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RESEARCH ARTICLE

"SCREENING OF CARDIOPROTECTIVE AND ANTI-OXIDANT ACTIVITY OF *RUELLIA TUBEROSA*"

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ABSTRACT

Objective: The aim of the current study was to evaluate the cardioprotective effect of the methanolic extract of Ruellia tuberosa on doxorubicin induced cardiotoxicity.
Methodology: For cardiotoxicity, thirty rats were evenly divided into 5 groups. Groups-1 served as Normal,Group-2 control drug treated group, Group-3 vitamin-E treated group, Group-4 test drug(low dose) treated group, Group-5 test drug(high dose) treated group, and treatment is given for 8 days, On 5th day need to induce cardio toxicity with 5 fluorouracil, then on 9th day need to sacrifices rat then blood samples is taken for lipid profile test, and the rat heart for histopathology were obtained under inhaled diethyl ether anesthesia**.**
Result: administration of 5 fluorouracil in control rats showed a significant increase serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), and decrease in high density lipoprotein (HDL).Rats treated with hydromethanolic extract of Ruellia tuberosa (250 mg/kg and 500 mg/kg) showed decreased TC, TG, LDL and increases HDL levels. The histopathological studies also showed that the plant extract significantly minimized the damage induced by % fluorouracil.
Conclusion: Thus, Ruellia tuberosa provide cardio protection against 5 fluorouracil induced MI in rates.

INTRODUCTION

Acute myocardial infarction is the medical name for a heart attack. A heart attack is a life-threatening condition that occurs when blood flow to the heart muscle is abruptly cut off, causing tissue damage. This is usually the result of a blockage in one or more of the coronaryarteries. A blockage can develop due to a buildup of plaque, a substance mostly made of fat, cholesterol, and cellular waste products (Lopez and Murray, 198). Myocardial infarction (MI) occurs when there is myocardial necrosis due to prolonged imbalance between the myocardial oxygen supply and demand of the myocardium (Petrich *et al.,* 1996). Myocardial infarction is said to be part of a spectrum of diseases known as Acute Coronary Syndromes (ACS). The diseases that make up the spectrum are unstable angina, acute myocardial infarction, and sudden cardiac death (Thygesen, 2007). Among the various proposed mechanisms, the accumulations of free radicals have been implicated in the pathophysiology of acute myocardial infarction (Zhou *et al.,* 2008). It is the one of serial cause of the death in US and other developed countries. Main risk factors for the MI is the atherosclerosis of coronary artery1, calcium reduction, generation of free radicals, oxidative metabolism of catecholamines, these oxidative products impact on the cardiac myocytes membrane and also depress the cardiac contractile function, prior to which damage in the mitochondria, sarcotubular system and contractile functions (Rona, 1985). Fluorouracil (5-FU) is an anticancer medicine that works by slowing or stopping cell growth (Beni-suef, 2016). Fluorouracil is part of a group of chemotherapy drugs known as antimetabolites (Grem, 1997). The antimetabolite 5-Fluorouracil (5-FU), an analogue of uracil, and its pro-drugs are widely used antineoplastic agents for the treatment of gastrointestinal cancers, breast, gynecological as well as head and neck tumors (Zhang *et al.,* 2008). Due to its structure, 5-FU interferes with nucleoside metabolism and can be incorporated into ribonucleic acid (RNA) and deoxyri bonucleic acid (DNA), leading to cytotoxicity and cell death (Arias *et al.,* 2008; Rich *et al.,* 2004). When combined with radiation therapy, improved local control and survival rates have been reported in a variety of malignancies, compared to radiotherapy alone (Tessa *et al.,* 2009). However, 5-FU indiscriminate mechanism of action targets not only cancer cells, but all rapidly dividing cells within the body (Keller *et al.,* 1996). Fluoroacetate enters the Krebs' cycle and converts into fluorocitrate, which inhibits the enzyme aconitase (Gradishar and Vokes, 1990) causing citrate accumulation, disruption of the tricarboxylic acid cycle and severe impairment of energy production within themyocytes (Rashid *et al.,* 2014). The pathogenesis of 5-FU induced cardiotoxicity may involve cellular damage due to the oxidative stress and the induction of apoptosis (Casale *et al.,* 2004). 5-FU availability for intracellular anabolism mainly depends on tissue drug catabolism. After administration, 5-FU follows different metabolic destinations: more than 80% of the dose is inactivated by biotransformation primarily in the liver, approximately 15–20% is eliminated in the urine and only a

