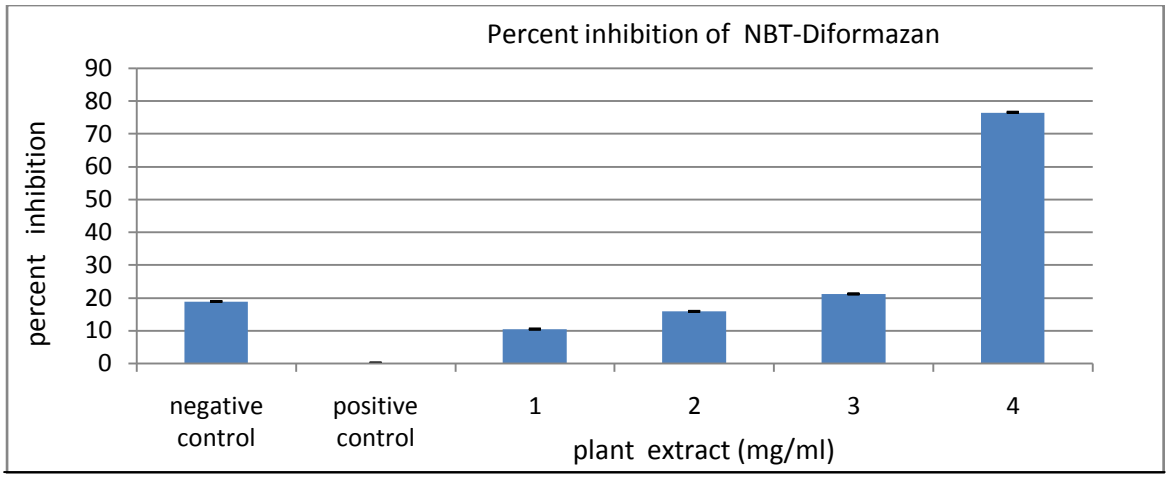


NEGATIVE CONTROL-only RBC + NBT diformazan formation
POSITIVE CONTROL – only NBT diformazan

Graph 12 .*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract inhibition of NBT – diformazan formation (in percentage) increases.

2d. EFFECTS OF METHANOLIC EXTRACT OF *Cordia dichotoma* ON SOD LEVELS IN HEMOLYSATE.

Concentartion Of Extract(mg/ml)	PERCENT INHIBITION
Positive control	0
Negative control	18.77
1	10.35
2	15.78
3	21.05
4	76.43

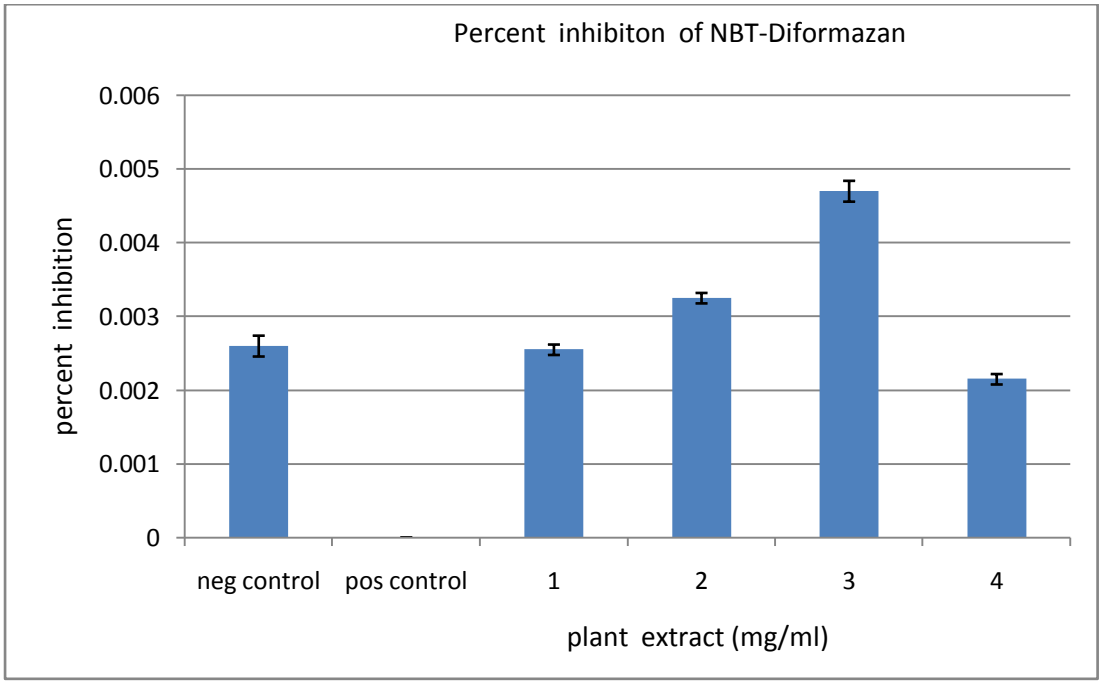


NEGATIVE CONTROL-only RBC + NBT diformazan formation
POSITIVE CONTROL –NBT only diformazan.

Graph 13.*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract inhibition of NBT – diformazan formation (in percentage) increases.

2e. EFFECTS OF AQUEOUS EXTRACT OF *Prosopis cineraria* ON SOD LEVELS IN HEMOLYSATE.

Concentration Of Extract(mg/ml)	PERCENT INHIBITION
Positive control	0
Negative control	.0026
1	.00255
2	.00325
3	.0047
4	.00215

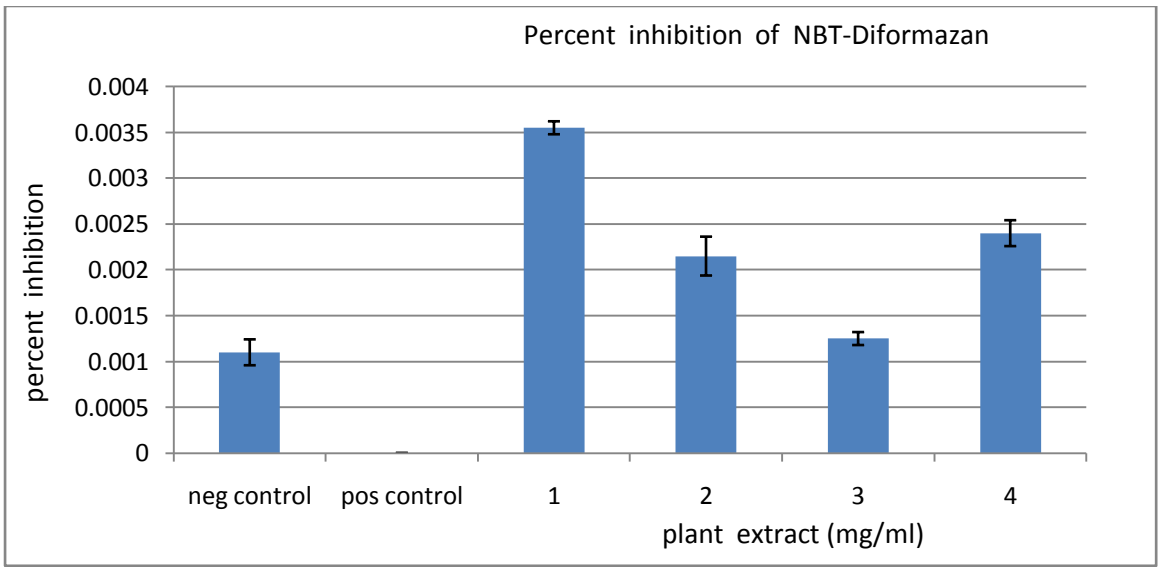


NEGATIVE CONTROL-only RBC + NBT diformazan formation
POSITIVE CONTROL – only NBT diformazan

Graph 14.*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract inhibition of NBT – diformazan formation (in percentage) increases at a conc. Of 3mg/ml but decreases again at 4 mg/ml.

2e. EFFECTS OF AQUEOUS EXTRACT OF *Rhus myserensis* ON SOD LEVELS IN HEMOLYSATE.

