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# EFFECT OF METHANOL EXTRACT OF *DELONIX REGIA* ON FREE RADICAL SCAVENGERS AND LIPID PROFILE OF WISTAR ALBINO RATS

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## ABSTRACT

Methanol extract of *Delonix regia* was tried at two doses (250mg and 500mg per kg body weight) for its effect on free radical scavengers and lipid profile of albino rats. Serum levels of superoxide dismutase (SOD) and catalase (CAT) were significantly (P<0.05) lower in the animals that received extract (both doses) than both controls. Also, GSH levels were lower in the animals that received the extract than both controls. However, malondialdehyde (MDA) levels were significantly elevated (P<0.05) in the rats that received the extract when compared with the controls. Vitamins C and E levels did not display dose-dependence and did not differ significantly (P>0.05) from those of the controls. The mean HDL and LDL levels in both groups of rats did not differ significantly (P<0.05) at 250mg but at 500mg, HDL level was significantly lowered and LDL remained constant. Irrespective of dosage, the total cholesterol and triacylglycerol levels of the experimental rats did not differ from those of the controls. The *Delonix regia* extract displayed mild antioxidant effect and little elevation of HDL and other bad lipids.

**KEYWORDS:** *Delonix regia*, malondialdehyde (MDA), mild antioxidant effect.

## 1.0 INTRODUCTION

Free radical scavenger is a vitamin, mineral, or enzyme that is able to destroy free radicals. The term "free radical" refers to a molecule that has one or more unpaired electrons. This makes them very unstable, and they move through the blood stream, taking electrons from other cells or giving away unpaired ones. By doing so, free radicals causes cell damage that has been linked to a host of diseases including heart disease and cancer.

Mammallian cells possess elaborate defense mechanisms for radical detoxification. Key metabolic steps are superoxide dismutase (SOD),catalase (CAT), and glutathione peroxidase (GPX), which destroy toxic peroxides. In addition to antioxidant enzymes, non-enzymatic molecules, including thiols, and disulfide-bonding play important role in antioxidant defense systems. Some of the compounds are of exogenous nature and are obtained from food, such as α-tocopherol, β-carotene, and ascorbic acid, and such micronutrient elements as zinc and selenium (Halliwell *et al.*,1998).If cellular constituent do not effectively scavenge free radicals, they lead to disease conditions to the cell membranes and DNA, including oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutations leading to cancer, degenerative, and other diseases (Cerutti. 1991*).*

Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades (Devasagayam; *et al,* 2004). This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased (Deiana. 1999).

Several herbs and spices have been reported to exhibit antioxidant activity, including thyme, nutmeg, turmeric, white pepper, chili pepper, ginger and several Chinese medicinal plant extracts (Kikuzak *et al,* 1991). The majority of the active compounds are flavonoids, isoflavones, flavones, anthocyanins coumarins, lignans, catechins and isocatechins. In addition to the above compounds found in natural foods, Vit. C and E, β-carotene and α-tocoherol are known to possess antioxidant potential (prior *et al,* 2003). The role of free radical scavenger is to hunt down unstable molecules and destroy them before they can cause significant cell damage within the body.

Everybody has free radicals. They are a natural part of life, although some people have more than others. Factors like being overweight or smoking cigarettes may put a person at higher risk for having large number of free radicals. In a normal body, these unstable cells are destroyed by the immune system via white blood cells. However, the average immune system cannot destroy all free radicals without assistance. For this reason, it is highly important to eat a diet rich in foods with multiple free radical scavengers. This enhances immune function and keeps the unstable cell level at tolerable limits.

### 1.1 LIPIDS PROFILE

Serum lipids consist mostly of lipoproteins-spherical macromolecular complexes of lipids and specific proteins (apolipoproteins). A lipid profile is a direct measurement of three blood components. Cholesterol, triacygcerols, and high-density lipoproteins (HDL). Cholesterol and triacyglycerol are transported in the blood by combination of lipids and proteins called lipoproteins. (Olson, 1998). Factors such as age, sex and genetics can influence lipid profile, including diet, level of physical activity, level of diabetes control and smoking status (USDA, 2008).

### 1.2 LIVER-THE TARGET ORGAN

The liver is the largest organ of the human body weighing approximately 1500g and is located in the upper part of the abdomen on the right kidney and intestines and beneath the diaphragm. The liver performs many essential functions, including.

i. To manufacture proteins, including albumin (to help maintain the volume of blood) and blood clothing factors.
ii. To synthesize, store and process fats including fatty acids (used for energy) and cholesterol.
iii. To metabolize and store carbohydrates (used as the source for sugar in blood).
iv. To form and secrete bile that contains bile acids to aid in the to aid in the intestinal absorption of fats and the fat soluble vitamins A,D,E and K; to eliminate, by metabolizing or secreting the potentially harmful biochemical products produced by the body, such as bilirubin, from the breakdown of protein, and environmental toxins (Tzanakakis, 2002).

#### 1.2.1 HEPATOTOXICITY

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Navarro , et al., 2006). The chemicals that cause liver injury are

called hepatotoxins or hepatotoxicants. Certain drugs may cause liver injury when introduced even with the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes biliary epithelial cells and or liver vasculature (Jereb , *et al.*, 2006) other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g, microcystins) and herbal remedies can also induce hepatotocity.

### 1.3 OBJECTIVE OF THE STUDY

The pigment extracts and the successive extract prepared from dried leaves of *Delonix regia* were evaluated for their ability to scavenge free radicals through *in vitro* chemical and biological models.

## CHAPTER TWO

## LITERATURE REVIEW

### 2.0 INTRODUCTION

*Delonix regia*, *syn*. *Poinciana regia*, is an ornamental tree planted in avenues, gardens and roadsides in all the warmer and damper parts of india. *Delonix regia* as one of the medicinal plant used in Karachi (Pakistan). It has been used in the folk medicine systems of several civilizations like for the treatment of constipation, inflammation, arthritis, hemiplagia , leucorrhoea and b rheumatism. Flowers of *Delonix regia* have been used as traditional herbal remedies for gynecological disorders and they are also used as tablet binder.

*Delonix regia* has been reported to have broad-spectrum antibacterial and antifungal activities in flowers, anti-inflammatory and analygesic activities in flowers and bark and it also has been reported to have hepatoprotective, antimicrobial, and antioxidant activity.