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small fraction remains available to exert its anti-tumor action (Álvarez *et al.,* 2012) Fluorouracil was patented in 1956 and came into medical use in 1962 (Heidelberger *et al.,* 1957). It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system (Longley *et al.,* 2003). 5-FU acts in several ways, but principally as a thymidylate synthase (TS) inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication. Thymidylate synthase methylates deoxyuridine monophosphate (dUMP) to form thymidine monopho sphate (dTMP). Administration of 5-FU causes a scarcity in dTMP, so rapidly dividing cancerous cells undergo cell death via thymineless death (Álvarez *et al.,* 2012). Calcium folinate provides an exogenous source of reduced folinates and hence stabilises the 5-FU-TS complex, hence enhancing 5-FU's cytotoxicity (Pardeshi *et al.,* ?). The world health organization (WHO) estimates that about 80% of people living in developing countries depends almost exclusively on traditional medicines for their primary health care needs (Yang Mekar ditamanku). *Ruellia tuberosa* is geneous of flowering plant.Family-Acantheacea (Mmbb Bristow *et al.,* 1978). Its native range is in Central America but presently it has become naturalized in many countries of tropical South and Southeast Asia (Sailaja and Bharathi). Ruellia used as a "cooling" agent, for urinary problems and high cholesterol. In traditional medicine, used as anthelmintic; for joint pains and muscle strain. Also used as abortifacient. Root is used against kidney diseases and whooping cough (Alam *et al.,* 2009). Ethanol extract of Ruellia tuberosa showed antinociceptive and anti-inflammatory activities with maximum time response against thermal stimuli similar to that of diclofenac (Rajan *et al.,* 2012) Cardioprotective have been reported to be present in Ruellia tuberosa as it contains flavonoids like cirsimarin, cirsiliol 4'-glucoside, sorbifolin, pedalitin (Sayantan *et al.,* 2013). Study evaluated the anti-carcinogenic activity and antioxidant property of R. tuberosa methanol leaf extract on HepG2 cell line (Durre Shahwar *et al.,* 2011).

MATERIALS AND METHODS

Collection of Plant Material

The leaf of plant *Ruellia tuberosa* was collected from Thirupati forest region, Thirupathi district, Andhra Pradesh, India in the month of September, 2017, the plant species were authenticated by Dr.K. Mahadev Chetty, Assistant Professor, Department of botany, Sri venkateswara university, Thirupati, Andhara Pradesh India. The plant was identified by a botanist and voucher specimen was deposited in Rajiv Gandhi University Of Health Science and copy has been preserved for, future reference at Karnataka College Of Pharmacy, Department Of Pharmacology, the collected leaf was washed thoroughly with water to remove adhering soil, mud and debries. Then the leaf was dried in shade at room temperature, then the plant material was powdered with blender, the powder was stored in an airtight container and protected from light.

Preparation of extract

250 gm powdered plant material was subjected to successive extraction in a reflux condenser using 1.5 litre methanol for 3 hour at temperature of 80^{0c},separate the supernatant layer and remaining portion mixed with 1 litre of methanol and heated at 80^{0c} for another 1 and half hour again separate the supernatant layer and remaining portion is mixed with 1 litre methanol and heated at 80^{0c} for another 1 and half hour and separate the supernatant layer, finally mix all the three layers of extract and final product is evaporated to dryness to get constant weight.

Experimental Design

Experimental animals

Wister albino rat having weight (150-200gm) were purchased from NIMHANS Bangalore. They were housed, six per poly propylene cage with paddy husk bedding. Animal will maintain under standard laboratory conditions at room temperature (25°C± 2°C) with 12 hr. light / dark cycle. The animals were provided with pellet chow and water. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of Karnataka College of pharmacy, Bangalore.

Animals Are Divided Into 5 Groups Each Group Have Six Animals.