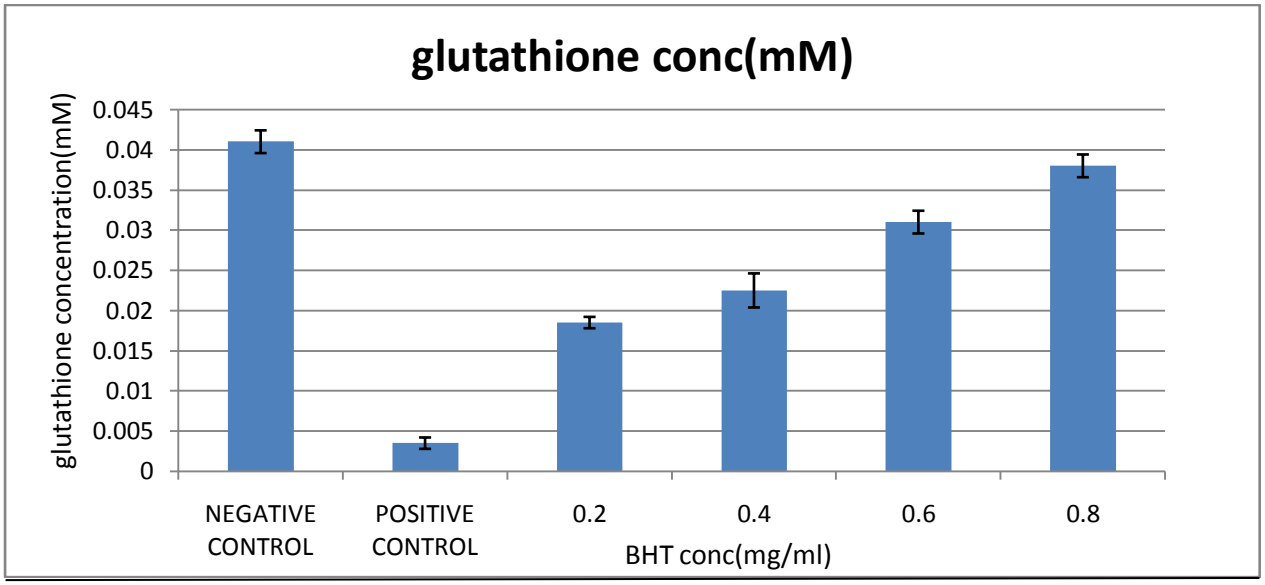
Concentartion Of Extract(mg/ml)	PERCENT INHIBITION
Positive control	0
Negative control	.0011
1	.00355
2	.00215
3	.00125
4	.0024



NEGATIVE CONTROL-only RBC + NBT diformazan formation
POSITIVE CONTROL – only NBT diformazan

Graph 15.*In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis , this graph represents the mean of 3 observations , with increase in conc. of plant extract inhibition of NBT – diformazan formation (in percentage) increases.

3) GLUTATHIONE

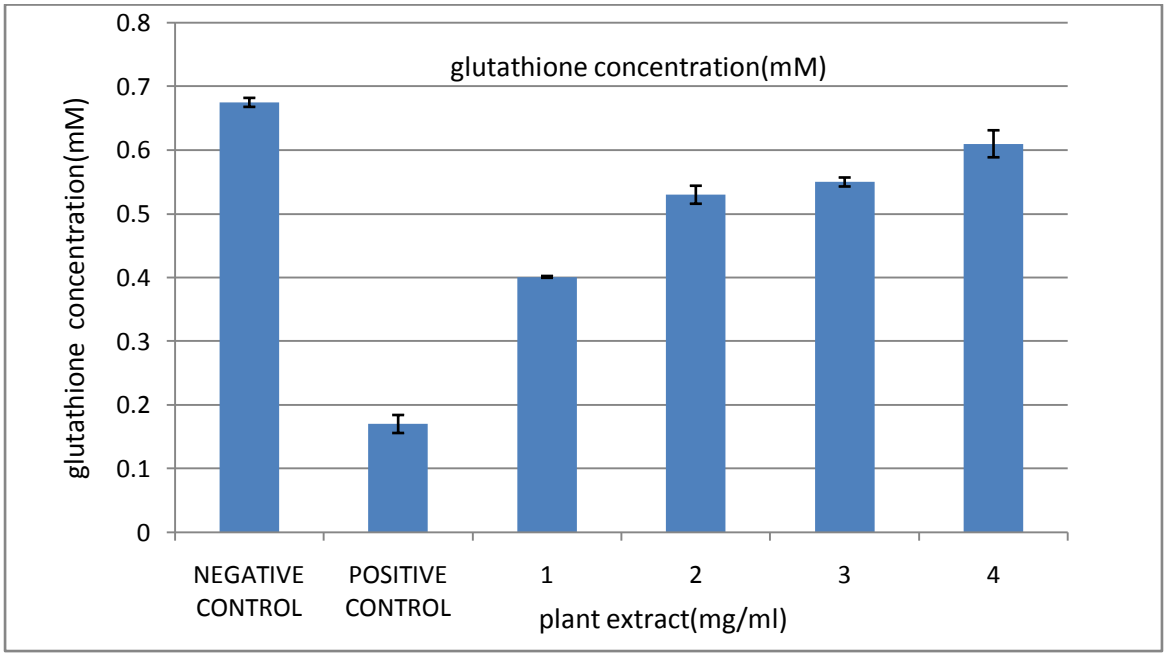


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 16. *In vitro* effect of varying conc. of BHT (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc. of plant extract glutathione level increases.

3a) Effect of aqueous extract of *Stevia rebaudiana* on glutathione levels in erythrocytes

Plant extract concentration(mg/ml)	Glutathione level(mM)
Negative control	0.67
Positive control	0.17
1	0.40
2	0.53
3	0.55
4	0.61

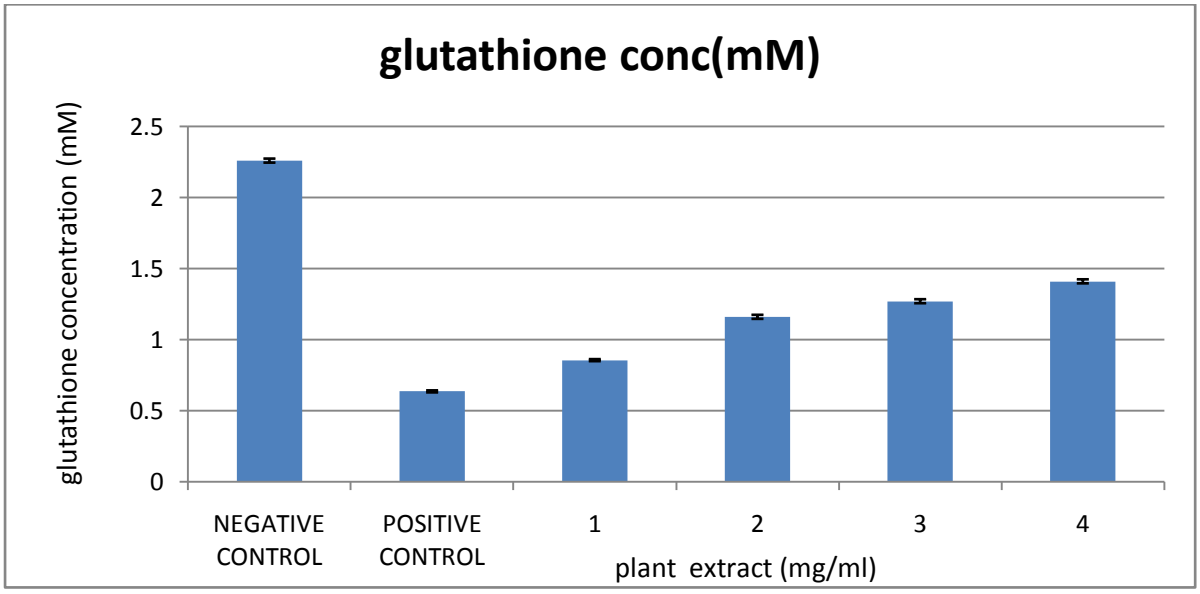


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 17. *In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations , with increase in conc. of plant extract glutathione level increases.