***Delonix regia* plant (flame of the forest)**

## 2.1 BOTANICAL DESCRIPTION

Royal Poinciana grows to 9-15m in height but its elegant, wide-spreading, umbrella-like canopy can be even wide.

### 2.1.1 BIOPHYSICAL LIMITS

*Delonix regia* seems to tolerate many types of soils from clay to sandy but it prefers sandy soils. Means annual temperature and rainfall for this plant should be 14-26$^0$C and over 700mm respectively. An altitude for this plant should be 0-2000m. The plant demands light. It grows weakly and sparsely under shade.

### 2.1.2 GEOGRAPHICAL DISTRIBUTION

Royal Poinciana is a native to Madagascar, but is now rare in the wild. It is widely cultivated and may be seen in adorning avenues, parks and estates in tropical areas throughout the world.

### 2.1.3 TAXONOMICAL CLASSIFICATION

**Class:** Dictoledons

**Subclass:** Rosidea;

**Order:** Fabales;

**Family:** Leguminosae;

**Subfamily:** Caesalpinioidea;

**Tribe:** Caesalpinieae;

**Genus:** *Delonix*;

**Specific epithet:** *regia*- (Hook) Raf;

**Botanical name:** *Delonix regia* (Hook) Raf (Anonymous et al., 2012).

### 2.1.4 VERNACULAR NAMES

**English:** Flamaboyant, flamboyant flame tree, flame of the forest, flame tree, gold mohur, gul mehr, pabock flower, royal Poinciana; Arabic goldmore, French: flamboyant, Poinciana, royal burnese: sainban, Spanish: acacia roja, swahil: mjohoro, Mkakay: Hindi glumohr. Mayirkonra, panjadi, telugu (Pacheco. *et al.*, 2010).

### 2.1.5 TRADITIONAL USES OF *DELONIX REGIA*

The extract of delonix regia is known to have medicinal properties (Hamdoon et al., 2009). This plant is used in several countries to prepare extracts with antimicrobial and antifungal

activities (Sammour *et al.*, 1992) seeds of *Delonix regia* contains flavonoids and are used as wound healing agent in household (Vidyasagar *et al.*, 2007).

**LEAVES**

The compound leaves have a feathery appearance and are a characteristic light, alternate, bright green. They are doubly pinnate. Each leaf is 30-50cm long and has 20 to 40 pairs of primary leaflets on it, and each of these is further divided into 10-20 pairs of secondary leaflets (CheL *et al,* 2010).

**FRUITS (PODS)**

Green and flaccid when young, turning to dark brown, hard woody pods, 30-50cm long, 3.8cm shic, 5-7.6cm broad, ending in a short break when mature, with many horizontally partitioned seed chambers inside, indehiscent, finally splitting into two parts (Arora *et al.*, 2010).

**BARK**

Smooth, grayish-brow, slightly cracked and having many lenticels, inner bark is light brown (Rosado. *et al.*, 2010).

**WOOD**

Soft and white in colour (Lakshmi Vijai 1987).

**ROOT**

Shallow (Pacheco *et al.*, 2010).

**FLOWERING SEASON**

April to July (Arora *et al.*, 1982).

**FRUIT SEASON**

August to October (Sen *et al.*, 2010).

### 2.1.6 REPORTED PHYTOCONSTITUENT

Stem bark: Flavonoids, alkaloids, saponins, sterols, stigmasterols, carotene, hydrocarbons phytotoxins β-sitosterol, lupeol (Lakshmi et al., 1987), P-methoxybenzaldehyde, isolupeol, carotene, hydrocarbons phytotoxins, and phenolic acid (Shanmukha *et al.*, 2011).

**ROOT BARK**

Glycosides, tannins, alkaloids, steroids, terpenoids and carbohydrates (Sama et al., 2011).

**FLOWERS**

Flavonoids, tannins, saponins, steroids (Parekhj, *et al.*, 2007), carotenoids (Lycopene, phytene, neolycopene, $\delta$ - lycopene and Y – lycopene, phenolic acid (gallic acid protocatehuic acid, salicyclic acid), anthocyanins (cyaniding-3-glucoside and cyaniding -3-gentiobioside and β-stitosterol (Adje *et al.*, 2008).

**LEAVES**

Lupeol, phenolic acid (gallic acid, protocatehuic acid and salicyclic acid) and β – sitosterol (Anwa *et al.*, 2011).

**SEEDS**

Saponins and galactomannons (Jungalwala et al.,, 1962).

### 2.1.7 REPORTED BIOLOGICAL ACTIVITIES

Many biological activity have been reported of *Delonix regia*. These are as follows:

### 2.1.8 ANTI-DIARRHOEAL ACTIVITY

The flowers of *Delonix regia* plant have been reported to have in vivo anti-diarrhoeal activity. The experimental models were castor oil induced diarrhea, prostaglandin $E_2$ induced enteropooling and charcoal induced gastro intestinal motility test in Wistar albino rats. The 90% ethanolic extract of *Delonix regia* flower was used for activity. The flower of *delonix regia* shows the dose dependent antidiarrhoeal effect in all the treated groups (Shiramane et al., 2011).

### 2.1.9 ANTI-INFLAMMETORY ACTIVITY

The powdered leaves of the Delonix regia were used for the anti-inflammatory acivity. The models for anti-inflmamatory activity were the carrageenan-induced ran paw edema and cotton pellet granuloma. The ethanolic extract of leaves of *Delonix regia* shows significant activity at 400mg /kg in the both models when compared with standard group (Shewale *et al.*, 2011).

## 2.2 HEPATOPROTECTIVE ACTIVITY

The study was designed to evaluate the beneficial effect of methanol extract of aerial parts of *Delonix regia* in $CCL_4$ induced liver damage rats. The metabolic extract of aerial parts of *Delonix regia* possesses hepatoprotective activity against $CCL_4$ induced hepatotoxicity in rats (Ahmed et *al.*, 2011).