Group-1: Normal (normal saline for 8 days)

Group-2: I.P. injection of 5 fluorouracil only on 5th day (150mg/kg)

Group-3: Vitamin-E () (for 8 days) before 1 hr of IP injection of 5-FU+I.P. injection of 5 fluorouracil (150mg/kg) only on 5th day

Group-4: Ruellia tuberosa extract, low dose (250mg/kg) before 1 hr of IP injection of 5-FU (for 8 days) +I.P. injection of 5 fluorouracil (250mg/kg) only on 5th day

Group-5: Ruellia tuberosa extract, high dose (500mg/kg) before 1 hr of IP injection of 5-FU (for 8 days) +I.P. injection of 5 fluorouracil (150mg/kg) only on 5th day

Here, to control group normal saline was given at the dose of 10 ml/kg and to standard drug vitamin-E treatment group vitamin-E was given at the dose of 100 mg/kg and to RT treatment group(low dose) 250 mg/kg RT was given and to RT treatment group(high dose) 500 mg/kg RT was given. All above drugs was given for seven days by oral route. Then on 5th day 5-fluorouracil was given at the dose of 150 mg/kg by IP route.

Biochemical assays

On the 8th day, the rats were fasted overnight. On the 9th day the fasted rats were sacrificed under diethyl ether anesthesia and blood samples were collected into plain sample bottles. Blood samples were collected via retro-orbital puncture or by cardiac puncture with 21G needle mounted on 5ml syringe. The animals were analysed according to standard methods for effect of the extract on various biochemical parameters of rats such as TC, TG, LDL and HDL.

Histological studies

On the 8th day, the rats were fasted overnight. On the 9th day the fasted rats were sacrificed under diethyl ether anesthesia and portion of heart and liver of rats was collected from all group rats (normal, disease control, vit-E, low dose of RT, high dose of RT) and fixed in 10% formalin (10 ml of formaldehyde added to 90 ml of water). Then it was send for histopathological study to diagnostic centre.

Table 1.

SR.	Group	Total cholesterol (mg/dl)	Triglycerides (mg/ml)	HDL (mg/dl)	LDL (mg/dl)
1.	Normal	90.5±0.763	79.67±1.22	56.5±1.607	80.33±1.453
2.	Control	165±0.763###	155±1.544###	46.17±2.088###	150±1.204###
3.	Standard	116.3±1.542***	97.17±2.892***	49.67±1.82***	80.83±2.442***
4.	Low dose	126±2.04***	107±2.769***	53.83±1.47***	96.67±2.431***
5.	High dose	112.5±1.784***	103.7±2.85***	51.33±1.498***	91.83±2.088***

Value are expressed as mean±SEM and n=6. ###P<.001 when compared to normal.
***P<.001when compare to disease control.

Stastical Analysis

Results were expressed as the Mean ±standard error means S.E.M.). The comparison of data within groups was performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by Dunnett's test. A probability level of less than 1 % ($P < 0.001$) was considered significant. Statistical analysis was performed using Graph Pad prism.

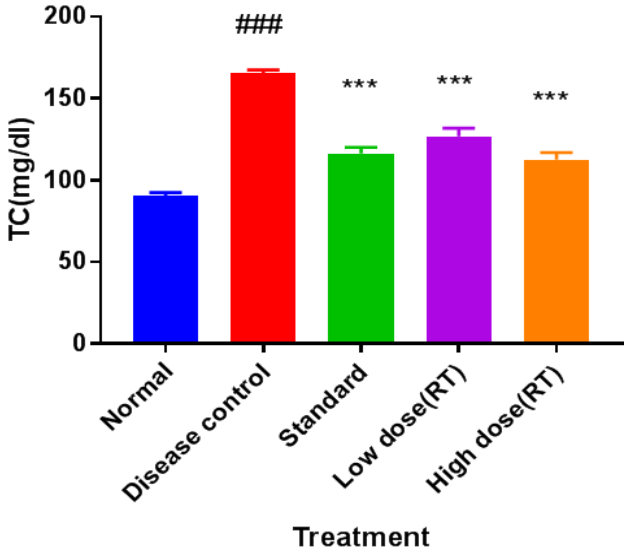

Fig. 1. Effect of 5-FU, Vit E and Ruellia tuberosa on serum total cholesterol level

