EVALUATION OF EFFECT OF RED REISHI MUSHROOM *(GANODERMA LUCIDUM)* ON SEROTONIN INDUCED CARCINOID HEART DISEASE IN RATS

A dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

CHENNAI - 600032.

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY
IN
PHARMACOLOGY

Submitted by

Reg. No : 261426068

Under the guidance of

Dr. K. M. SUDHA.M.D.,

INSTITUTE OF PHARMACOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI- 600003

APRIL 2016

CERTIFICATE

This is to certify that the dissertation entitled "**EVALUATION OF EFFECT OF RED REISHI MUSHROOM *(GANODERMA LUCIDUM)* ON SEROTONIN INDUCED CARCINOID HEART DISEASE IN RATS**" submitted by the **Register number 261426068** in partial fulfillment of the requirements for the award of Degree of Master of Pharmacy in Pharmacology by The Tamilnadu Dr. M.G.R Medical University, Chennai is a bonafide work done by her in Institute of Pharmacology, Madras Medical College, Chennai during the academic year 2015-2016.

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Dr. B. VASANTHI M.D.,
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Dr. K. M. SUDHA.M.D.,
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CONTENTS

I. INTRODUCTION

Carcinoid heart disease in human is a rare condition that occurs as a part of carcinoid syndrome, a systemic disorder mediated by elevated circulating levels of vasoactive substances, including serotonin, histamine, bradykinin, tachykinins produced by a rare metastatic neuroendocrine malignancy carcinoid.

Carcinoid syndrome is characterized by a triad of symptoms such as flushing, diarrhoea and bronchospasm that occur in association with hepatic metastases. High level of vasoactive substance, particularly 5 HT in the right side of the heart causes progressive fibrotic endocardial plaque. Features of thickened immobile tricuspid and pulmonary valves with stenosis and regurgitant lesions are highly suggestive of carcinoid heart disease. It manifests as right heart failure.

Elevation of urinary 5 Hydroxy Indole Acetic Acid (5 – HIAA) levels is highly specific and moderately sensitive for diagnosis of carcinoid syndrome and it is confirmed by echocardiography and cardiac MRI[1].

Serotonergic drugs cause valvular fibrosis similar to that seen in patients with carcinoid heart disease.

In animals serotonin produced cardiac changes as that of human carcinoid heart disease. The free fraction of serotonin in blood is probably the biologically active substance causing carcinoid syndrome and carcinoid heart disease[2].

In rats exposed to long term administration of serotonin, increased cell proliferation and thickening of the heart valves that resembles the changes reported in patients with carcinoid heart disease are observed. Serotonin increases cell

proliferation in several cell types, including valvular interstitial cells and vascular smooth muscle cells.

There is evidence for activation and nuclear translation of mitogen- activated protein kinases and other proliferative pathways by serotonin. Rective oxygen species, especially superoxide (O_2), appear to participate in serotonin induced mitogenesis. Antioxidants has been found to prevent the mitogenic effects of serotonin[3].

The herbal drugs have been used throughout the world and have raised greater attention in recent times, because of their diverse nature of curing disease, safety and high level of tolerance compared to conventional medicine[4]. Red reishi mushroom (*Ganoderma lucidum)* has been used in traditional Chinese medicine for more than 4000 years[5].

It has been known to have numerous pharmacological effects including immune modulating, anti- inflammatory, anti cancer, anti diabetic effect. It inhibits mitogen protein kinase enzyme, increases anti – oxidative enzyme activity and exhibits direct free radical scavenging activity.

It has been found to be effective in coronary heart disease, chronic bronchitis, hypertension. In addition to its diuretic and ACE inhibitory effect, the cardiac protective activity by reducing lipid peroxidation has also been established[6].

No scientific studies have been carried out so far to evaluate its effect on carcinoid heart disease. Hence the present study to evaluate the effect of *Ganoderma lucidum* on inhibiting serotonin induced carcinoid heart disease by its antioxidant property has been attempted.

II. DISEASE PROFILE:

Carcinoid heart disease (CHD, Hedinger syndrome), an unique manifestation, has been described in up to 60% of patients with both Neuro Endocrine Tumors and Carcinoid Syndrome, typically inducing abnormalities of the right side of the heart .

CHD occurs most frequently in patients with Neuro Endocrine Tumors originating in the small bowel (72%) followed by NETs of the lung, large bowel, pancreas, appendix, ovarian origin and gonads[7].

Nearly 50% of patients exhibiting the carcinoid syndrome will develop carcinoid heart disease (CHD) with fibrotic endocardial plaques and associated heart valve dysfunction that classically involves the tricuspid valve. Advanced changes of tricuspid valvular disease rather than tumor dissemination, is the cause of death in approximately one-third of these patients[8].

Serotonin is presumed to be the catalyst for the cardiac fibrotic process. Nearly 95% of patients present with right-sided heart valve disease, characterized by tricuspid insufficiency and pulmonary stenosis and the subsequent development of pulmonary hypertension. Left-sided cardiac disease may be seen in up to 10% of patients, and is commonly associated with angina and coronary vasospasm[9].

PATHOPHYSIOLOGY:

Carcinoid heart disease is characterized by thickening of the tricuspid and pulmonary valves result in regurgitation and /or stenosis of the affected valves. Any or all of the cardiac valves can be affected, with tricuspid regurgitation being the most frequently observed pathology.

The characteristic pathological findings are endocardial plaques of fibrous tissue that may involve the tricuspid valve, pulmonary valve, cardiac chambers, vena cava, pulmonary artery, and coronary sinus. The fibrous reaction may involve not only the valve leaflets, but also the subvalvular apparatus including the tendinous chords and papillary muscles of the tricuspid valve, and more rarely the mitral valve in cases with left sided involvement.

The fibrous tissue in the plaques results in distortion of the valves leading to either stenosis, regurgitation, or both. The preferential right heart involvement is most likely related to inactivation of the vasoactive substances by the lungs. In the 5–10% of cases with left sided valvular pathology, one should suspect either extensive liver metastases, bronchial carcinoid, or a patent foramen ovale[10,11].