3b) Effect of methanolic extract of *Stevia rebaudiana* on glutathione level in erythrocytes

Plant extract concentration (mg/ml)	Glutathione concentration(mM)
Negative control	2.27
Positive control	0.63
1	0.85
2	1.16
3	1.27
4	1.41

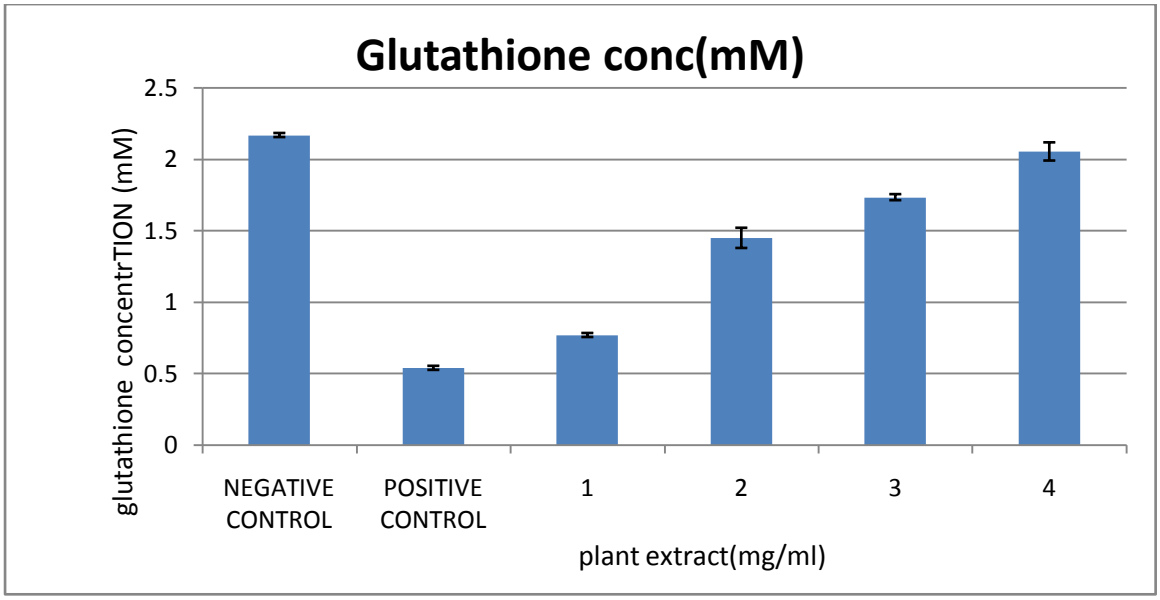


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

Graph 18 *In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc. of plant extract glutathione level increases.

3c) Effect of acetone extract of *Stevia rebaudiana* in erythrocytes

Plant extract concentration(mg/ml)	Glutathione level(mM)
Negative control	2.17
Positive control	0.54
1	0.77
2	1.45
3	1.75
4	2.05

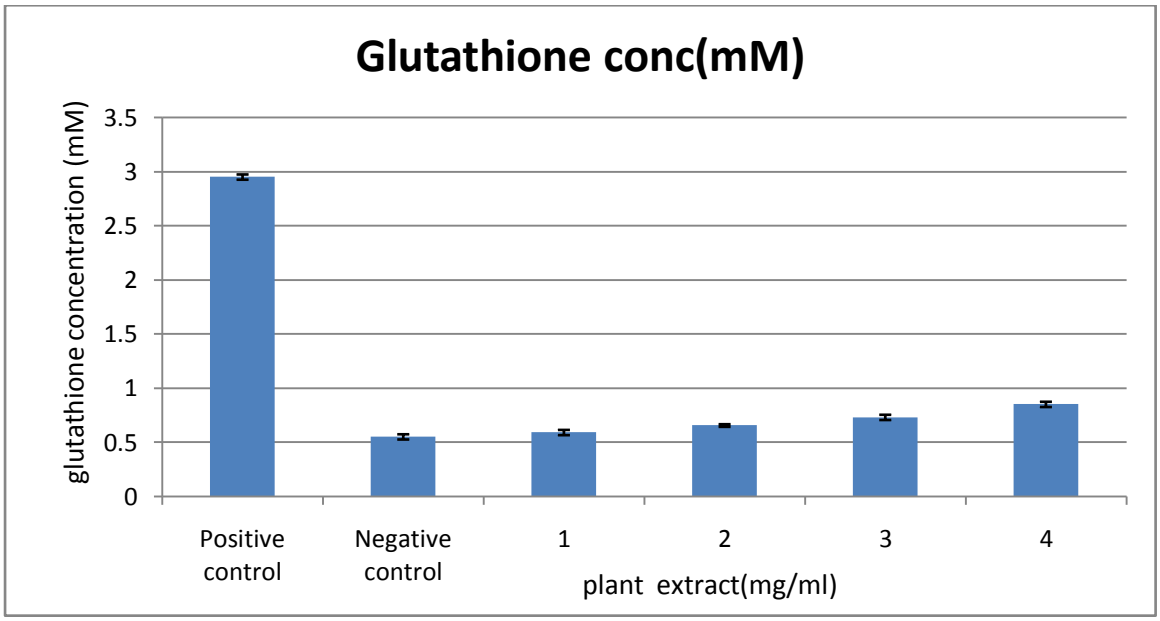


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

GRAPH 19. *In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc. of plant extract glutathione level increases.

3d) Effect of aqueous extract of *Prosopis cineraria* in erythrocytes

Plant extract concentration (mg/ml)	Glutathione level(mM)
Negative control	2.975
Positive control	0.55
1	0.59
2	0.65
3	0.73
4	0.85

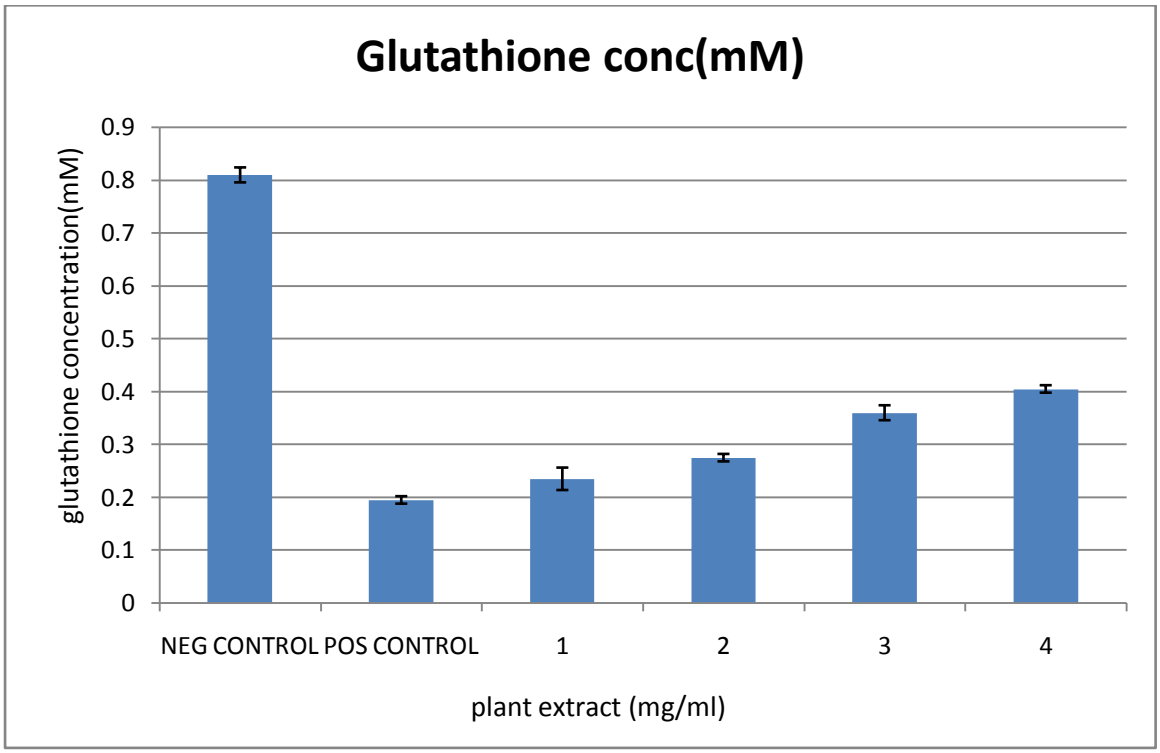


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

GRAPH 20. *In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc. of plant extract glutathione level increases.