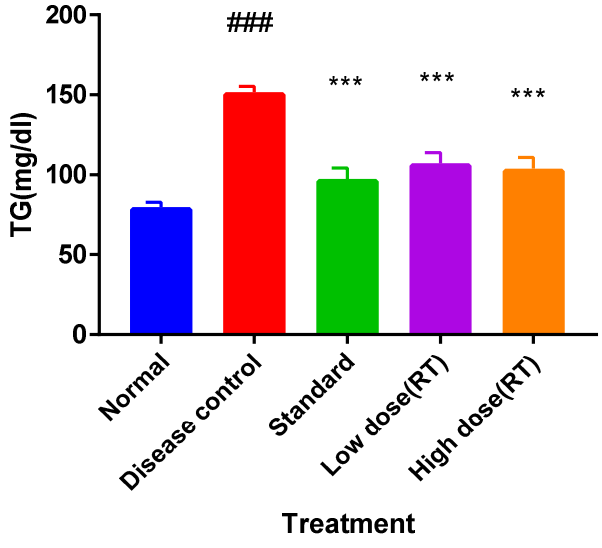

Fig. 2. Effect of 5-FU, Vit E and Ruellia tuberosa on serum triglyceride level

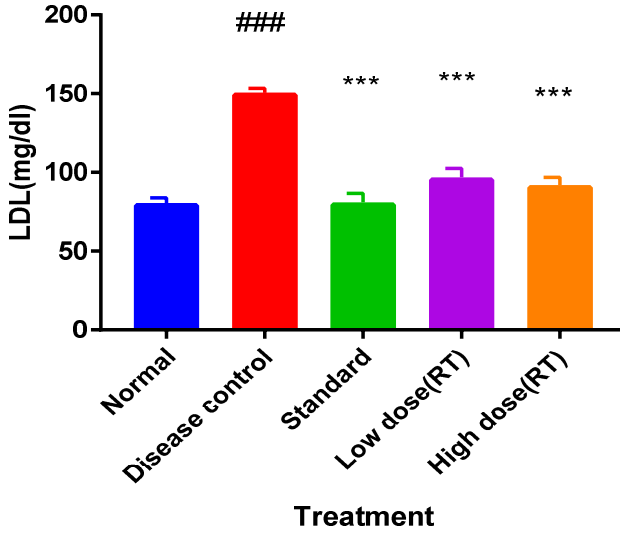

Fig. 3. Effect of 5-FU, Vit E and Ruellia tuberosa on serum total LDL level

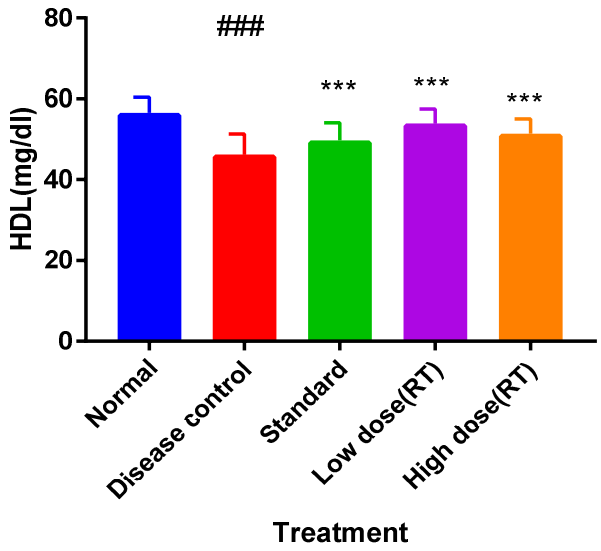

Fig. 4. Effect of 5-FU, Vit E and Ruellia tuberosa on serum total HDL level

Histopathology of 5 fluorouracil induced myocardial necrosis

Heart: Normal

Microscopy: Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers (Fig.8a, Arrow). The cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils (Fig.8b, Arrow). The interstitial space appears within normal limits.