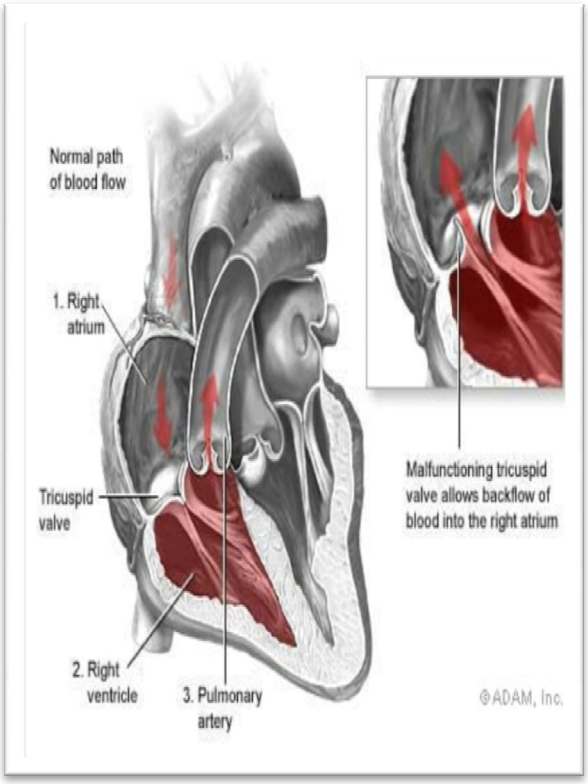


Fig: 1

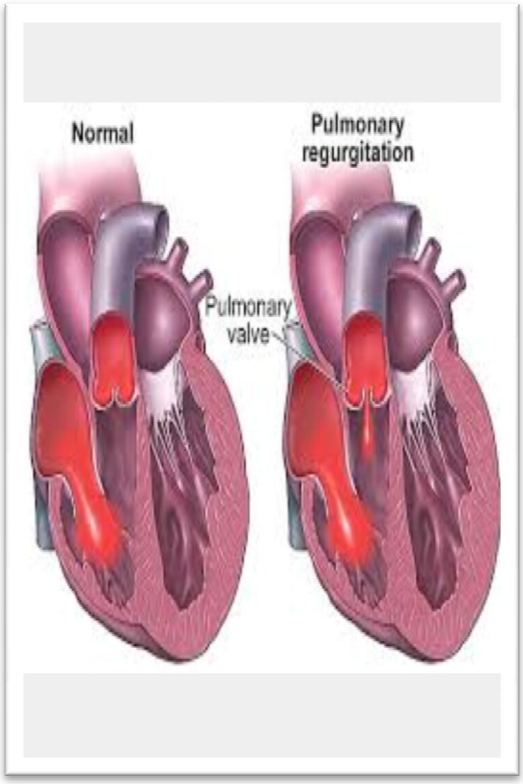


Fig: 2

The precise mechanisms responsible for the development of CHD remain obscure. The disease is thought to be multifactorial and mediated by a variety of vasoactive substances secreted by the tumor, including 5-HT (serotonin), prostaglandins, histamine, bradykinin, and other substances with fibroblast proliferative properties such as tachykinins (substance P, neurokinin A, neuropeptide K) or transforming growth factor-β (TGF-β), which finally lead to the deposition of plaques on the endocardial surfaces of valve leaflets, subvalvular apparatus (chordae and papillary muscles) and cardiac chambers and occasionally within the intima of the pulmonary arteries and the aorta.

These plaque-like deposits are composed of myofibroblasts, smooth muscle cells, extracellular matrix (ECM) components (collagen and myxoid ground

substance), and an endocardial cell layer. These deposits usually involve primarily the right side of the heart (in ~90% of cases), specifically the downstream side of the valve leaflets, i.e. the ventricular aspect of the tricuspid valve and the pulmonary arterial side of the pulmonary valve .

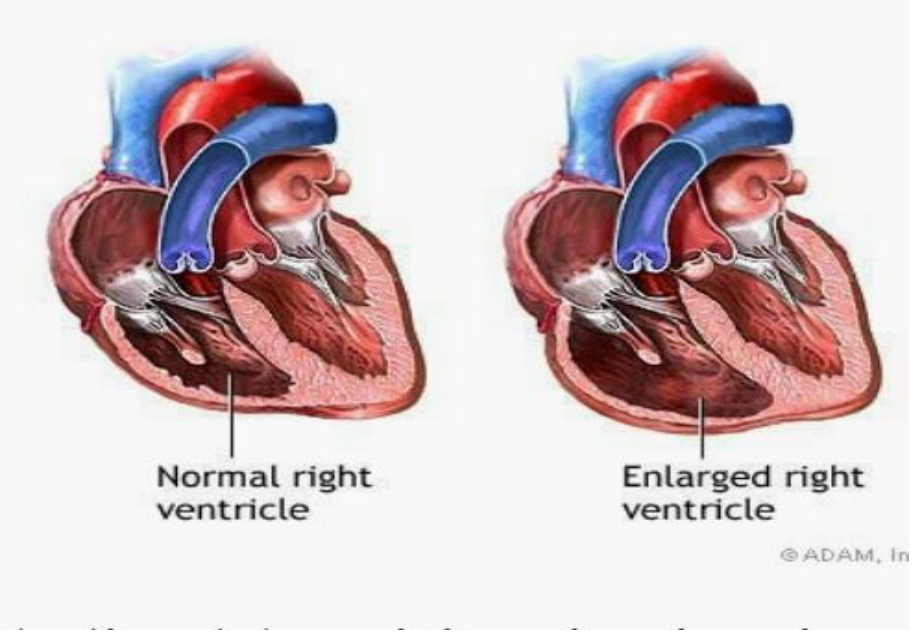


Fig: 3 Enlarged right ventricle

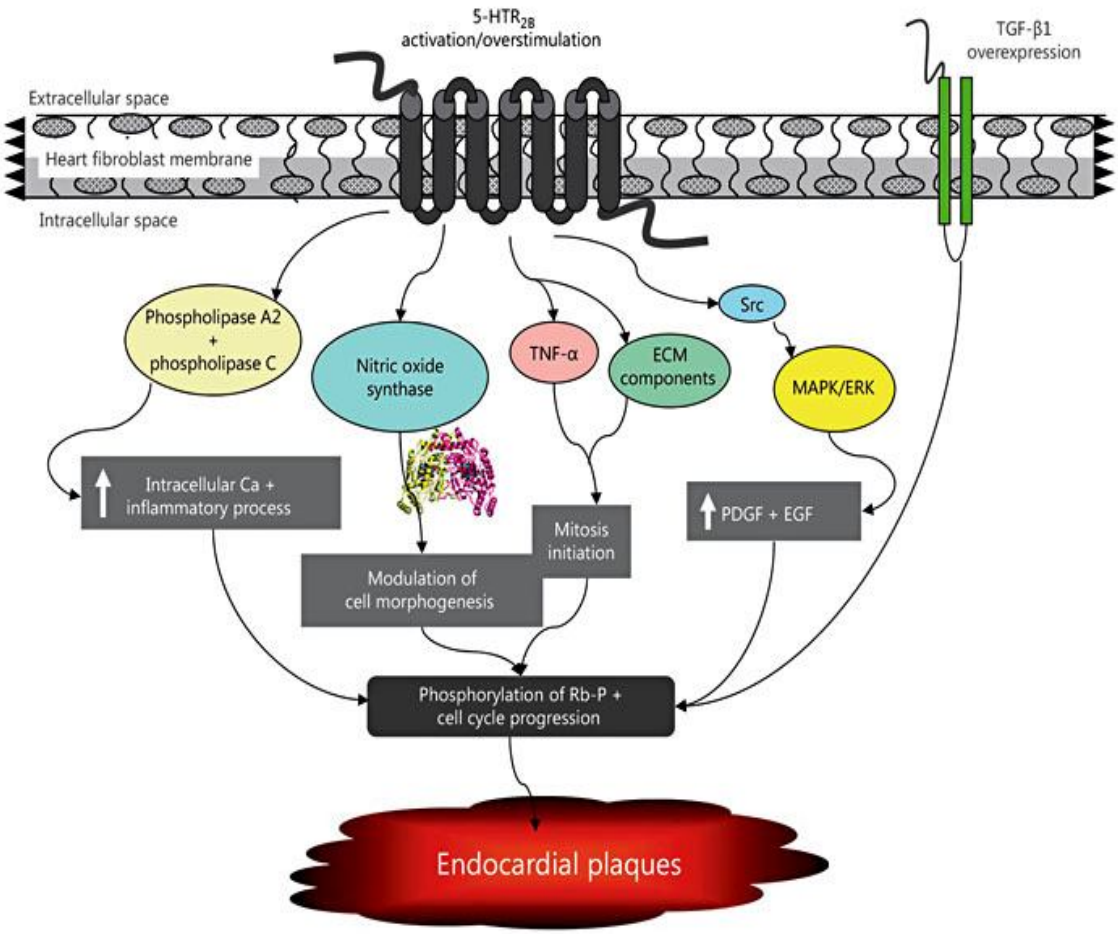
Simultaneous involvement of both the tricuspid and pulmonary valves strongly suggests CHD as the likely diagnosis, demonstrating a pathognomonic appearance. In the pulmonary valve the plaques are deposited on the leaflets, leading to the adherence of pulmonic leaflets to the pulmonary arterial endocardium and resulting in a mixture of valvular stenosis and regurgitation, whereas in the tricuspid valve regurgitation tends to be predominant as the plaques involve mainly the subvalvular apparatus[12].