### 2.2.1 WOUND HEALING ACTIVITY

Wound healing activity study was done to investigate the wound healing properties of *Delonix regia* in experimental animal models. The ethanolic and aqueous extracts of *Delonix regia* flowers were prepared to study the effect on wound healing. The animals used were

Wistar albino rats. The wound models were incision and excision wound. Wound healing was assessed by the rate of wound contraction, period of epithelization, tensile strength, (Skin breaking strength) and estimation of the hydroxyproline content of skin. The ethanolic and aqueous extracts significantly promoted the healing process (Amit *et al.,* 2012).

### 2.2.2 LIVER FUNCTIONS TEST

Liver function test are groups of blood tests that gives information about the state of the patient's lever (Lee,2009). The parameters measured include albumin, bilirubin, liver transaminases (AST and ALT), total protein and others. AST and ALT are useful biomarkers of liver injury in a patient with some degree of intact liver functions. (Johnson *et al* 1999). Most liver disease causes only mild symptoms initially, but it is vital that these diseases be detected earlier.

### 2.2.3 ASPARTATE TRANSAMINASE (AST)

AST also called serum glutamic oxaloacetic transaminase (SGOT) is similar to ALT in that it is another enzyme associated with the liver parenchymal cells. It is raised in acute liver-damage, but it is also present in red blood cells, and cardiac also skeletal muscle, and is therefore not specific to the liver. The ratio of AST and ALT is sometimes useful in differentiating between causes of liver damage, and AST has been used as a cardiac marker.

### 2.2.4 ALANINE AMINO TRANSAMINASE (ALT)

ALT is a cytosolic enzyme that catalyzes the transfer of amino group from alanine to keto-glutarate to form pyruvic acid and glutamate respectively, and is the commonly used marker for hepato cellular injury (prati,et al 2002). AST/ALP elevation favours liver cell necrosis as a mechanism over cholestasis.

### 2.2.5 ALBUMIN

Albumin is protien made specifically by the liver, and can be measured cheaply and easily. It is the main constituent of total protein (the remaining from albumins).

Albumin levels are decreased in liver disease, such as cirrhosis. It is also decreased in nephritic syndrome, where it is lost through the urine. The consequence of low albumin can be edema since the intra-vascular oncotic pressure becomes lower than the extra-vascular space. An alternative to albumin measurement is pre-albumin which is better at detecting

acute changes (half-life of albumin and pre-albumin is -2weeks and 2days respectively).Hussian et., al 2012 reported albumin (P<0.0001).

## CHAPTER THREE

## MATERIALS AND METHODS

### 3.0 AUTHENTICATION OF PLANT SAMPLE

The leave of *Delonix regia* were collected from the roadside of Michael Okpara University of Agriculture Umudike during September 2014. The Delonix regia leaves were authenticated by professor Garuba of the department of plant science and biotechnology in MOUAU.

### 3.1 PLANT EXTRACTION

100g of fresh leave (i.e leaflets) were washed with water, dried under shed and crushed into coarse powder and was subjected to complete extraction with 100ml of methanol (Methanolic extract, ME). Completion of extraction was directly related to the extent that chlorophyll is removed into the solvent and when the tissue debris, on repeated extraction, is completely free of green colour. After extraction, the solvent was distilled off and extract was concentrated on heating mantle to a dry residue.

### 3.2 ANIMALS

Wistar albino rats of the same sex (female) weighing between (100kg-160kg) were housed in polypropylene cages and were fed on standard laboratory diet and water adlibitum. Animals were fasted overnight before commencing the experiment but had free access to water. All the drugs (standard and test as well as vehicle were administered per-orally using insulin syringe.

### 3.3 EXPERIMENTAL DESIGN

Sixteen female Wistar albino rats were used for the study. The crude extract of *delonix regia* was dissolved in deionize water before the treatment. The female Wistar albino rats were divided into four groups of four each. The first two groups being the test groups (A and B) received 250mg/kg and 500mg/kg methanolic extract of *Deolnix regia* orally respectively, and the last two groups (C and D) being the normal and nagative control groups that received water and feed only. The animals were kept for 10days. All the animals used for this study were initially subjected to hepatocyte damage using hexane and chloroform in the ratio of 4:6 at 2.5ml per rat.

## 3.4 BIOCHEMICAL ANALYSIS

### 3.4.1 CHOLESTEROL

Cholesterol is a waxy steroid metabolite found in the cell membrane and transported in the blood serum of all animals. It is essential structural component of mammalian cell membranes, where is required to establish proper membrane permeability and fluidity. In addition cholesterol is an important component for the manufacture of bile acids, steroid hormones, and several fat-soluble vitamins (Smith, 1991). Since cholesterol is essential for all animal life, it is primarily synthesized from simpler substances within the body. However, high levels in blood circulation, depending on how it is transported within lipoproteins, are strongly associated with progression of atherosclerosis. The body therefore compensate for cholesterol intake by reducing the amount synthesized. Cholesterol is recycled and it is excreted by the liver via bile into digestive tract. Typically about 50% of the excreted cholesterol is reabsorbed by the small bowel back into the blood stream (Olson, 1998).

### 3.4.2 TRIACYLGLYCEROLS

Triacylglycerol is an ester composed of a glycerol bound to three fatty acid (Hemat, 2003). It is the main constituent of vegetable oil and animal fats. Triacyglycerols are formed from a single molecule of glycerol combined with three molecules of fatty acid. The glycerol molecule has three hydroxyl (OH) groups. Each fatty acid has a carboxyl group ($COOH^-$). The enzyme pancreatic lipase acts at the ester bond, hydrolyzing the bond and releasing the fatty acid. (Daley *et al*., 2004). In triacyglycerol form, lipids cannot be absorbed by the duodenum. Fatty acids, monoglycerides (one glycerol, one fatty acid) and some diglycerides are absorbed by the duodenum once the triacylglycerols have been broken down (Balch, 2006). Most natural fats contain a complex mixture of individual triacylgycerols (AHA, 2009). Usually, triacylgycerols cannot pass through cell membrane freely. Special enzymes break down triacyglycerols into free fatty acids and glycerol. Fatty acids can then be taken up by cells via the fatty acid transporters (Balch, 2006).