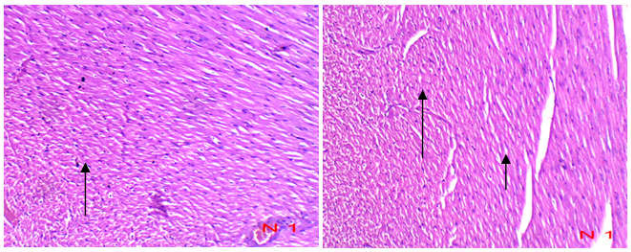

Figure 8(a):[H&E, x400] Figure-8(b):[H&E, x400]

Heart: Control

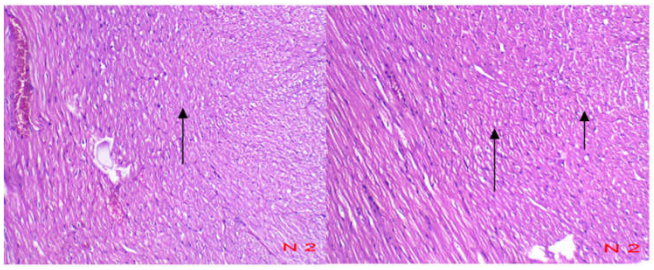

Figure 9.(a) [H&E, x400] Figure 9. (b)[H&E, x400]

Microscopy: Section studied from the myocardium shows focally haphazard arrangement of the cardiac muscle fibers (Fig.9b, Arrow). The cardiac muscle fibers show focal loss of integrity of myocardial cell membrane, myofibrillar structure with focal loss of striations and loss of continuity with adjacent myofibrils (Fig.9a, arrow). The interstitial space appears mildly increased at focal areas.

Heart: Vitamin E Group

Microscopy

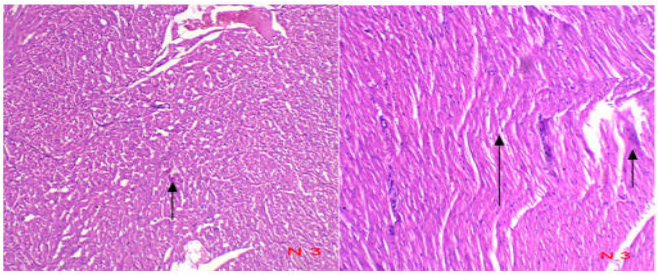

Figure10. (a)[H&E, x400] Figure 10. (b)[H&E, x400]

Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers (Fig.10b, Arrow). The cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with focal loss of striations and loss of continuity with adjacent myofibrils (Fig.10a, arrow).

Heart: Low dose [Test Drug]

Microscopy

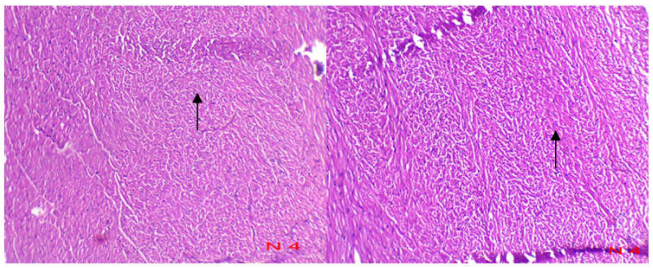

Figure 11(a). [H&E, x400] Figure-11(b)[H&E, x400]

The interstitial space appears increased at few areas. Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers (Fig.10b, Arrow).

Heart: High Dose [Test Drug].

Microscopy

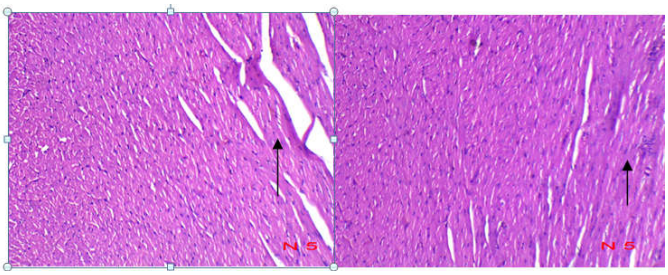

Figure 12(a)[H&E, x400] Figure-12(b)[H&E, x400]

The cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with focal loss of striations and loss of continuity with adjacent myofibrils (Fig.10a, arrow). The interstitial space appears increased at few areas. Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers [Fig.11b, Arrow]. The cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with focal l oss of striations and loss of continuity with adjacent myofibrils [Fig.11a, arrow]. The interstitial space appears intact. Section studied from the myocardium shows intact arrangement of the cardiac muscle fibers.