The complex mechanism of cardiac plaque formation in CHD is considered to be multifactorial. Initial animal studies postulated that the variety of vasoactive substances secreted by the NET may exert paraneoplastic effects. For example, bradykinin has been reported to induce endocardial injury, the resulting fibrosis representing a healing response of the endocardium. Moreover, tachykinin was described as a pro-proliferative agent for the endocardial fibroblasts, thereby inducing plaque formation.

Nowadays, there is a strong body of evidence implying that serotonin plays a major role in stimulating fibroblast growth and fibrogenesis. It is well known that urinary 5-hydroxyindoleacetic acid (5-HIAA), the serotonin metabolite which reflects the amount of serotonin production, is significantly higher in patients with CHD compared with those without cardiac involvement[12,10].

THE SIGNAL TRANSDUCTION EFFECTS INDUCED BY THE 5-HT_{2B} RECEPTOR ACTIVATION IN DIFFERENT CELLS AND TISSUES ARE COMPLEX AND MAY INCLUDE THE FOLLOWING:

- Stimulation of phospholipase C and phospholipase A2 .
- Stimulation of nitric oxide synthase.
- Mitosis initiation together with the increase in the secretion of inflammatory cytokines such as TNF-α and ECM components .
- Activation of MAPK (mitogen-activated protein kinase). Phosphorylation of the cytoplasmic tyrosine kinase Src and activation of ERK (extracellular-regulated kinase) .
- Phosphorylation of retinoblastoma protein (Rb-P) and cell cycle progression.
- Over expression of TGF-β1: the 5-HT_{2B} receptor works in concert with the angiotensin II type 1 receptor (AT1R) to mediate hypertrophic signalling in cardiac fibroblasts. The agonist signalling of these receptors has been shown to induce an increase in the synthesis and up regulation of the cytokine TGF-β1, known to stimulate fibroblasts to produce ECM proteins; TGF-β1 is over expressed in CHD lesions and seems to be a major mediator in the tissue changes related to the valvular disease.



The signal transduction effects induced by the 5-HT_{2B} receptor activation. PDGF = Platelet-derived growth factor; EGF = epidermal growth factor; Rb-P = phosphorylation of retinoblastoma protein[12].

DIAGNOSIS:

The key investigations for the diagnosis of carcinoid heart disease is transthoracic echocardiography[13,14,15].

Basic screening with a 12 lead ECG. Between 30–50% of ECGs are normal; non-specific ST segment changes and sinus tachycardia are the most common abnormal findings and p pulmonale or right bundle branch block may also be seen on occasion.

TREATMENT:

Patients with cardiac involvement tend to have higher circulating concentrations of 5-HIAA and more advanced disease. The principles of management of patients with carcinoid heart disease can be divided into the treatment of right heart failure, pharmacotherapy to reduce the secretion of tumour products, and surgical/interventional treatment of valvar pathology.

HEART FAILURE MANAGEMENT

- Right heart failure can be successfully treated with a combination of loop diuretics and digoxin. Often, loop diuretics alone are enough to achieve sufficient fluid loss, but if additional diuresis is required, the judicious coadministration of a thiazide diuretic usually produces the desired effect.

- Digoxin is believed to help with right ventricular contractility although the data on pure right sided heart failure(without concomitant lung disease or pulmonary hypertension) are confirmed [12,13].

III. DRUG PROFILE:

Fig 5 **Fig:6 Pure Red Reishi Mushroom**

Kingdom: Fungi

Division: Basidiomycota

Class: Agaricomycetes

Order:*Polyporales*

Family:*Ganodermataceae*

Genus:*Ganoderma*

Species: *Ganoderma lucidum(Curtis)P. Karst*

Official Latin Name: *Ganoderma lucidum* ,

Biological Scientific Name:*Ganoderma* lucidum.[16,6]

COMMON NAMES

United States: Reishi mushroom (Herbs of Commerce), Ganoderma.

China: Ling zhi, ling zhi cao, ling chih, hong lingzhi, chi zhi (*Ganoderma lucidum*);

he ling zhi, zi zhi (*Ganoderma japonicum*) (Mandarin).

Japan: Reishi, mannentake; rokkaku reishi (antler form).

Korea: Young ji.

Vietnam: Ling chi[17].

Botanical Source: The fruiting body of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

Parts Used: The fruiting body of the mushroom .

Harvest and Processing:

Collect fruiting bodies in autumn; remove soil, sand and other foreign matter; dry in shaded areas or under the sun.

Fig 7

Distribution:

Reishi mushrooms grows wild on decaying logs and tree stumps in the coastal provinces of China. It occurs in six different colours, but the red variety is most commonly used and commercially cultivated in north America, China, Taiwan[17,18].

The reishi mushroom is a purplish-brown fungus with long stalk, brown spores, and a fan-shaped cap with a shiny, varnish-coated appearance, Reishi grow on decaying wood or tree stumps, preferring the Japanese plum tree and found on oak. Cultivation of reishi is long, complicated process.

The reishi grows in 6 colors,each thought to have different charaterstics and known as

- Aoshiba (blue reishi)
- Akashiba (red reishi),
- Kishiba (yellow reishi),
- Shiroshiba (white reishi),
- ,Kuroshiba (black reishi),
- Murasakishiba (purple reishi).

Active constituents:

Ganoderma lucidum produces a group of triterpenes, called ganoderic acids, which have a molecular structure similar to steroid hormones. It also contains other compounds often found in fungal materials, including polysaccharides (such as beta- Lucan), coumarin, mannitol, canthaxanthin, lanostan and alkaloids. Sterols isolated from the mushroom

include ganoderol, ganoderenic acid, ganoderiol, ganodermanontriol, lucidadiol, and ganodermadiol[6].

THERAPEUTIC USE:

Immunopotentiator:

Lingzhi accelerates the production of interlukin-2 from helper T cells and potentiates the induction of different types of anti-tumor cells, such as NK cells and cytotoxic macrophages, in addition to the induction of interferon production.

It stimulates the immune system to activate both B-lymphocytes and T-lymphocytes natural T- killer cells, improving immune surveillance. It can be effective againt food allergies and is valuable in the treatment of Epstein –Barr virus infection.