### 3.4.3 LOW-DENSITY LIPOPROTEIN (LDL)

Low-density lipoprotein (LDC) is one of the five major groups of lipoproteins (chylomicrons, VLDL, IDL, HDL, LDL) that enables lipids like cholesterol and triacyglycerols to be transported within the water-based blood stream. Since higher levels of LDL particles can promote medical problems like cardiovascular disease, they are often called based cholesterol particular studies have shown that LDL particle vary in size and density and that a pattern

that has more small dense LDL particulars, called pattern B, equates to a higher risk factor for coronary heart disease than those pattern with more of the larger and less dense LDL particles (pattern A). This is because the smaller particles are more easily able to penetrate the endothelium (Segrest *et al.*, 2001).

### 3.4.4 HIGH-DENSITY LIPOPROTEIN (HDL)

High-density lipoproteins (HDL) are one of the major classes serum of lipoprotains. (chylomicrons, VLDL, IDL, LDL), that enables lipids like cholesterol and triacyglycerols to be transported within the water-based blood stream. In healthy individuals, about 30% of blood cholesterol is carried by HDL-C, (AHA, 2009). A high level of HDL-C seems to protect against candiovascular diseases and low HDL cholesterol level (less than 40mg/dl) or about 1mm/L increase the risk for heart disease. Cholesterol contained in HDL particles is considered beneficial for the cardiovascular health, in contrast to "bad" LDL cholesterol.

## 3.5 ANTIOXIDANTS

Antioxidant compounds plays important role in our body due to favourable effects on human health consumption of food containing phytochemical with potential antioxidant properties can reduce the risk of human disease (temple, 2000). Vegetable oils contain natural antioxidants. Chain breaking antioxidants are highly reactive with free radicals and form stable compounds that do not contribute to the oxidation chain reaction (EI-Diwani et al., 2009). Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (Boxin *et al.*, 2002). Antioxidant is a chemical that delays the start or slows the rate of lipid oxidation reaction. It inhibits the formation of free radical and hence contributes to a stabilization of the lipid sample. Natural antioxidants fruits and vegetables and they have attracted a great deal of public and scientific attention ( EI-Diwani et al., 2009). Vegetable oils contain natural antioxidants and the most common are tocopherols, which are hindered phenolic chain breaking antioxidants are highly reactive with free radicals and form stable compounds that do not contribute to the oxidation chain reaction (E.I-Diwani, 2009).

### 3.5.1 GLUTATHIONE (GSH)

Glutathione is a cysteine-containing peptide found in most form of aerobic lifes. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. Due to its high concentration and

its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants. (Fahey et al., 2001).

### 3.5.2 VITAMIN E (Tocopherol)

Vitamin E is a collective name for a set of eight related tocopherol and tocotrieonols, which are fat –soluble vatamins with antioxidant properties (Herrera et al2001). Of these, α-tocopherol has been most studied as it has the highes bioavailabity,with the body preferentially absorbing and metabolizing this form (Brigelius et al 1999) . It has been claimed that the α- tocopherol form is the most important lipid-soluble antioxidant ,and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction .(Herrera et al 2001). This removes the free radicals intermediate s and prevents the propagation reaction from continuing. Glutathione peroxidase (GPX4) is the only known enzyme that efficiently reduces lipid-hydroperoxidase within biological membrane.(Seiler *et al* 2008).

### 3.5.3 SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutase is an endogenous and first-line-of-defence enzyme that eliminates superoxide by catalyzing its dismutase into $O_2$ and $H_2O_2$(Okado et al 2001). Superoxide radicals ($O_2^-$) is generated as by-product in aerobic organisms from a number of physiological reactios and redox reactions in cell (Andrea et al 1989).It can react with hydrogen peroxide to produce hydroxyl radicals ($OH^-$),one of the most reactive molecules in the living cells. Hydroxyl radicals can cause the peroxidation of membrane lipids.superoxide peroxidase is a well known antioxidant enzyme which can convert superoxide radical to peroxide (Henry *et al.,* 2006). Superoxide dismutase mainly acts by quenching of superoxide ($O_2^-$)

$2O_2^- + 2H \xrightarrow{SOD} H_2O_2 + O_2$ (Vivek and Surendra, 2006).

### 3.5.4 CATALASE

Catalases (EC 1.11.1.6) are the class of enzymes,which catalyze the decomposition of hydrogen peroxide to oxygen and water, and these ubiquitous enzymes have been isolated and purified from different natural sources including animal tissues, plant, and microorganisms. Catalase convert hydrogen peroxide to oxygen and water (Tetyana et al.,2005). Catalase works with the protein glutathione to reduce hydrogen peroxide and ultimately produce water ($H_2O$)

$$2H_2O_2 \xrightarrow{CAT} H_2O + O_2$$

### 3.5.5 MALONDIALDEHYDE (MDA)

Malondialdehyde (MDA) is formed during lipid peroxidation of polyunsaturated fatty acids, (PUFAs) by the action of human platelet thromboxane synthetase on prostaglandins $PGH_2$, $PGH_3$, and $PGG_2$, and by the action of polyamine oxidase and amine oxidase on sperminer.

MDA is a dialdehyde and is often thought of as a very reactive molecule.However, under physiological conditions (pH 7.4), MDA exists as an enolate enolate anion (- O – CH=CH-CHO), a form that is fairly reactive, forming Schiff base with molecules containing a free amine group. Under more acidic conditions (pH<4), however, β-hydroxyacrolein (HO-CH=CH-CHO) (β HA) is the predominant form. Proteins are much reactive with MDA than free amino acids forming a variety of adducts and cross-links. MDA can also react with DNA bases producing a variety of mutagenic compounds. Furthermore, MDA has the potential induce amino-imino-propen cross-links between complimentary strands of DNA and can also cause the formation of DNA-protein cross links.

MDA is metabolized in the liver to malonic acid semialdehyde. This is unstatble and spontaneously decomposes to acetaldehyde that is then converted to acetate by aldehyde dehydrogenase and finally to carbondioxide and water. Some MDA eventually ends up as acetyl – CoA.

### 3.6 DETERMINATION OF HIGH DENSITY LIPOPROTEIN (HDL)

The high density lipoprotein (HDL) was determined by the method invented by grove, (1979) to obtain serum HDL concentration.

**PRINCIPLE**

The principles involve the precipitation of VLDL and LDL in a sample with phosphotungstate and magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined.

**PROCEDURE**

**Precipitation**

- The various samples and reagents were pipetted into labeled centrifuge tubes.

  Sample/standard 0.2ml

  Reagent (A) 0.5ml.