Antioxidant study,

Table 2.

reatment	Catalase(µg/mg of protein)	SOD(µg/mg of protein)	LPO(µg/mg of protein)	(Mmol/min/mg of protein)
Normal	18±4.00	1±0.003	3.86±0.05	67.17±6.355
Control	13±3.00###	0.3025±0.0055##	5.61±0.400###	46.73±2.53##
Standard(Vitamin E)	15±3.00***	0.861±0.033**	4.395±0.415***	63.02±3.92**
Low dose of RT 250mg/kg	14±3.00***	0.7215±0.0005**	4.84±0.14***	57.9±2.8**
High dose of RT 500mg/kg	15.5±3.5**	0.886±0.005**	4.31±0.389***	61.29±4.485**

Value are expressed as mean±SEM and n=6. ***P<0.001, ###P<0.001, ##P=0.003, **P=.005 and when compared to normal.

These cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils (Fig.12a, Arrow). The interstitial space appears intact (Fig.12b, Arrow).

Antioxidant activity of catalase

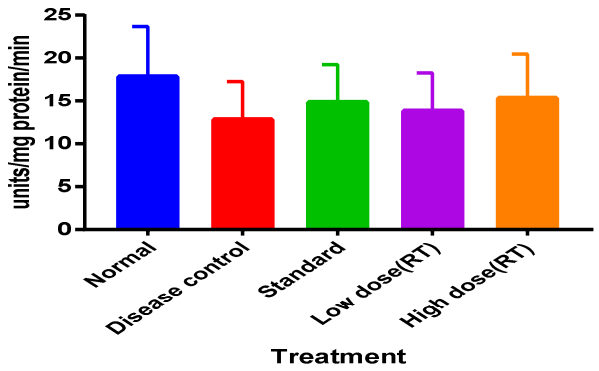

Fig. 1. Effect of 5-FU, Vit E and Ruellia tuberosa on catalase P*=0.001 and **P=0.003**

Antioxidant activity of SOD

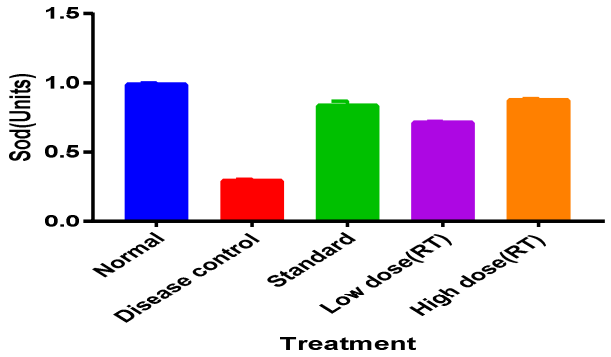

Fig. 2. Effect of 5-FU, Vit E and Ruellia tuberosa on SOD p*=0.001 and **P=0.006**

DISCUSSION

Ruellia tuberosa act as antioxidants. Based on these assumptions on *Ruellia tuberosa* was used to study antioxidant and cardioprotective activity.

Antioxidant activity of LPO

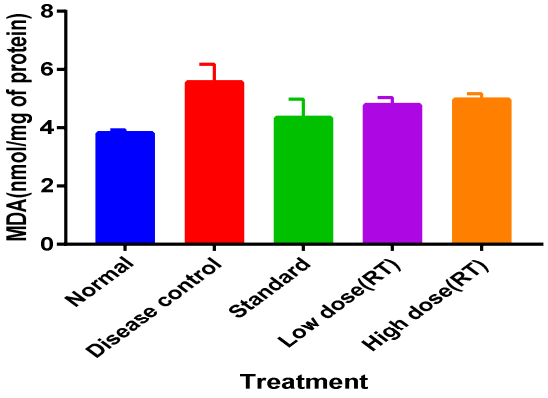

Fig.3. Effect of 5-FU, Vit E and Ruellia tuberosa on LPO **P=0.005 and ***p=<0.001

It has been well established that nutrition plays an important role in the etiology of hypercholesterolemia and atherosclerosis.