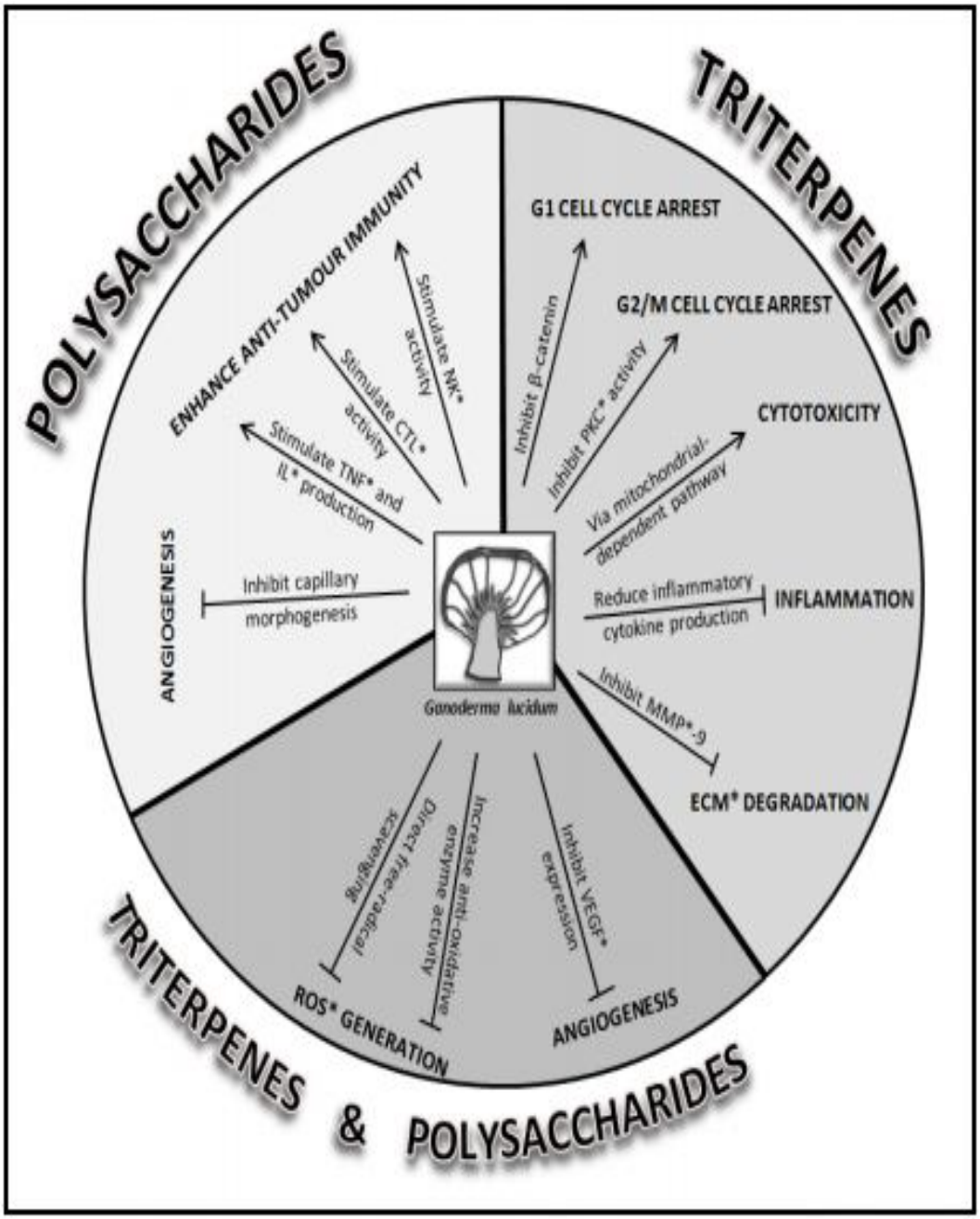
Cancer:

Anticancer effects of reishi have been reported largely from *in vivo* experiments, and data from clinical trials have been published. It is generally accepted that the anticancer effects are due to immune enhancement , and may be exhibited from diverse chemical constituents in reishi.

Cardiovascular effects:

Red reishi Mushroom (Ling Zhi) helps to promote the function of the heart. The effect of reishi on the cardiovascular system has been investigated. Regulation of high blood pressure were reported to be attributed to the ganoderic acids. Angiotensin-converting enzyme-inhibiting triterpenes from reishi have been described. Inhibition of cholesterol biosynthesis, enhanced antioxidase activity, decreased platelet aggregation, and reduced lipid peroxidation have been

demonstrated in animal and *in vitro* experiments. It is also found to be useful in congestive heart failure.



Diabetes:

In animal experiments, ganopoly affected carbohydrate metabolism and promoted insulin secretion. In a clinical trial of patients with type 2 diabetes,

ganopoly 1,800 mg 3 times daily reduced postprandial glucose values. The glucans ganoderan A and B (glucans) inhibited hyperglycemia in clinical studies[16,18].

Hepatitis:

In vitro and *in vivo* animal experiments, hepatoprotection by extracts of ganoderma against induced liver damage has been demonstrated. Polysaccharide ganopoly therapy for 6 months resulted in normalization of aminotransferase levels in 33% and cleared serum hepatitis B surface antigen in 13% of trial participants compared with control.

Rheumatoid Arthritis:

The effect of reishi on the immune system has been studied *in vitro* experiments. In an experiment using synovial fluid from patients with rheumatoid arthritis, researchers demonstrated an inhibitory effect of a polysaccharide extract on the proliferation of synovial fibroblasts, possibly via the nuclear factor-kappa B transcription pathway. Two clinical trials have been conducted involving patients with rheumatoid arthritis. Both trials used a combination of lingzhi plus San-Miao-San, therefore making it difficult to attribute the positive outcomes to the individual agents.

Antiviral effects:

Polysaccharides isolated from reishi have been proven effective *in vitro* against herpes simplex virus types 1 and 2. Reishi isolates also have been tested against other viral strains, including influenza A, and demonstrated effectiveness against their replication.

Contraindications:

Contra indications have not been identified .

Pregnancy/Lactation:

Information regarding safety and efficacy in pregnancy and lactation is lacking.

Interactions: None well documented[19,20].

IV. REVIEW OF LITERATURE:

1. Rebecca Dobson *et al* (2015) reported the Association of a Panel of Biomarkers with the presence and severity of carcinoid Heart Disease: A cross-sectional study of patients with neuro endocrine tumours with documented liver metastases and/or carcinoid syndrome. Serum was analyzed for Chromogranin A, Chromogranin B and N-terminal pro Brain Natriuritic Peptide (NT-proBNP). Plasma was analyzed for Neurokinin A and 5-Hydroxyindoleacetic acid (5HIAA). Echocardiography was used to determine the presence and severity of carcinoid heart disease. NT-proBNP and plasma 5HIAA are both sensitive and specific biomarkers for the presence of carcinoid heart disease whereas only NT-proBNP is moderately correlated with disease severity[21].

2. Wiebke Janssen *et al* (2015) 5-HT2B Receptor Antagonists Inhibit Fibrosis and Protect from RV Heart Failure. To investigate the effects of Terguride (5-HT2A and 2B receptor antagonist) or SB204741 (5-HT2B receptor antagonist) on right heart function and structure upon pulmonary artery banding (PAB) in mice. *Methods.* Seven days after PAB, mice were treated for 14 days with Terguride (0.2□mg/kg bid) or SB204741 (5mg/kg day). Right heart function and remodeling were assessed by right heart catheterization, magnetic resonance imaging (MRI), and histomorphometric methods. Chronic treatment with Terguride or SB204741 reduced right ventricular fibrosis and showed improved heart function in mice after PAB[22].

3. Buda A *et al* (2012) reported the Primary insular carcinoid of the ovary with carcinoid heart disease: High level of 5-HIAA has a rule in the development and progression of the carcinoid heart syndrome and could lead the right tricuspid valvular involvement[23].
4. Alexandra KeKewska *et al* (2012) reported the antiserotonergic properties of terguride in Blood vessels, Platelets, and valvular Interstitial cells. Kinetic studies on the effects of terguride in pulmonary arteries showed that the rate to reach drug-receptor equilibrium for terguride was fast. Pretreatment with terguride inhibited 5-HT-induced amplification of ADP-stimulated human platelet aggregation (IC_{50} 16 nM). In porcine valvular interstitial cells, 5-HT-induced activation of extracellular signal-regulated kinase (ERK) 1/2, an initiator of cellular proliferation and activity, was blocked by terguride as shown by Western blotting[24].
5. Silva scott R *et al* (2011) reported the Effect of PTEN on Serotonin Synthesis and Secretion from the Carcinoid Cell Line BON. PTEN was inhibited by pharmacological and molecular approaches, and the resultant secretion of serotonin and expression of tryptophan hydroxylase (TPH1), the rate-limiting enzyme in serotonin synthesis, was assessed. Inhibition of PTEN *in vitro*, with concomitant increased Akt signaling, resulted in decreased secretion of serotonin, as well as decreased serotonin synthesis, as confirmed by reduced expression of TPH1. Inhibition of PTEN in BON cells in an animal model resulted in decreased serum serotonin. By inhibiting signaling through Akt, PTEN indirectly promotes serotonin synthesis and secretion[26].
6. Droogmans *et al* (2009) reported the Cyproheptadine prevents pergolide - induced valvulopathy in rats, Echocardiographic and histopathological