- The samples and reagents were mixed and allowed to sit for 10 minutes at room temperature.

- Then the samples and reagent were centrifuged at about 400rpm for 10 min.
- The supernatant was carefully collected with micro pipette.
- The reagent (B) was brought to room temperature 20 to $25^0$C

**CALCULATION**

The HDL cholesterol concentration in the sample is calculated using the general formula.

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \text{ x concentration of standard}$$

**3.7 LOW DENSITY LIPOPROTEIN (LDL)**

LDL – cholesterol can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS)in the presence of polyetheyleneglycerol monomethyl ether.

**PROCEDURE**

i. The precipitant solution and the sample were mixed thoroughly and allowed to stand for 15 minutes at room temperature (20-$25^0$c).

ii. Then the sample and reagent were centrifuged at $37^0$C for 15 minutes.

iii. The cholesterol concentration was determined in the supernatant.

**CALCULATION**

LDL – cholesterol (mg/dl) = Total cholesterol (mg/dl) - 1.5 x supernatant cholesterol (mg/dl)

**3.8 DETERMINATION OF TRIACYLGLYCEROL (TAG)**

**COLOMETRIC METHOD**

The triacylglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4- aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

**PRINCIPLE**

$$\text{Triacylglycerol} + H_20 \xrightarrow{\text{Lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{GK}]{} \text{glycerol} - 3 \text{ phosphate} + \text{ADP}$$

$$\text{Glycerol-3- phosphate} + 0_2 \xrightarrow{\text{GPD}} \text{dihydroxyacetone} + \text{phosphate} + H_20_2$$

$$2H_20_2 + \text{4-aminophenazone} + \text{4- chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + \text{HCL} + 4H_20.$$

**PROCEDURE**

- The samples and reagents were pipetted into cuvette.
- The reagents and various samples were mixed and incubated for 10 minutes at 20- $25^0$C or 5 minutes at $37^0$C.
- The absorbance of the sample and standard were read against the reagent blank within 60 minutes.

**CALCULATION**

When using a standard

Triacylgycerol concentration = $\frac{A_{sample}}{A_{standard}}$ Standard Conc. = mmol/l (mmol/l )

$\frac{A_{sample}}{A_{standard}}$ Standard Conc. = mg/dl (mg/dl)

**3.9 DETERMINATION OF GLUTATHIONE**

**Principle**

Glutathione peroxidase catalyzes hydrogen peroxide by the oxidation of GSH according to the following reaction

$$2GSH + H_20_2 \xrightarrow{GR} GSSG + H_20$$

$$GSSG + NADPH \xrightarrow{GPX} 2GSH + NADP.$$

**PROCEDURE**

Fifty microliters of RBC is mixed with 1ml cyanodilution mixture and then shaken. To 0.02ml of diluted RBC are added to 500 microliter of potassium.

**3.10 DETERMINATION OF CATALASE**

Catalase were determined using the enzymatic analysis method 3rd ed. (bergmeyer et al., 1983).

**Principle:** The ultraviolet absorption of hydrogen peroxide can be easily measured at 240nm. On the decomposition of hydrogen peroxide with catalase, the absorption decreases with time and from this, decrease catalase activity can be measured.

**PROCEDURE**

Red blood cell lysate is prepared by adding 1.2ml of distilled water to 0.2ml of RBC. Then five hundred. Fold dilution of RBC lysate by phosphate buffer is made before the determination of catalase activity. Immediately following the addition of 1ml phosphate buffer (blank) or hydroxide peroxide solution into 2ml RBC diluted lysate, the change of absorbance of RBC against blank at 240nm is recorded every 15 seconds for 1 minute on a spectrophotometer. The activity of catalase is calculated by using catalytic concentration (umil/L) = $(0.23*\log A_1A_2)/0.00693$.

Where $\wedge_1$ is $\wedge$ 240 at t = 0,

$\wedge_2$ is $\wedge$ 250 at t = 15 second.

### 3.11 DETERMINATION OF SUPEROXIDE DISMUTASE (SOD)

Superoxide disputase (SOD) was determined using the method Xin *et al.*, (1991).

**Principle**

Superoxide dismutase (SOD) reduces superoxide to hydrogen peroxide. The theory of this method is based on the competition between SOD activity and iodonitrotetrazolium violet in reacting with superoxide, which is generated by xanthine oxidase (XOD) reaction. The reactions are demonstrated below:

a. xanthine x $0_2$ $\xrightarrow{XOD}$ uric acid + $0_2$

b. $O_2 + O_2 + 2Ht \xrightarrow{SOD}$ Hydrogen peroxide + $O_2$

c. $O_2$ + Violet $\longrightarrow$ oxidized detector (generate colour).

**PROCEDURE**

To test tube, pipette 0.9ml of distilled water 0.1ml of sample, 0.1ml of this mixed with 0.9ml of carbonate buffer and 75Nl of oxanthine oxidase added. The absorbance is determined at 50nm for 3 min at 20 seconds interval. The changing rate of absorbance is used to determined superoxide dismutase activity.

### 3.12 DETERMINATION OF VITAMIN C (Ascorbic acid)

Ascorbic acid was assayed using the dinitrophenyl hydrazine method of carasy (1970).

**Principle**

Ascorbic acid is oxidized and converted to dikitogutamic acid in strong acid solution and the plasma separated from the blood cells. These plasma samples were analyzed as soon as possible.

**PROCEDURE**

10g of EDTA was dissolved in distilled water and the volume made up to 100ml, to each tube was added 0.05ml of EDTA for 5 ml of blood. This was allowed to evaporate to dry salt at room temperature before they were used.

### 3.13 DETERMINATION OF MALONDIALDEHYDE (MDA)

Malondialdehyde was determined spetrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al., (1993).

**Principle**

Malondialdihyde (MDA) reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution. Absorbed maximally at 532 nm. MDA + 2TBA - MDA: TBA adduct + $H_20$.