Hypercholesterolemia involves heterogenous disorders of lipid metabolism characterized by elevated levels of plasma total cholesterol and LDL. Although several factors, such as a diet high in saturated fats and cholesterol, age, family history, hypertension, and lifestyle play a significant role in causing heart failure, the high levels of cholesterol particularly TC, TG and LDL cholesterol is mainly responsible for the onset of CHD.

Antioxidant activity of Glutathione Peroxidase(GPs)

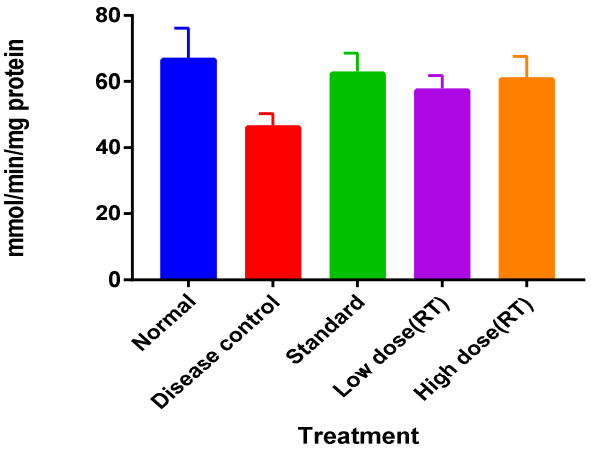

Fig.4. Effect of 5-FU, Vit E and Ruellia tuberosa on GPs ***p=<0.001

Fluorouracil - induced myocardial necrosis in rats

In this study animal feed with normal diet for 7 days. Then 5FU is given on 5th day, on the 9th day the fasted rats were sacrificed under diethyl ether anesthesia and blood samples were collected into plain sample bottles. Blood samples were collected via retro-orbital puncture or by cardiac puncture with 21G needle mounted on 5ml syringe. The animals were analyzed according to standard methods for effect of the vitamin-E, and extract on various biochemical parameters of rats such as TC, TG, LDL and HDL. Administration of 5-FU in control rats showed a significant increase serum Total Cholesterol (TC), Triglycerides (TG), low density lipoprotein (LDL) and decrease in High density lipoprotein (HDL). Rats treated with standard cardio protective drug (10 mg/kg), vitamin-E (100 mg/kg), methanolic extract of *Ruellia tuberosa* (250 mg/kg and 500 mg/kg) showed decreased TC, TG, LDL and increases HDL levels.

Antioxidant property

Ruellia tuberosa has shown a dose dependent increase in anti-radical activity. However, the anti-radical activity of RT was much less when compared to ascorbic acid. Cardioprotective activity of *Ruellia tuberosa* may be partially due its free radical scavenging activity. Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA. Therefore, studying the scavenging activity of plant extract on different radical is one of the most important ways of the mechanism of antioxidant activity. The hydroxyl radical is an extremely reactive oxidizing radical that will react with most biomolecules at diffusion controlled rates.

It is extremely short half-life but is capable causing damage within a small radius of its site of production. A single hydroxyl radical can result in the formation of many molecules of lipid hydroperoxide in the cell membrane, which may severly disrupt its function and leads to cell death. Lipid peroxidation, which involves the series of free radical-mediated chain reaction processes, is also associated with several type of biological damage. Therefore, much attention has been focused on the use of natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals. The antioxidant potency of *Ruellia tuberosa* was investigated in comparision with known antioxidant ascorbic acid (AA) following in-vitro studies. Antioxidant activity of ascorbic acid was well established. The quantity of radicals such as superoxide, lipidperoxide, hydroxyl radicals was compared to known antioxidant ascorbic acid. The plants revealed the presence of flavonoids, steroids, glycosides, alkaloids and they were reported to possess antioxidant activity and cardioprotective activity.

Conclusion

From the experimental studies carried out, extract of leaves of *Ruellia tuberosa* at two different administered doses (250 mg/kg and 500 mg/kg) showed dose dependent cardioprotective and antioxidant activity. The higher dose 500 mg/kg showed significant protection compared to lower dose 250 mg/kg. The cardioprotective and antioxidant effect may be due to the presence of cardiac glycoside and flavonoids. Further studies need to be carried out to isolate the potential chemical constituents of flavonoid of leaves of *Ruellia tuberosa* and to find its mechanism of action in the treatment.

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