study. 50 male Wistar rats received daily intraperitoneal injections of pergolide (0.5 mg/kg, n = 14), pergolide (0.5 mg/kg) combined with cyproheptadine (10 mg/kg, n = 12), cyproheptadine (10 mg/kg, n = 12), or no injections (control, n = 12) for 20 wk. Echocardiography was performed blindly at baseline and at 10 and 20 wk followed by pathology. At baseline, no differences between groups were found with echocardiography. At 20 wk, aortic regurgitation was present in all pergolide-treated animals, whereas it was less frequently observed in the other groups ($P < 0.0001$). For the other valves, this difference was less pronounced. On histopathology, not only aortic but also mitral valves were thicker, myxoid, and exhibited more 5-HT(2B)R-positive cells in pergolide-treated animals compared with the other groups[27].

7. Ricardo. A *et al* (2009) reported the Serotonin produces monoamine oxidase-dependent oxidative stress in human heart valves. serotonin induces oxidative stress in human heart valves, and examined mechanisms by which serotonin may increase reactive oxygen species. Superoxide ($O_2^{\cdot-}$) was measured in heart valves from explanted human hearts that were not used for transplantation. $O_2^{\cdot-}$ levels (lucigenin -enhanced chemo luminescence) were increased in homogenates of cardiac valves and blood vessels after incubation with serotonin[28].
8. Rajamannan NM *et al* (2007) reported the Cell proliferation in carcinoid valve disease: a mechanism for serotonin effects. Serotonin is a powerful mitogen for valvular subendocardial cells. The mitogenic effect is at least partly mediated via 5HT1b receptors. Subendothelial cell proliferation is significantly elevated in human carcinoid valves *In vivo*. The data suggest a

mechanism whereby serotonin may contribute to valvular proliferation in carcinoid heart disease[28].

9. Steven Droogmans *et al* (2007) reported the *In vivo* model of drug –induced valvular heart disease in rat, pergolide – induced valvular heart disease demonstrated with echocardiography and correlation with pathology.thirty male wister rats were given daily injection of pergolide serotonin and vehicle for 5 month, echocardiography demonstrated the presence of aortic regurgitation in serotonin and in pergolide compared with normal. Histological examination revealed the presence of diffused thickened and myxoid aortic, mitral and tricuspid valves in serotonin and pergolide animal as seen in VHD[29].
10. Bjorn.I.Gustafsson MD *et al* (2005) reported the Long-term serotonin administration induces heart disease in rats. rats dosed with serotonin develope changes similar to those seen in human carcinoid heart disease. Ten Sprague -Dawley rats were given serotonin injections subcutaneously once daily for 3 months, controls were given saline. A long-lasting hyperserotoninemia with a >10-fold increase in both platelet-poor plasma and dialysate from the femoral muscles appeared. The animals developed clinical signs such as flushing and loose stools. After 3 months, 6 of 10 rats given serotonin had pathological echocardiographs. Two animals had a combination of aortic and pulmonary valve insufficiency, 1 had isolated aortic valve insufficiency, and 3 had isolated pulmonary valve insufficiency. Histopathological examination revealed shortened and thickened aortic cusps and carcinoid like plaques characterized by a collection of myofibroblasts within an extracellular matrix of collagen ground substance[30].

11. Karl Engelman M.D et al (2005) reported the Inhibition of Serotonin Synthesis by Para-Chlorophenylalanine in Patients with the Carcinoid Syndrome. the role of 5-hydroxytryptamine (serotonin, 5HT) in the pathophysiology of the carcinoid syndrome, and especially in flush production, has been seriously questioned,[2] the fact remains that increased production of serotonin is a hallmark of the condition. If it were possible effectively to inhibit the formation of this pharmacologically potent amine in patients with carcinoid tumours, a likely result would be a better understanding of the clinical significance of serotonin. A possibility of achieving this goal appeared with the studies of Koe and Weissman showing that para-chlorophenylalanine (PCP) is a potent and selective depletor of tissue serotonin in animals[31].
12. Sandeepa Musunuru *et al* (2005) reported A Mouse Model of carcinoid syndrome and Heart disease. Seventeen nude mice underwent intrasplenic injection of human pancreatic carcinoid BON cells (10^7) and then were euthanized 9 weeks later. Murine livers were analyzed by immunohistochemistry. Murine hearts were sectioned and the surface area of the right heart valves determined. Blood was also collected and analyzed for platelet serotonin by ELISA.[32]
13. J M Zuetenhorst *et al* (2004) reported the Role of natriuretic peptides in the diagnosis and treatment of patients with carcinoid heart disease. Carcinoid heart disease (CHD) occurs in 20-70% of the patients with metastatic well-differentiated Neuro Endocrine Tumours (NET). To evaluated whether natriuretic peptides (ANP or NT-proBNP) are useful in early detection of CHD. Blood samples from 32 patients with NET were compared

with cardiac ultrasound follow-up. CHD was defined as thickening of the tricuspid valve in the presence of grade III-IV/IV tricuspid valve regurgitation. CHD was found in nine out of 32 patients (28%), all with symptoms of the carcinoid syndrome compared to 65% in the 23 patients without CHD (P=0.04). Median levels of NT-proBNP and 5-HIAA were significantly higher in patients with CHD (894 ng l(-1) and 815 micromol 24 h(-1)) compared to those without (89 and 206 ng l(-1), P<0.001 and P=0.007). No significant differences were detected in ANP levels (P=0.11). Dilatation of the right atrium and ventricle as well as thickening of the tricuspid valve and degree of regurgitation were statistically significant correlated with NT-proBNP levels. The accuracy of NT-proBNP in the diagnosis of CHD was higher than that of ANP. A significantly better survival was observed in case of normal NT-proBNP values. In conclusion, NT-proBNP is helpful as a simple marker in the diagnosis of CHD[33].

14. Zuetenhorst *et al* (2003) reported the Carcinoid heart disease: the role of urinary 5-hydroxyindoleacetic acid excretion and plasma levels of atrial natriuretic peptide, transforming growth factor-beta and fibroblast growth factor. Serotonin excretion plays a role in the development of carcinoid heart disease (CHD), but the exact pathogenesis is not known. In the current study, To evaluated 24-hour urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion, as well as plasma levels of transforming growth factor-beta (TGF-beta), fibroblast growth factor (FGF), and atrial natriuretic peptide (ANP) in patients with and without CHD determined by ultrasound examination. Urine and plasma samples were obtained for 37 patients and cardiac ultrasound was performed during follow-up in 1999 and 2000. Median 5-HIAA excretion

was calculated for the period between diagnosis and ultrasound examination.[34]