3e) Effect of methanolic extract of *Prosopis cineraria* on glutathione in erythrocytes

Plant concentration(mg/ml)	Glutathoine concentration(mM)
Negative control	0.81
Positive control	0.19
1	0.24
2	0.27
3	0.36
4	0.40

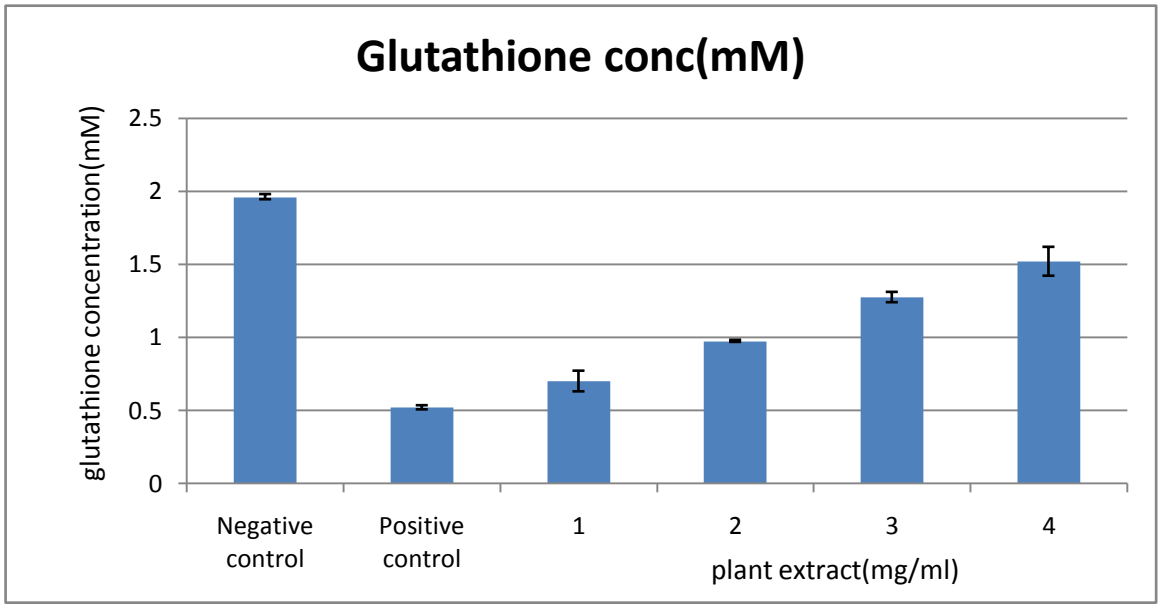


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

GRAPH 21. *In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations , with increase in conc. of plant extract glutathione level increases

3f) Effect of methanolic extract of *Cordia dichotoma* on glutathione in erythrocytes

Plant extract concentration (mg/ml)	Glutathione concentration(mM)
Negative control	1.97
Positive control	0.53
1	0.7
2	0.975
3	1.275
4	1.52

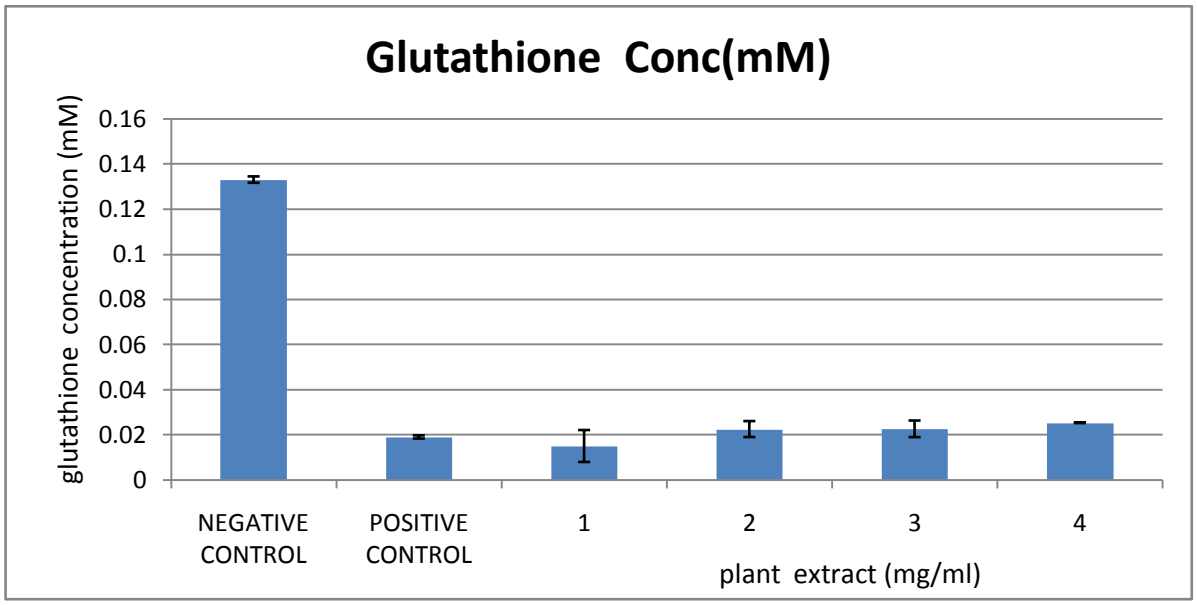


NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H_2O_2

GRAPH 22. In *vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc. of plant extract glutathione level increases

3g) Effect of aqueous extract of *Rhus myserensis* on glutathione in erythrocytes

Plant extract concentration (mg/ml)	Glutathione concentration(mM)
Negative control	0.133
Positive control	0.019
1	0.015
2	0.023
3	0.025
4	0.026



NEGATIVE CONTROL – only RBC
POSITIVE CONTROL - RBC + H2O2

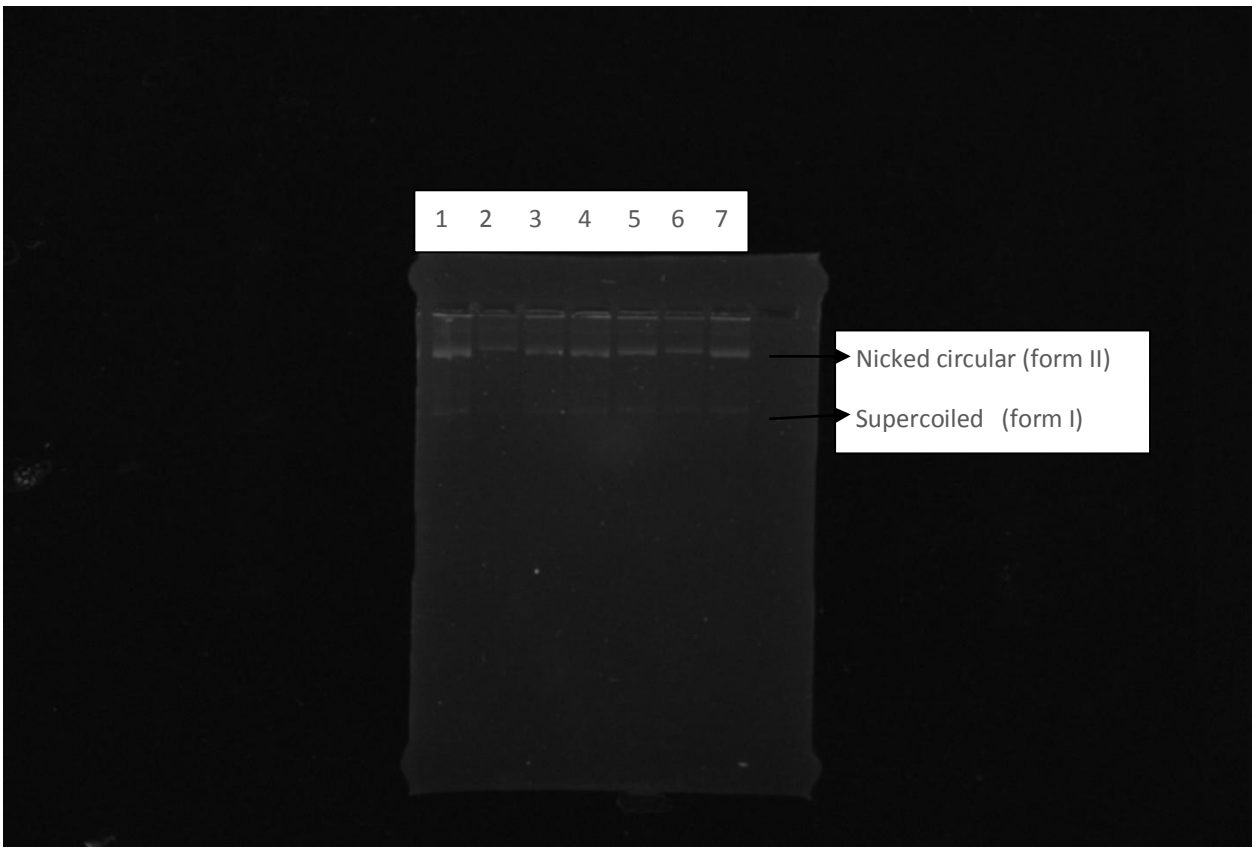
Graph 23. *In vitro* effect of varying conc. of plant extract (mg/ml) on "x" axis, this graph represents the mean of 3 observations, with increase in conc. of plant extract glutathione level increases