**PROCEDURE**

- To a test tube ad 0.1ml of sample + 0.9ml of distilled water + 0.5ml of 25% TCA + 0.5ml of 1% TBA in 0.3% Na0H.
- Incubate at $95^0$C for 40 min.
- Add 0.1ml of 20% SDS (Sodium dodecyl sulphate).
- Determine absorbance at 532 and 600nm against blank.

$$\% \text{ TBARS} = \frac{A\,532 - A\,600}{0.5271 \times 0.1} \times \frac{100}{1}$$

Slope from standard curve = y = 0.5208 x (mg/ml)

### 3.14 DETERMINATION OF VITAMIN E

**PROCEDURE**

- Pipette 0.1ml of the serum into a test tube.
- Add 1ml of 0.2% ferric chloride and 1ml of alcoholic 0.5% and α-dipyridyl solution.
- Shake
- Dilute to 5ml with distilled water
- Measure the absorbance at 520nm.

### 3.15 DETERMINATION OF TOTAL CHOLESTEROL (CHOL)

The serum total cholesterol (Allian *et al.*,1974) to obtain the total serum cholesterol.

**Principle**

The cholesterol is determined after enzymatic hydrolysis and oxidation.

The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase

Cholesterol ester + $H_20$ $\xrightarrow{\text{Cholesterol}}$ Chol + fatty acids

Cholesterol + 0 $\xrightarrow[2]{\text{cholesterol}}$ cholestone -3-one + $H_20_2$

$2H_20_2$ + phenol + 4-Aminoantipyrine $\xrightarrow{\text{Peroxide}}$ quinoeimine + $4H_20$

**PROCEDURE**

- Reagents were brought to room temperature.
- The various samples and reagents were pipetted into labeled cuvette

| 1 | **Reagent blank SO** | **SI** | **Sample** |
|---|---|---|---|
| $ddH_20$ | 50µl | - | - |
| Standard | - | 50 µl | - |
| Sample | - | - | 50 µl |
| Reagent | 50 µl | 50 µl | 50 µl |

- The various samples were mixed thoroughly and incubated at $37^0$ C for 10 min.
- The absorbance (A) of the standard and sample at 500nm against the reagent blank within 60 min.

**MANUAL CALCULATION**

The cholesterol in the serum were calculated using the formula

$$\frac{\Delta A \text{ sample} \times \text{Concentration of standard}}{\Delta A \text{ standard}}$$

**CHAPTER**

**RESULT**

**4.0 STATISTICAL ANALYSIS**

Data obtained were analyzed statistically using statistical package for social science (SPSS) (version 17.0). The data are expressed as mean ± standard deviation using bar charts comparisons were made between the control animals that received the methanol extract of

250mg/kg and 500mg/kg of Delonix regia using paired T-test. The significance difference was accepted at ($p<0.05$)

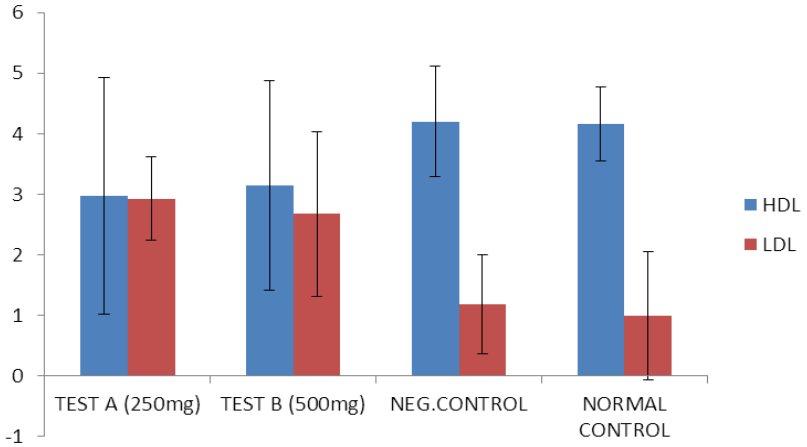


**Fig 1. The effect of ME of *Delonix regia* on test and control**

From fig. 1 above ,the mean value result of the 250mg extract (2.97 ± 1.95) and 500mg extract (3.14 ± 1.73) on HDL has a non- significant ($P>0.05$) increase and increase respectively when compared with the negative control (4.20± 0.91) as well as non-significant ($P>0.05$) increase and decrease respectively when compared with the normal control (4.16 ± 0.16).

Also the mean value of 250mg extract (2.93±0.64) and 500mg extract
(2.68 ± 1.36) on LDL has a non-significant ($P>0.05$) increase and decrease when compared with the negative control (1.18±0.82) as well as non-significant ($P>0.05$) increase and decrease when compared with the normal control (0.99 ±1.06).

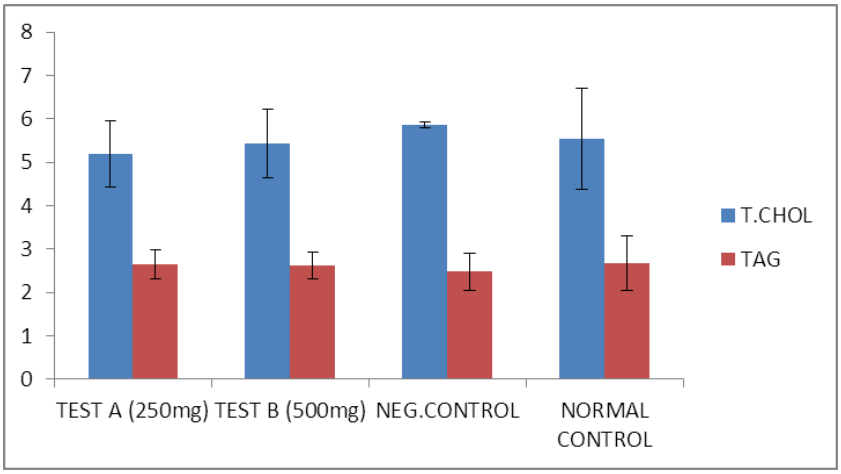


**Fig. 2: The effect of ME of *Delonix regia* on test and control (T.CHOL and TAG)**

From fig. 2 above ,the mean value result of the 250mg extract (5.19 ± 0.77) and 500mg extract (5.43 ± 0.80) on Total Cholesterol has a non- significant (P>0.05) increase and decrease respectively when compared with the negative control (5.86± 0.58) as well as non-significant (P>0.05) increase and decrease respectively when compared with the normal control (5.54 ± 1.157).