15. Jacob E. Møller, M.D, Ph.D *et al* (2003) Factors Associated with Progression of Carcinoid Heart Disease sample included 71 patients with the carcinoid syndrome who underwent serial echocardiographic studies performed more than one year apart and 32 patients referred directly for surgical intervention after an initial echocardiographic evaluation. A score for carcinoid heart disease was determined on the basis of an assessment of valvular anatomy and function and the function of the right ventricle. An increase of more than 25 percent in the score between studies was considered suggestive of disease progression. Tumor progression was assessed on the basis of abdominal computed tomographic scans and changes in the level of urinary 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin[35].
16. Rosenzweig - Lipson S *et al* (2002) serotonin induces up regulation of transforming growth factor-β in aortic valve interstitial cells via 5-HT_2 receptors and that transforming growth factor-β stimulates glycosaminoglycan production in sheep aortic valve interstitial cells.[36]
17. Watanabe T *et al* (2001) Serotonin potentiates angiotensin II - induced vascular smooth muscle cell proliferation. investigated the growth promoting activities of two potent vasoactive substances, angiotensin II (Ang II) and serotonin (5-HT), on cultured rabbit VSMCs[37].
18. Watanabe T *et al* (2001) reported the serotonin potentiates angiotensin II--induced vascular smooth muscle cell proliferation. Vascular smooth muscle cell (VSMC) proliferation is a key feature in the development of atherosclerosis and restenosis after angioplasty, which can occur in response

to many different humoral and mechanical stimuli. To investigated the growth promoting activities of two potent vasoactive substances, angiotensin II (Ang II) and serotonin (5-HT), on cultured rabbit VSMCs. Growth-arrested VSMCs were incubated with serum-free medium containing different concentrations of Ang II in the presence or absence of 5-HT. [3H] thymidine incorporation into VSMC DNA was measured as an index of cell proliferation. Ang II and 5-HT stimulated DNA synthesis in a dose-dependent manner with a maximal effect at 1.75 microM for Ang II (202%) and 50 microM for 5-HT (205%)[38].

19. Di Lazio S *et al* (2000) reported the Carcinoid Heart Disease, Carcinoid syndrome originates from metastatic carcinoid tumours localized in the gastrointestinal system, pancreas, biliary vessels, bronchi, ovaries, and testes; it is characterized by flushing, diarrhoea, bronchoconstriction, and fibrous endocardial plaques in the heart. Cardiac involvement is detected by echocardiography in over 50% of patients with this syndrome. Right-sided valvular heart disease occurs frequently in patients with carcinoid syndrome, involving most commonly the tricuspid and pulmonary valves. Involvement of the left-sided valves rarely occurs[39].
20. Paul Egermayer *et al* (1999) reported the Role of serotonin in the pathogenesis of acute and chronic pulmonary hypertension. The pathophysiology of pulmonary hypertension cannot be fully understood in terms of a traditional single cause and effect model. The concept of a balance of factors is probably more helpful. Under different circumstances three general, often interrelated, types of response are apparent, vasodilation/vasospasm, mitogenesis/cytostasis, and thrombosis/fibrinolysis.

Although the vascular response to insult often appears relatively stereotyped, causes are invariably multifactorial. The role of serotonin in other varieties of pulmonary hypertension has been unjustly neglected[40].

21. Hurst RD *et al* (1995) reported the Octreotide inhibition of serotonin-induced ileal chloride secretion. It inhibits 5HT-stimulated electrogenic chloride secretion at the mucosal level. Additionally this inhibitory effect of octreotide is likely mediated by activation of the inhibitory subunit of membrane-bound GTP-binding regulatory proteins. These results thus provide experimental evidence in support of the ability of SMS to ameliorate the carcinoid diarrhoea by a direct effect on stimulated mucosal ion secretion[41].
22. L Lundin *et al (1988)* reported the Carcinoid heart disease: Relationship of circulating vasoactive substances to ultrasound-detectable cardiac abnormalities ,Cardiac ultrasound investigation of 68 prospectively studied patients with histologically proven midgut carcinoid tumours showed right heart disease in 66%. The abnormal findings included morphologic and functional aberrations of the tricuspid valve in 52% and 83%, respectively, right atrial and ventricular enlargement in 53% and 30%, and paradoxical systolic septal contractions in 19%. The patients with the most pronounced right heart disease had significantly higher (p less than .01) plasma levels of the tachykinins neuropeptide K and substance P as well as higher (p less than .001) urinary excretion of the serotonin metabolite 5-hydroxyindoleacetic acid. These patients also had the most extensive tumor disease. The occurrence of echocardiographic abnormalities of the left heart was similar to that in healthy individuals of the same age, but abnormalities were less frequent among the patients with severe right heart disease. The severity of

cardiac involvement does not seem to be related to the duration of carcinoid disease but more to the extent of the disease, i.e., higher plasma levels of serotonin and tachykinins.[42]

23. Jacob E. Møller, MD *et al* (2005) reported the Prognosis of Carcinoid Heart Disease, Analysis of 200 Cases Over Two Decades. The long-term prognosis of patients who develop carcinoid heart disease and the effect of cardiac surgery on outcome are not well established. In this retrospective study, identified 200 patients with carcinoid syndrome referred for echocardiography in whom the diagnosis of carcinoid heart disease was confirmed. Patients were divided into 3 groups of similar size according to the date from first diagnosis of carcinoid heart disease. Group A comprised patients diagnosed from 1981 through June 1989; group B, diagnosed July 1989 through May 1995; and group C, June 1995 through 2000. The end point was all-cause mortality. Median survival was significantly lower in group A (1.5 years, 95% CI 1.1 to 1.9 years) compared with groups B (3.2, 95% CI 1.3 to 5.1 years) and C (4.4, 95% CI 2.4 to 7.1 years; P=0.009). In a multivariate model adjusted for treatment and clinical characteristics, the risk of death in groups B (hazard ratio 0.67, 95% CI 0.46 to 0.99, P=0.04) and C (hazard ratio 0.61, 95% CI 0.39 to 0.92, P=0.006) was significantly reduced relative to group A. Cardiac surgery was performed in 87 patients. When cardiac surgery was included as a time-dependent covariate in a multivariate analysis, it was associated with a risk reduction of 0.48 (95% CI 0.31 to 0.73, P<0.001), whereas the time period of diagnosis was no longer significant. The prognosis of patients with recognized carcinoid heart disease

has improved over the past 2 decades at our institution. This change in survival may be related to valve replacement surgery[43].

24. P A Pellikka *et al* (1993) reported the Carcinoid heart disease. Clinical and echocardiographic spectrum in 74 patients. The echocardiographic, Doppler, and clinical features of the 74 patients (56%) with echocardiographic evidence of carcinoid heart disease are described. Among these patients, 97% had shortened, thickened tricuspid leaflets. Tricuspid regurgitation was present in all 69 patients with carcinoid heart disease who underwent Doppler examination, and it was of moderate or severe degree in 62 patients (90%)[44].

25. Howard RJ, Drobac M, Rider WD *et al.*(1962) Carcinoid heart disease: Diagnosis by two-dimensional echocardiography[45].

REVIEW OF *GANODERMO LUCIDUM*:

1. Lasukova TV *et al* (2015) reported the Cardio protective activity of *Ganoderma lucidum* Extract during total ischemia and reperfusion of isolated heart. The cardio protective effects of *Ganoderma lucidum* extract were examined in experiments with global ischemia (45 min) and reperfusion (30 min) of isolated and perfused rat heart. The course of preventive administration of the extract in a dose of 400 mg/kg for 15 days diminished necrotic death of cardiomyocytes and reduced reperfusion contracture. *Ganoderma lucidum* extract demonstrated antioxidant properties. Cardio protective properties of *Ganoderma lucidum* extract are largely determined by its antioxidant properties[46].
2. Zengenni Liang *et al* (2014) Chemical Characterization and Antitumor Activities of Polysaccharide Extracted from *Ganoderma lucidum.* In which polysaccharide (GLP) is a biologically active substance reported to possess anti-tumor ability.