- **RESULTS SUMMARY**

Table: 6

PLANT EXTRACT (mg/ml)	MDA (INHIBITION OF MDA LEVEL)	SOD (INHIBITION OF NBT DIFORMAZAN FORMATION)	GLUTATHIONE (RESTORATION OF GLUTATHIONE LEVEL)
Stevia rebaudiana **(AQUEOUS)**	33.56%	50.56%	77.98%
Stevia rebaudiana **(METHANOLIC)**	4.88%	-	51.64%
Stevia rebaudiana **(ACETONE)**	48.53%	40.70%	69.35%
Prosopis cineraria **(AQUEOUS)**	0.06%	.014%	23.63%
Prosopis cineraria **(ACETONE)**	44.34	-	36%
Prosopis cineraria **(METHANOLIC)**	18.95%	27.1%	39.19%
Rhus myserensis **(AQUEOUS)**	.03%	.0046%	17.01%
Cordia dichotoma **(METHANOLIC)**	12.15%	30.90%	56.7%

7) Protection of plasmid pBR322 DNA by plant extracts



(Agarose conc. – 1.2 %)

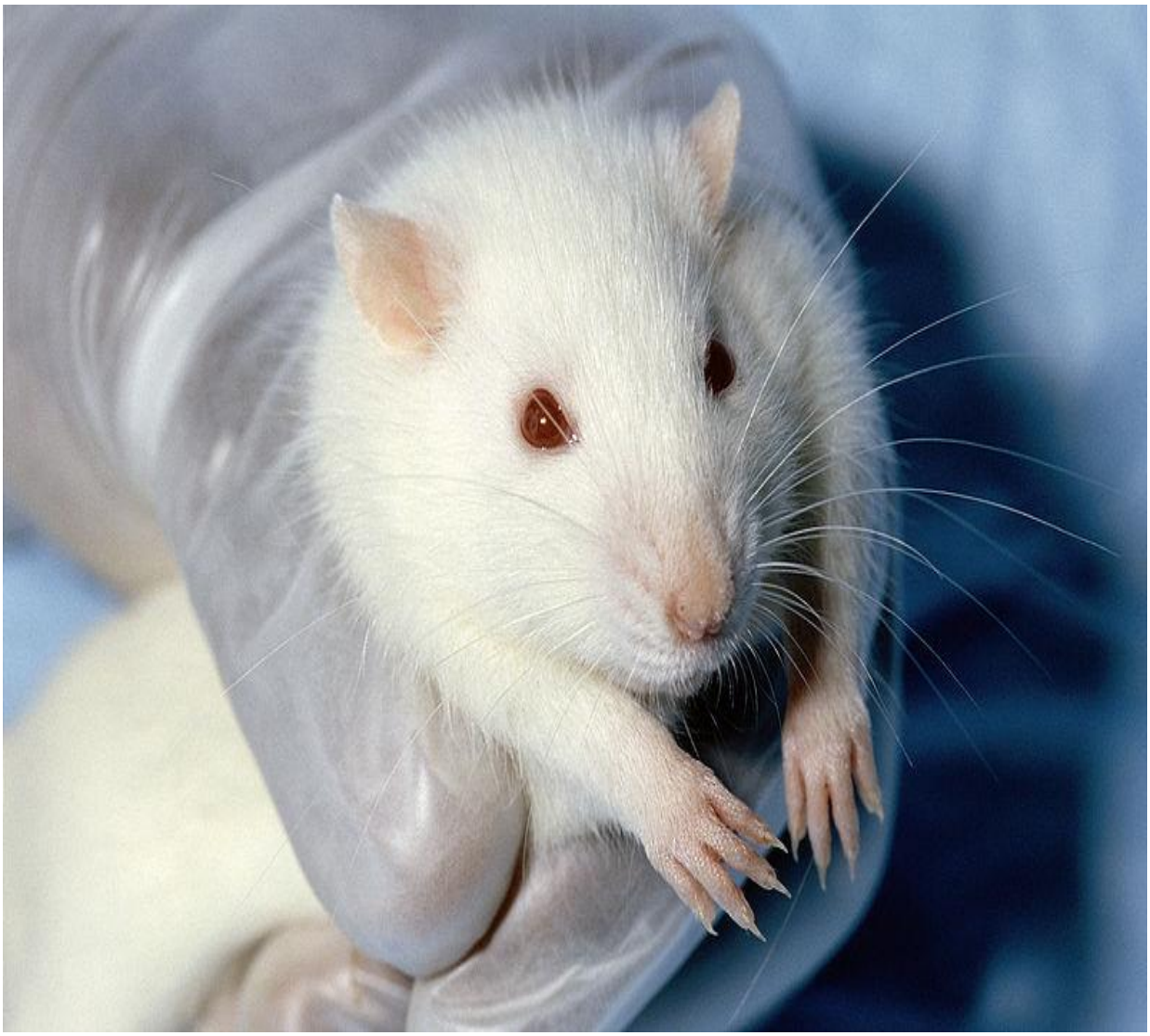
Agarose gel electrophoresis pattern of plasmid pBR322 DNA treated with 30% H_2O_2 in the presence and absence of different concentrations of plant extracts (8 mg / ml)

pBR322 plasmid

1) pBR322 plasmid
2) pBR322 plasmid + H_20_2
3) pBR322 plasmid + H_20_2 + *Stevia rebaudiana* aqueous
4) pBR322 plasmid + H_20_2 + *Stevia rebaudiana* methanolic
5) pBR322 plasmid + H_20_2 + *Stevia rebaudiana* acetone
6) pBR322 plasmid + H_20_2 + *Prosiopis cinereria* acetone
7) pBR322 plasmid + H_20_2 + *Cordia dichotoma* methanolic

- ***In – VIVO* RESULTS**

4) LIVER FUNCTION TESTS



- **Lindane induced liver toxicity in wistar rats**

Param-eters	GGT (U/L)	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	ALBUMIN (g/dl)
0 day	3.28	8.14	5.8	40.8	7.89
5 day	6.56	20.3	16.2	48.96	5.97
10 day	42.64	40	35	73.44	4.9
15 day	55.76	58	50	100	4.6
20 day	101.8	60.36	62.30	120	4.3
25 day	112.4	65.43	68.53	125	4
30 day	115.4	67.40	70	127	3.9