Also the mean value of 250mg extract (2.64±0.34) and 500mg extract (2.60 ± 0.31) on TAG has a non-significant (P>0.05) increase and decrease when compared with the negative control (2.48 ± 0.43) as well as non-significant (P>0.05) increase and decrease when compared with the normal control (2.67 ±0.63).

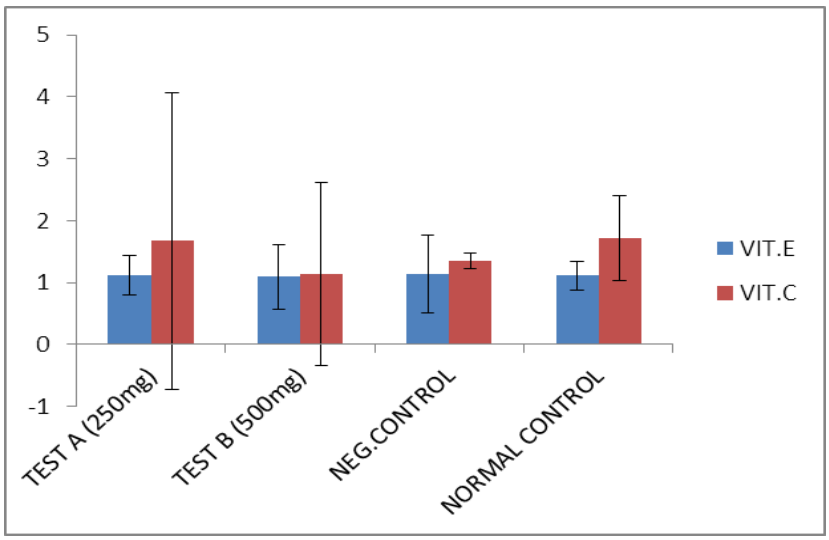


**Fig. 3: The effect of ME of *Delonix regia* on test and control (VIT.C and VIT.E)**

From fig. 3. Above, the mean value result of the 250mg extract (1.11± 0.03) and 500mg extract (1.09± 0.03) on VIT. E has a non- significant (P>0.05) decrease and increase respectively when compared with the negative control (1.13± 0.06) as well as non-significant (P>0.05) increase and decrease respectively when compared with the normal control (1.12 ± 0.24).

Also the mean value of 250mg extract (1.67 ±2.39) and 500mg extract (1.13 ± 1.48) on VIT.C has a non-significant (P>0.05) increase and decrease when compared with the negative control (1.35±0.13) as well as non-significant (P>0.05) increase and decrease when compared with the normal control (1.72 ±0.69).

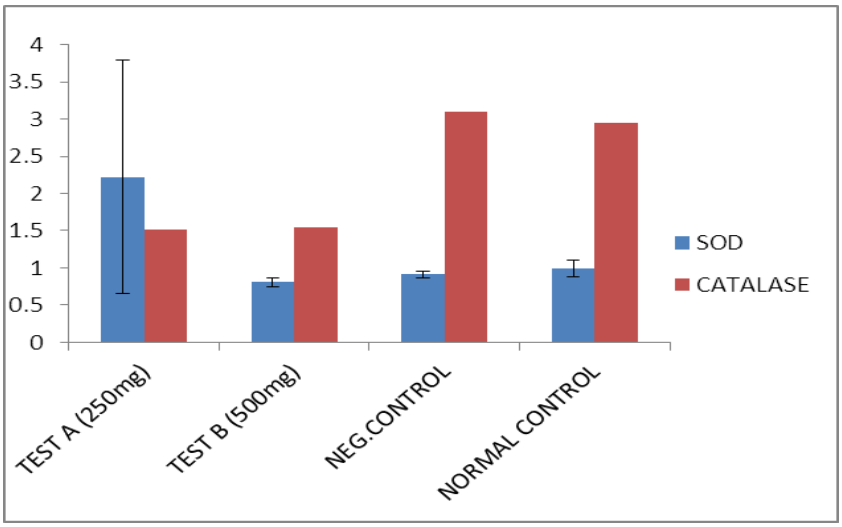


**Fig.4: The effect of ME of *Delonix regia* on test and control (SOD and CAT)**

From fig. 4. Above, the mean value result of the 250mg extract (2.22± 1.57) and 500mg extract (0.81± 0.06) on SOD has a non- significant (P>0.05) increase and decrease respectively when compared with the negative control (0.91± 0.05) as well as non-significant (P>0.05) decrease and increase respectively when compared with the normal control (0.99 ± 1.11).

Also the mean value of 250mg extract (1.52 ±1.22) and 500mg extract (1.54 ± 1.23) on VIT.C has a non-significant (P>0.05) decrease and increase when compared with the negative control (3.09±0.61) as well as non-significant (P>0.05) decrease and increase when compared with the normal control (2.95 ±2.17).

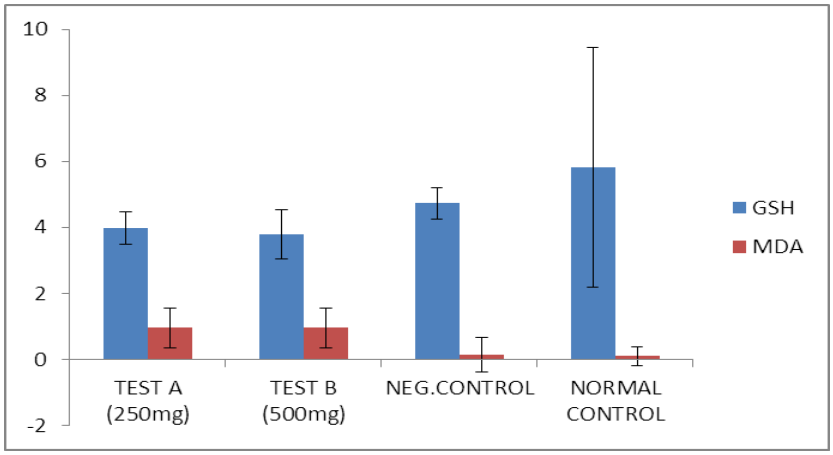


**Fig.5: The effect of ME of *Delonix regia* on test and control (MDA and GSH)**

From fig. 5. Above, the mean value result of the 250mg extract (3.99± 0.49) and 500mg extract (3.79± 0.74) on GSH has a non- significant (P>0.05) increase and decrease

respectively when compared with the negative control (4.73± 0.48) as well as non-significant (P>0.05) increase and decrease respectively when compared with the normal control (5.80 ± 3.64).