The mechanisms of GLP-stimulated apoptosis are still unclear. This study aims to determine the inhibitory and apoptosis-inducing effects of GLP on HCT-116 cells. They found that GLP reduced cell viability on HCT-116 cells in a time- and dose-dependent manner, which in turn, induced cell apoptosis. The observed apoptosis was characterized by morphological changes, DNA fragmentation, mitochondrial membrane potential decrease, S phase population increase, and caspase-3 and -9 activation. Furthermore, inhibition of c-Jun *N*-terminal kinase (JNK) by SP600125 led to a dramatic decrease of the GLP-induced apoptosis. Western blot analysis unveiled that GLP up-regulated the expression of Bax/Bcl-2, caspase-3 and poly (ADP-ribose) polymerase (PARP). These results demonstrate that apoptosis stimulated by GLP in human colorectal cancer cells is associated with activation of mitochondrial and mitogen -activated protein kinase (MAPK) pathways.[47]

3. Sun-he jang *et al* (2014) reported the hepatoprotective evaluation of *Ganoderma lucidum* pharmacopuncture: *In vivo* studies of ethanol-induced acute liver injury. Apoptotic changes are inhibited by the addition of antioxidants and glp, suggesting that oxidative stress is involved in the release of cytochrome c, which precedes apoptosis in hepatocytes exposed to ethanol. therefore, glp suppresses apoptosis by regulating mitochondrial-damage-mediated endogenous pathways, which could be one of the important mechanisms for preventing alcoholic-induced liver injury. in conclusion, the present study has revealed that glp protects against ethanol -induced hepatic injury in SD rats by modulating the activities of ethanol-metabolizing enzymes and attenuating oxidative stress[48].

4. Tran HB *et al* (2014) reported the Hypotensive effects and angiotensin-converting enzyme inhibitory peptide of reishi (*ganodermo lingzhi*) auto-digested extracted. Reishi's own proteases to hydrolyze its protein and obtained auto-digested reishi (ADR) extract. The extract was subjected to *in vitro* assays and administered to spontaneous hypertensive rats (SHRs) to determine its potential for use as a hypotensive medication. Bioassay-guided fractionation and de novo sequencing were used for identifying the active compounds. After 4 h administration of ADR, the systolic pressure of SHRs significantly decreased to 34.3 mmHg (19.5% change) and the effect was maintained up to 8 h of administration, with the decrease reaching as low as 26.8 mmHg (15% reduction-compare to base line a decrease of 26.8 mmHg is less than a decrease of 34.3 mmHg so it should give a smaller % reduction). Eleven peptides were identified and four of them showed potent inhibition against ACE with IC50 values ranging from 73.1 μM to 162.7 μM[49].

5. Deng pan *et al (2013)* reported the Antidiabetic, Antihyperlipidemic and Antioxidant Activities of a Novel Proteoglycan from *Ganoderma lucidum* Fruiting Bodies on db/db Mice and the Possible Mechanism *FYGL* was an effective antidiabetic agent by enhancing insulin secretion and decreasing hepatic glucose output along with increase of adipose and skeletal muscle glucose disposal in the late stage of diabetes. Furthermore, *FYGL* is beneficial against oxidative stress, thereby being helpful in preventing the diabetic complications[50].

6. Shih-Fen Liao *et al* (2013) reported the Immunization of fucose-containing polysaccharides from Reishi mushroom induces antibodies to tumor-associated Globo H-series epitopes. Carbohydrate-based vaccines have shown therapeutic efficacy for infectious disease and cancer. The mushroom *Ganoderma lucidum* (Reishi) containing complex polysaccharides has been used as antitumor supplement, They show that the mice immunized with a L-fucose (Fuc)-enriched Reishi polysaccharide fraction (designated as FMS) induce antibodies against murine Lewis lung carcinoma cells, with increased antibody-mediated cytotoxicity and reduced production of tumor-associated inflammatory mediators (in particular, monocyte chemoattractant protein-1)[51].

7. Sudheesh NP *et al* (2013) *Ganoderma lucidum* ameliorate mitochondrial damage in isoproterenol - induced myocardial infarction in rats by enhancing the actities of TCA cycle enzymes and respiratory chain complexes. Cardiac toxicity was assessed by determining the activities of creative kinase (CK) and lactate dehydrogenises (LDH) after subcutaneous injection of ISO (85 mg/kg) at an interval of 24h for 2 days. The animals were sacrificed 24h after last ISO administration. G. lucidum (100 and 250 mg/kg, pod.) was given to the rats once daily for 15 days prior to the ISO challenge. Similarly, α-Tocopherol (100mg/kg, p.o) was kept as the standard. To assess the extent of cardiac mitochondrial damage, the activities of Krebs cycle dehydrogenises and mitochondrial complexes I, II, III, and IV as well as the level of ROS and mitochondrial membrane potential (ΔΨmt) were evaluated. Administration of G. lucidum and α-tocopherol significantly protected the elevated activities of CK and LDH[52].

8. Bang-Jau You *et al* (2013) reported the A Novel Approach to Enhancing Ganoderic Acid Production by *Ganoderma lucidum* Using Apoptosis , This study investigated the role of apoptosis signaling on GA biosynthesis and presented a novel approach, namely apoptosis induction, to increasing GA production. Aspirin was able to induce cell apoptosis in *G. lucidum*, which was identified by terminal deoxynucleotidyl transferase mediated dUPT nick end labeling assay positive staining and a condensed nuclear morphology. The maximum induction of lanosta-7,9(11), 24-trien-3α-01-26-oic acid (ganoderic acid 24, GA24) production and total GA production by aspirin were 2.7-fold and 2.8-fold, respectively, after 1 day. Significantly lower levels of GA 24 and total GAs were obtained after regular fungal culture for 1.5 months. ROS accumulation and phosphorylation of Hog-1 kinas, a putative homolog of MAPK p38 in mammals, occurred after aspirin treatment indicating that both factors may be involved in GA biosynthetic regulation. However, aspirin also reduced expression of the squalene synthase and lanosterol synthase coding genes, suggesting that these genes are not critical for GA induction[54].

9. Michelle M *et al* (2011) reported the *Ganoderma lucidum* (Reishi) Inhibits Cancer Cell Growth and Expression of Key Molecules in Inflammatory Breast Cancer. effects of Reishi on viability, apoptosis, invasion, and its mechanism of action in IBC cells (SUM-149). Results show that Reishi selectively inhibits cancer cell viability although it does not affect the viability of noncancerous mammary epithelial cells[55]

10. Xue H *et al* (2010) reported the Effect of *Ganoderma lucidum* polysaccharides on hemodynamic and antioxidation in T2DM. Rats were fed high-fat diet for 4 weeks and then were injected STZ (30 mg x kg(-1)) to induce the type 2 diabetes mellitus (T2DM). Once the T2DM models were set successfully, rats were randomized into six groups: normal group (NG), group of diabetes mellitus (DMG), groups of low dosage (GLPs-LG), middle dosage (GLPs-MG), high dosage (GLPs-HG) and berberine (BerG). They received GLPs with different dosages (200, 400, 800 mg x kg(-1)) and berberine (30 mg x kg(-1)) continually for 16 weeks. At 16th week end, the following indices of rats were measured respectively: blood glucose, hemodynamic including LVSP, LVEDP, dp/dt(max) and dp/dt(max) and the contents of NO, SOD, MDA, GSH-Px, CAT in cardiac tissue. Besides, myocardial ultra structure was observed by electron microscope. Both the middle dosage and the high dosage of GLPs could low blood glucose effectively, and they could reduce LVEP but increase -dp/dt(max). Meanwhile, they could activate GSH-Px, CAT, SOD, NO, but reduce MDA in cardiac tissue and improve the myocardial ultrastructure[56].

11. Sudheesh NP *et al* (2010) reported the Therapeutic potential of *Ganoderma lucidum* (Fr.) P. Karst. against the declined antioxidant status in the mitochondria of post-mitotic tissues of aged mice. estimating the activities of manganese-superoxide dismutase (Man SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and catalase (CAT) as well as levels of reduced glutathione (GSH), lipid peroxidation, advanced oxidation protein products (AOPP) and reactive oxygen species (ROS) in the heart and brain mitochondria of aged mice after oral administration of ethanolic extract of G. lucidum (50 and 250mg/kg), once daily for 15 days. The effect was compared with that of aged and young control animals[57].