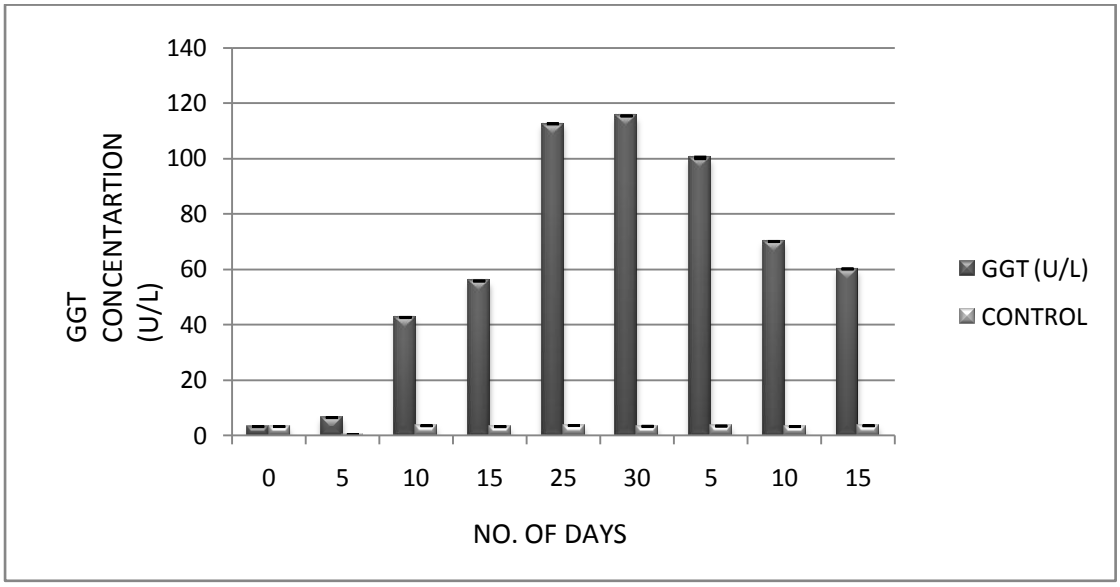
Table: 7

- **During treatment of plant extracts various parameters levels.**

DAYS	GGT (U/L)	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	ALBUMIN (g/dl)
5	100.16	40.12	65.02	120.5	4.24
10	70.06	30.16	55.12	115.6	4.73
15	60.10	15.38	30.27	96.8	5.24

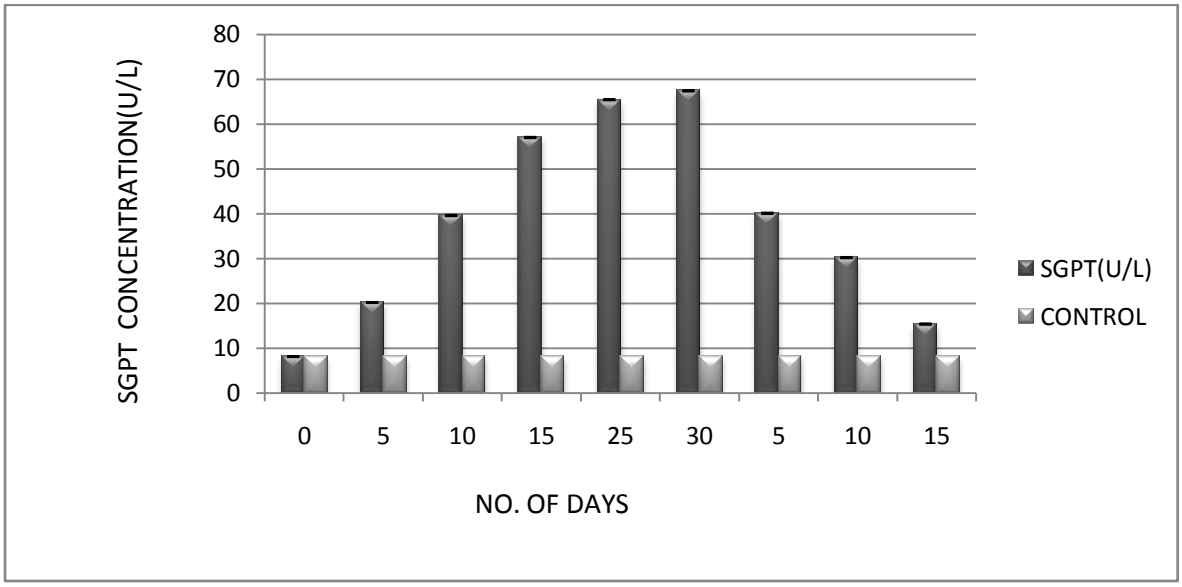
Table: 8

6.1 GGT



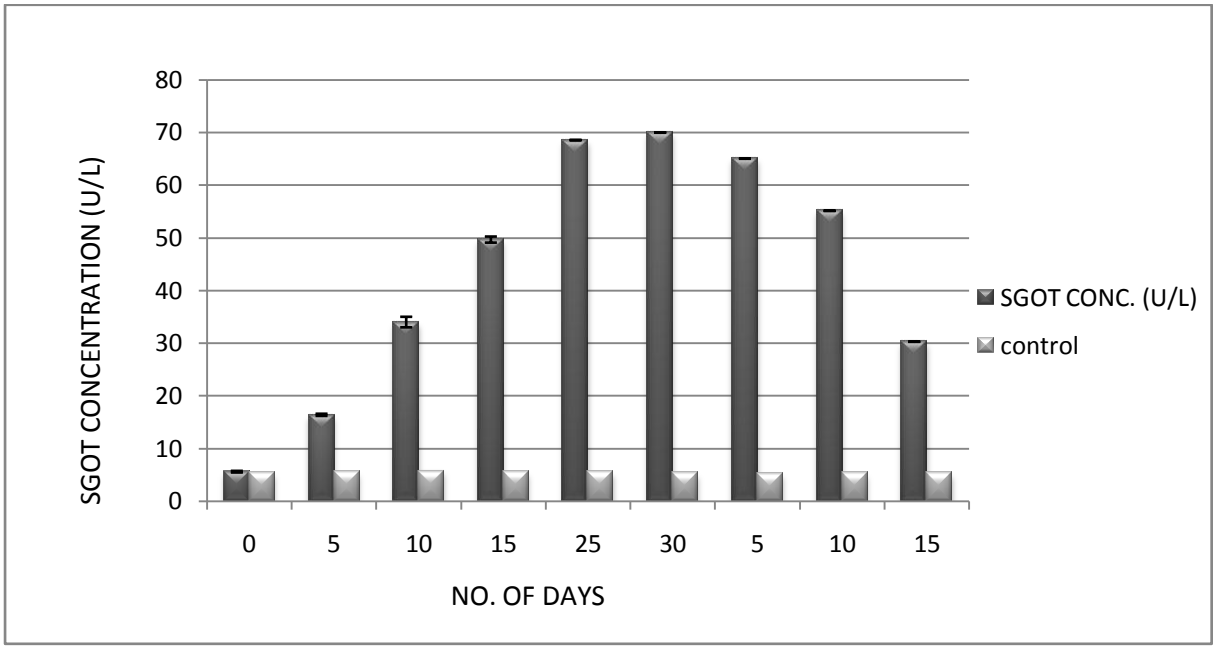
Graph24. This graph represents 15 days treatment and 30 days induction schedule by lindane , "X" represents no. of days from first 0 – 30 days is induction level of GGT , and after induction 5 – 15 days is of treatment by plant extract (*Stevia rebaudiana* leaves – acetone extract).

6.2 SGPT



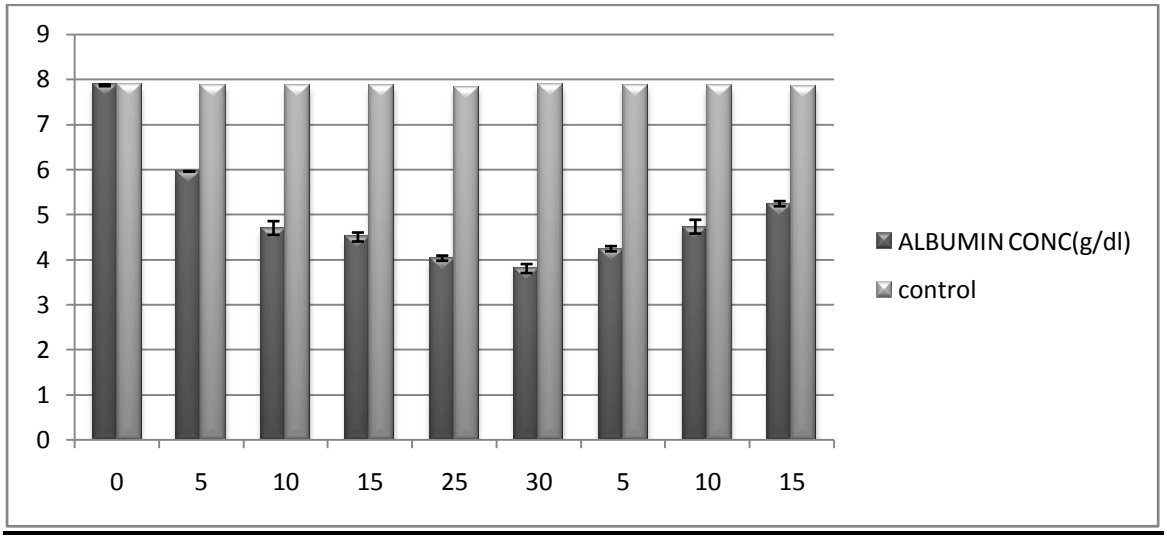
Graph25. This graph represents 15 days treatment and 30 days induction schedule by lindane , "X" represents no. of days from first 0 – 30 days is induction level of SGPT , and after induction 5 – 15 days is of treatment by plant extract (*Stevia rebaudiana* leaves – acetone extract).