Also the mean value of 250mg extract (0.96 ±0.59) and 500mg extract (0.95 ± 0.60) on MDA has a non-significant (P>0.05) increase and decrease when compared with the negative control (0.14±0.52) as well as non-significant (P>0.05) increase and decrease when compared with the normal control (0.10 ±0.03).

## CHAPTER FIVE

## 5.0 DISCUSSION

Free radical scavenger is a vitamin, mineral, or enzymes that is able to destroy free radicals. Mammalian cells possess elaborate detoxification. Key metabolite steps are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which destroy toxic perioxides. In addition to antioxidant enzymes, non-ezymatic molecules including thiols, thioredoxin, and disulfide-bonding play important roles in antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as α-tocopherol, β-carotene and ascorbic acid, and such micronutrient elements as zinc and selenium (Halliwel 1998).

If cellular constituents do not effectively scavenge free radicals, they lead to disease conditions to the cell membranes and DNA, including oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutation leading to cancer degeneratives, and other diseases (Cerulti 1994). The result of this study demonstrate that delonix regia is a rich source of potential antioxidant and such effect may be related to the negative control and normal control. The total cholesterol, HDL, LDL, TAG, MDA, GSH, VIT. E, SOD and catalase shows no significant (P <0.05) difference between these parameters in the wistar albino rats as compared to the negative control and the normal control.

The total cholesterol test of the mean and standard deviation wave (5.19 ± 0.77) in 250 mg test and (5.86 ± 0.58) in 250g negative control also (5.43 ± 0.80) in 500mg test and (5.54 ± 1.16) on 500 mg normal control. (P = 0.820) for 250mg negative control test (P = 0.591) for 250mg normal control test (P = 0.246) for 500mg test and negative control and (P = 0.944) for 500g mg test and normal control.

The triacylglycerol (TAG) test of mean and standard deviation were (2.64 ± 0.34) for 250mg test and (2.48 ± 0.43) for 250mg negative control also (2.60 ± 0.36) for 500mg test (2.67 ± 0.63). (P = 0.33) for 250mg test and negative control, (P = 0.91) for 250mg normal control, (P = 0.19) for 500mg test and negative control test and (P = 0.92) for 500mg test and normal control.

The HDL test of mean and standard deviation were (2.97 ± 1.95) for 250mg test and (4.20 ± 0.91) for 260mg negative control also (3.14 ± 1.73) for 500mg test and (4.16 ± 0.16). (P = 0.541) for 250 mg test and negative control, (P = 0.517) for 250mg test and normal control (P = 0.520) for 500mg test and negative control and (P = 0.294) for 500mg test and normal control.

The LDL test of mean and standard deviation were (2.93± 0.64) for 250mg test and (2.68 ± 1.36) for 250mg negative control also (1.18± 0.82) for 500mg test (0.99 ± 1.06). (P = 0.94) for 250mg test and negative control, (P = 0.2.3) for 250mg test normal control, (P = 0.98) for 500mg test and negative control and (P = 0.35) for 500mg test and normal control.

The MDA test of mean and standard deviation were (0.96 ± 0.59) for 250mg test and (0.95 ± .060) for 260mg negative control also (0.14 ± 0.52) for 500mg test and (0.10 ± 0.03). (P = 0.87) for 250 mg test and negative control, (P = 0.57) for 250mg test and normal control (P = 0.88) for 500mg test and negative control and (P = 0.56) for 500mg test and normal control.
The GSH test of mean and standard deviation were (3.99 ± 0.49) for 250mg test and (3.79 ± 0.74) for 250mg negative control also (4.73 ± 0.48) for 500mg test (5.80 ± 3.64). (P = 0.55) for 250mg test and negative control, (P = 0.59) for 250mg normal control, (P = 0.70) for 500mg test and negative control and (P = 0.61) for 500mg test and normal control.

The VIT.E test of mean and standard deviation were (1.11 ± 0.03) for 250mg test and (1.09 ± 0.05) for 260mg negative control also (1.13 ± 0.06) for 500mg test and (4.16 ± 0.16) (P = 0.86) for 250 mg test and negative control, (P = 020) for 250mg test and normal control (P = 0.24) for 500mg test and negative control and (P = 0.68) for 500mg test and normal control.
The VIT.C test of mean and standard deviation were (1.67± 2.39) for 250mg test and (1.13 ± 1.48) for 250mg negative control also (1.35 ± 0.13) for 500mg test (1.72 ± 0.69). (P = 0.81) for 250mg test and negative control, (P = 0.19) for 250mg normal control, (P = 0.81) for 500mg test and negative control and (P = 0.23) for 500mg test and normal control.

The SOD test of mean and standard deviation were (2.22 ± 1.59) for 250mg test and (0.81 ± 0.06) for 260mg negative control also (0.91 ± 0.05) for 500mg test and (0.99 ± 1.11). (P = 0.10) for 250 mg test and negative control, (P = 0.74) for 250mg test and normal control (P = 0.63) for 500mg test and negative control and (P = 0.92) for 500mg test and normal control.
The catalase test of mean and standard deviation were (1.52 ± 1.24) for 250mg test and (1.54 ± 1.23) for 250mg negative control also (3.09 ± 0.61) for 500mg test (2.95 ± 2.17). (P = 0.98) for 250mg test and negative control, (P = 0.08) for 250mg normal control, (P = 0.98) for 500mg test and negative control and (P = 0.10) for 500mg test and normal control.

A number of research studies have however shown statistically significant (P < 0.05) that the methanolic extract of *Delonix regia* hepatoprotective activity against chloroform and hexane induced hepatoxicity in wistar albino rats. Also, *Delonix regia* has been reported to have antioxidant, anti-arthritic, anti-ulcer and anti-inflammetory activity.

## 5.1 CONCLUSION

The results of the present study demonstrate that *Delonix regia* is a rich source of potential antioxidants and such effects may be related to their biochemical constituents in the leaves and flowers, major ones being lupeol phenolic acid, β-sistosterol, carotenoids, flavonoids and other polyphenolic compounds.

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