6.3 **SGOT**



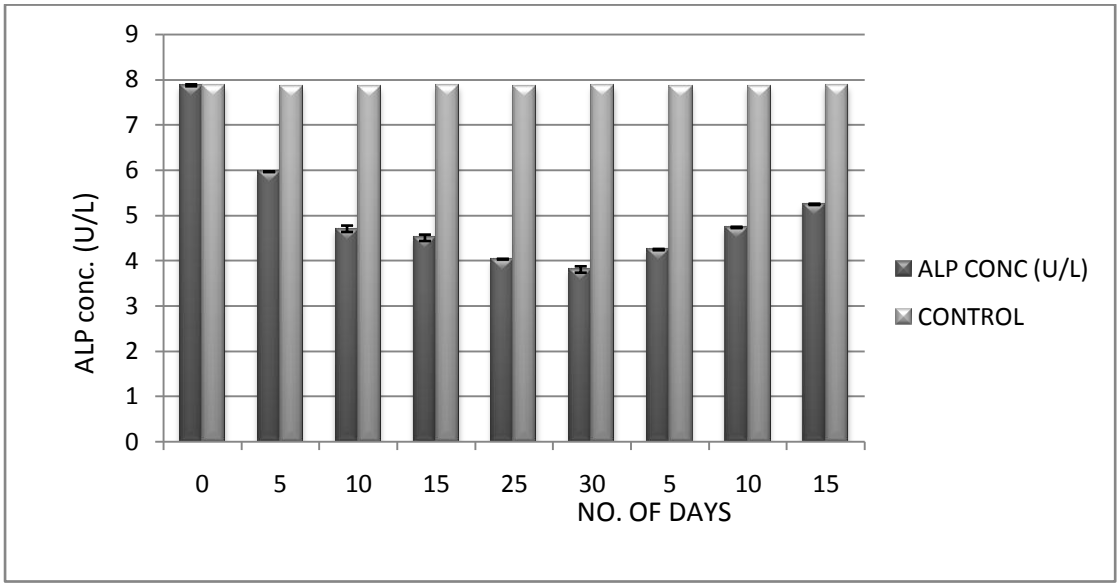
Graph26. This graph represents 15 days treatment and 30 days induction schedule by lindane , "X" represents no. of days from first 0 – 30 days is induction level of SGOT , and after induction 5 – 15 days is of treatment by plant extract (*Stevia rebaudiana* leaves – acetone extract).

6.4 ALBUMIN



Graph27. This graph represents 15 days treatment and 30 days induction schedule by lindane , "X" represents no. of days from first 0 – 30 days is induction level of ALBUMIN , and after induction 5 – 15 days is of treatment by plant extract (*Stevia rebaudiana* leaves – acetone extract).

6.5 ALP

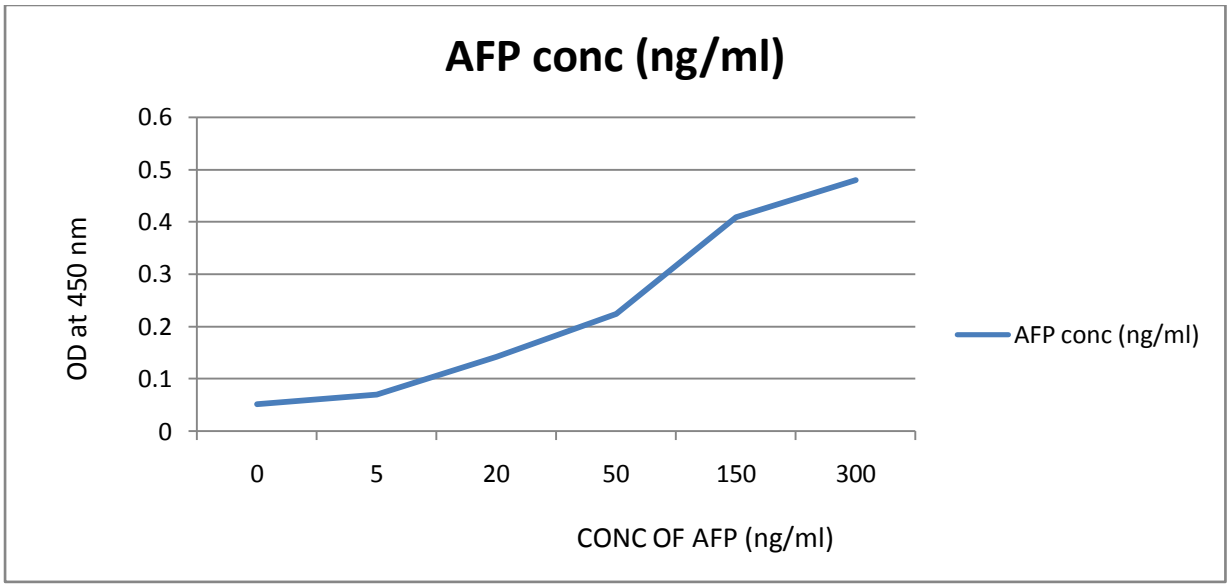


Graph28. This graph represents 15 days treatment and 30 days induction schedule by lindane , "X" represents no. of days from first 0 – 30 days is induction level of ALP , and after induction 5 – 15 days is of treatment by plant extract (*Stevia rebaudiana* leaves – acetone extract).

- **AFP STD**

Std afp conc given in kit	O.D at 450nm
0ng/ml	0.052
5ng/ml	0.070
20ng/ml	0.143
50ng/ml	0.224
150ng/ml	0.409
300ng/ml	0.480

Table: 9



Graph 29

- **Induction of afp by lindane**

Induction day	Concentration of AFP(ng/ml)
O day	0
5 day	1
10 day	2
15 day	3.8
20 day	4.5
25 day	5
30 day	6.1

Table: 10

- **Decreacing of AFP level by acetone extract.**

During treatment days	Concentration Of AFP (ng/ml)
5 days	3.8
10 days	2.0
15 days	1.0

Table: 11

DISCUSSION:

Natural antioxidants have biofunctionalities such as the reduction of diseases like DNA damage, mutagenesis, carcinogenesis, etc. and inhibitions of pathogenic bacteria growth, which are often associated with the termination. The antioxidant properties of plants can be correlated with oxidative stress defense in different human diseases including cancer, atherosclerosis, Alzheimer's and the ageing processes.

The aim of our present study is to examine the antioxidant ability of some plant extracts by performing various *in-vitro* and *in-vivo* assays and the results indicated a concentration dependent antioxidant ability of some plant extracts. In this study, the antioxidant activity of the Acetone, Methanol and Aqueous extracts of pods of *Prosopis cineraria*, peel and seed extracts of *Rhus myserensis* and *Cordia dichotoma* and leaves of *Stevia rebaudiana* was studied along with the standard BHT. The antioxidant activity of these plant extracts was evaluated in a series of *in vitro* tests on RBC by evaluating various antioxidative enzyme like SOD, MDA and Glutathione levels on the presence of oxidative stress induced by 0.4% H_2O_2 on RBC.

Cold extraction of these plant parts were prepared as hot extraction may led to damage to some of the important components of plants

and their evaluation of antioxidant and radical scavenging activity chemically was already done (Meena Rajesh dissertation thesis (2006), Dr Sarika Sinha). So we tried to evaluated prevention of oxidative stress on RBC by these plant extracts by studying various antioxidative parameters such as SOD, Glutathione and MDA. We also evaluated prevention of oxidative stress condition on pBR322 plasmid.

Oxidative stress causes generation of free radicals, radical like the hydroxyl radical will react indiscriminately with all components of the DNA molecule. Hence, the hydroxyl radical causes DNA damage by direct interaction in the generation of many of its genetic lesions. However, it can also cause DNA damage by other methods e.g. stimulating the rise of calcium intracellularly which in turn stimulates endonucleases to cause strand breaks and degradation products from the DNA molecule. DNA damage can produce a multiplicity of modifications in DNA including base and sugar lesions, strand breaks, DNA-protein cross-links and base-free sites.

In our study oxidative stress caused by H_2O_2 on plasmid led to opening supercoiled DNA to nick circular form and its reversal is found by some of our plant extract like methanolic extract of *Cordia dichotoma* seeds and acetone extract of *Stevia rebaudiana* leaves. So these plant extracts can be further studied at molecular level. *Stevia rebaudiana* (aqueous) extract and *Prosopis cineraria* (acetone) extract shows moderate activity and the remaining extracts were not effective.

Results of study on RBC showed that (table 6) *Stevia rebaudiana* leaves acetone extract showed profound antioxidative effect on MDA (48.53% inhibition of MDA levels), *Stevia rebaudia*na (aqueous). (33.56%) and *Prosopis cineraria* (acetone) (44.34%) showed moderate activity while *Prosopis cineraria* (methanolic) (18.95%), *Cordia dichotoma* (methanolic) (12.15%) showed less activity comparatively for MDA and *Rhus myserensis* (aq).(0.03%), *Prosopis cinereria* (aq) (.06%) and *Stevia rebaudiana* (methanolic) (4.88%) showed negligible activity for MDA assay.

For SOD assay *Stevia rebaudiana* (aq). extract (50.56%) showed highest activity, *Cordia dichotoma* (methanolic) (30.90%), *Prosopis cinereria* (methanolic) (27.1%), *Stevia rebaudiana* (aq).(50.56%) showed moderate activity , *Rhus myserensis* aqueous (0.0046%) and *Prosopis cineraria* aq.(.014%) showed negligible activity.

For Glutathione assay *Stevia rebudiana* (aqueous) extract shows highest activity (77.98%), *Stevia rebaudiana* (methanolic) (51.64%), *Stevia rebaudiana* (acetone) (69.35%), *Prosopis cineraria* (acetone) (32.25%) showed moderate activity and *Prosopis* cineraria (aq.) (23.63%), *Rhus myserensis* (aq).(17.01%), showed less activity(Table 6).

Lindane had been observed to have damaged the function of the tissue. Hence, there has been a drastic change in the serum profile of the animals. The serum enzyme, namely Alkaline Phosphatase, SGOT, SGPT, GGT was observed to increase drastically after the lindane treatment. The increase in the enzymes indicates the functioning of the hepatocytes has been disturbed, releasing the enzymes in circulatory system. Albumin is the solely produced by the liver. Treating animals with lindane, a gradual decrease in albumin was observed, indicating the dysfunction of the hepatocytes to generate albumin. During the treatment with the plant extracts, the activity of the serum enzymes was observed to decline. This indicates the repair of the damage tissue following the treatment.

In the present study , *Stevia rebaudiana* is checked on AFP levels during and after lindane induced toxicity , AFP is a very significant marker for liver toxicity, simultaneously serum parameters like GGT, SGOT, SGPT, ALBUMIN, ALP were also analyzed before and after lindane administration and their reversal is studied by *Stevia rebaudiana* acetone extract.

On the very first day all the parameters were checked, GGT (3.28 U/L), SGPT (8.14U/L), SGOT (5.8U/L)), ALP (40.8U/L), ALBUMIN (7.9 G/DL) they have been taken as control rats. On 30^{th} day values were, GGT raised to (115.4U/L), SGPT raised to (67.40U/L), SGOT raised to (17 U/L), ALP levels raised to (127 U/L) and ALBUMIN

levels drops to (3.9 G/DL) *ie* liver toxicity had induced as a cause of lindane dosage to rats.

After 30 day induction, treatment with acetone extract of *Stevia rebaudiana* was started and continued till 15 days, after that results were like GGT level drop to (60.10 U/L), SGPT levels drop to (15.38 U/L), SGOT levels dropped to (30.27 U/L), ALP levels dropped to (96.8 U/L), albumin levels raised to(5.24 U/L).

As AFP is a significant marker which if changes, may be a indication of initiation of hepatocarcinoma . On 1^{st} day AFP levels were (0 ng/ml), and on 30^{th} day AFP levels raised to (6.1 ng/ml) but after treatment with *Stevia rebaudiana* its levels drops down to (1 ng/ml) after 15 day.

In the present study there is a decrease in AFP level by treating with acetone extract of *Stevia rebaudiana* . So, it has further very good scope in the malignancy like cancer, tumors etc. as AFP level is a significant marker in liver cancer.

CONCLUSION:

Data in the present study showed that *Stevia rebaudiana* leaves acetone extract showed profound antioxidative effect on MDA (48.53% inhibition of MDA levels), *Stevia rebaudiana* (aq). (33.56%) and Prosopis cineraria (acetone) (44.34%) showed moderate activity while *Prosopis cineraria* (methanolic) (18.95%), *Cordia dichotoma* (methanolic) (12.15%) showed less activity comparatively for MDA and *Rhus myserensis* (aq). (0.03%), *Prosopis cineraria* (aq) (.06%) and *Stevia rebaudiana* (methanolic) (4.88%) showed negligible activity for MDA assay.

For SOD assay *Stevia rebaudiana* (aq). extract (50.56%) showed highest activity, *cordia dichotoma* (methanolic) (30.90%), *Prosopis cineraria* (methanolic) (27.1%), *Stevia rebaudiana* (aq).(50.56%) showed moderate activity , *Rhus* aqueous (0.0046%) and *Prosopis cineraria* aqueous(.014%) showed negligible activity.

For Glutathione assay *Stevia rebaudiana* (aq) extract shows highest activity (77.98%), *Stevia rebaudiana* (methanolic) (51.64%), *Stevia rebaudiana* (acetone) (69.35%), *Prosopis cineraria* (acetone) (32.25%) showed moderate activity and *Prosopis cineraria* (aq.) (23.63%), *Rhus myserensis* (aq).(17.01%), showed less activity.

They are promising plants for more detailed investigation of their antioxidant properties and application possibilities. The pods of *Prosopis cineraria* also possessed moderate radical scavenging abilities. Aqueous extracts of these plants were relatively weak antioxidants in comparison with methanol extracts, indicating that methanol is an ideal and effective solvent for extraction of antioxidants. The results obtained indicated the fruit peels and seeds as potential source of natural substances with antioxidant properties. In many cases, the fruit seeds and peels are the waste products of technological processes, hence their reusing as the antioxidant source, could bring measurable economical profits and contribute to reduction of pollutions introduced by fruit and vegetable industries into the environment.

In the study performed on wistar rats to check AFP levels and other serum enzyme levels, after liver toxicity induced by lindane for 1 month, acetone extract of *Stevia rebaudiana* leaves showed a profound activity and it lowers the levels of AFP, SGOT, SGPT, ALP, GGT and increases serum albumin levels which shows reversal of liver toxicity cause by lindane . So we can conclude that this extract has potential source and can be studied further for evaluating more of its properties.

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ABBREVIATIONS

AFP	Alpha Feto Protein
BHT	Butylated Hydroxytoluene
H_2O_2	Hydrogen Peroxide
SGOT	Serum Glutamate-Oxaloacetate transaminase
SGPT	Serum glutamate-pyruvate transaminase
GGT	Gamma Glutamate Transaminase
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
GSH	Reduced Glutathione
GSSG	Oxidised Glutathione
GR	Glutathione Reductase
MDA	Malonaldehyde
TBARS	Thiobarbituric Acid Reactive Substances
DDT	Dichlorodiphenyltrichloroethane
EPA	Environmental Protection Agency
RUP	Restricted Used Pesticides
γHCH	gamma hexachlorocyclohexane
ULV	Ultra low volume
NBT	Nitroblue terazolium
DTNB	5,5-Dithiobis-(2nitro-benzoic acid)

DMSO	Dimethyl sulphoxide
TCA	Trichloro acetic acid
TBA	Thiobarbituric acid
BSA	Bovine serum albumin
SDS	Sodium dodecyl sulphate
TE	Tris EDTA
LB	Luria Broth
BCG	Bromo cresol green