12. Keith R Martin *et al* (2010) Both common and specialty mushrooms inhibit adhesion molecule expression and *in vitro* binding of monocytes to human aortic endothelial cells in a pro-inflammatory environment, Human aortic endothelial cells (HAEC) were incubated overnight with control media with dimethylsulfoxide (DMSO) vehicle (1% v/v) or containing DMSO extracts of whole dehydrated mushrooms (0.1 mg/mL), which included *Agaricus bisporus* (white button and crimini), *Lentinula edodes* (shiitake), *Pleurotus ostreatus* (oyster), and *Grifola frondosa* (maitake). Monolayers were subsequently washed and incubated with medium alone or containing the pro-inflammatory cytokine IL-1β (5 ng/mL) for 6 h to upregulate pro-atherosclerotic adhesion molecules (AM). AM expression was assayed by ELISA and binding of U937 human monocytes pre-loaded with fluorescent dye was determined[58].

13. KK Janardhanan *et al* (2005) reported to evaluate the protective effect of *Ganoderma lucidum* extract against doxorubicin-induced cardiotoxicity. Administration of 3 doses of doxorubicin,6 mg/kg body weights, i.p. per each dose, alternative days, showed clear signs of cardio toxicity in rats. The drug enhanced serum creatine kinase (CK) activity and lipid peroxidation in tissue drastically. The drug also induced significant decrease in GSH level and activities of CAT, SOD and GPx. Administration of methanolic extract of G.lucidum (500 and 1,000 mg/kg body weight) significantly increased the level of GSH and activities of CAT, SOD and GPx. Activity of CK was significantly lowered in a dose dependent manner. The treatment also caused significant decrease in lipid peroxidation (MDA). The results thus indicated that methanolic extract of Ganoderma.lucidum prevented oxidative stress caused by doxorubicin administration and the increase in serum CK activity and lipid peroxidation in the

tissue. The experimental findings suggest the therapeutic potential of G.luciduma adjuvant in cancer chemotherapy[59].

16. Woo CW *et al* (2005) reported the *Ganoderoma lucidum* inhibits inducible nitric oxide synthase expression in macrophages. To investigate the effect of G. lucidum on iNOS-mediated NO production in macrophages. Human monocytic cell (THP-1) derived macrophages were incubated with lipopolysaccharide (LPS) for 24 h. Such treatment significantly stimulated NO production (253% versus the control). Such a stimulatory effect was resulted from increased iNOS mRNA expression (270% versus the control) and iNOS activity (169.5% versus the control) in macrophages. The superoxide anion level was also elevated (150% versus the control) in LPS-treated macrophages. G. lucidum may exert a therapeutic effect against atherosclerosis via ameliorating iNOS-mediated NO overproduction in macrophages[60].

17. Wong KL *et al* (2004) reported the Antioxidant activity of *Ganoderma lucidum* in acute ethanol-induced heart toxicity. The mice were divided into six groups with ten animals in each group. Ganoderma lucidum, at doses of 10, 25 and 50 mg/kg (pod) was administered. Superoxide anions were assayed by UV spectrophotometer using the cytochrome C reduction method. The results of this study showed that *Ganoderma lucidum* exhibited a dose-dependent antioxidative effect on lipid peroxidation and superoxide scavenging activity in mouse heart homogenate. Additionally, this result indicated that heart damage induced by ethanol shows a higher malonic dialdehyde level compared with heart homogenate treated with *Ganoderma lucidum*. It is concluded that the antioxidative activity may therefore contribute to the cardioprotective effect of *Ganoderma lucidum*, and may therefore protect the heart from superoxide induced damage[61].

18. You YH *et al* (2003) reported the Antioxidant effect of Ganoderma polysaccharide peptide, Copper was used as oxidant to induce low lipoprotein (LDL) oxidative modification, and alloxan was given i.v. to induce reactive oxygen species (ROS) injury in mice. GLPP decreased oxidation of LDL and the relative electrophoretic mobility (REM) of oxidative product of LDL. After GLPP was given i.p. for 20 days, the concentration of malondialdehyde (MDA) in serum and heart of mice was decreased. The GSHpx enzyme activity was increased, while the SOD level was decreased. The catalase (CAT) levels were not significantly changed by GLPP[62].

V. AIM & OBJECTIVE:

- To evaluate the antioxidant effect of red reishi mushroom (*Ganoderma lucidum*) by *in vitro* methods.

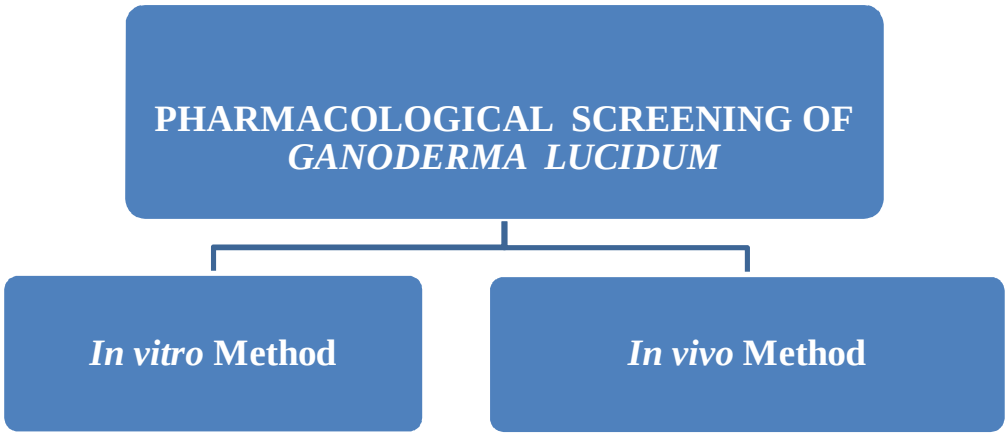
- To evaluate the inhibitory effect of *Ganoderma lucidum* on serotonin induced carcinoid heart disease in rats.

VI. PLAN OF WORK:

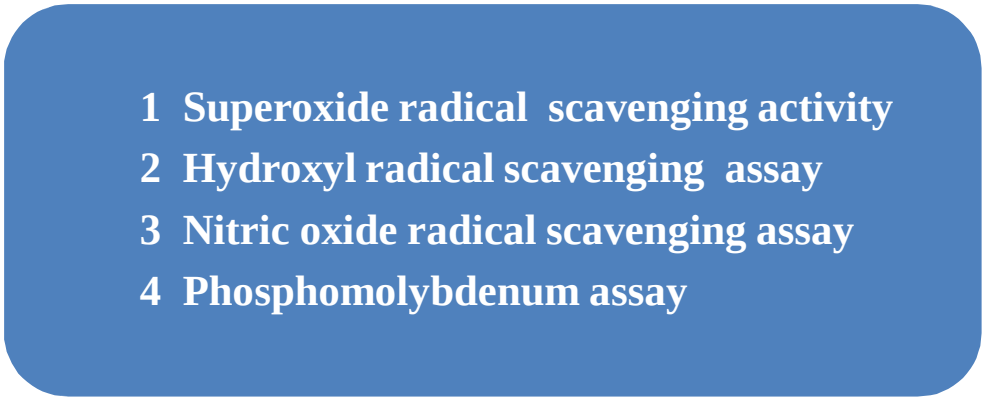
Serotonin creatinin sulphate complex purchased from sigma Aldrish company in Germany.

Pure red reishi mushroom procured form United States of America.

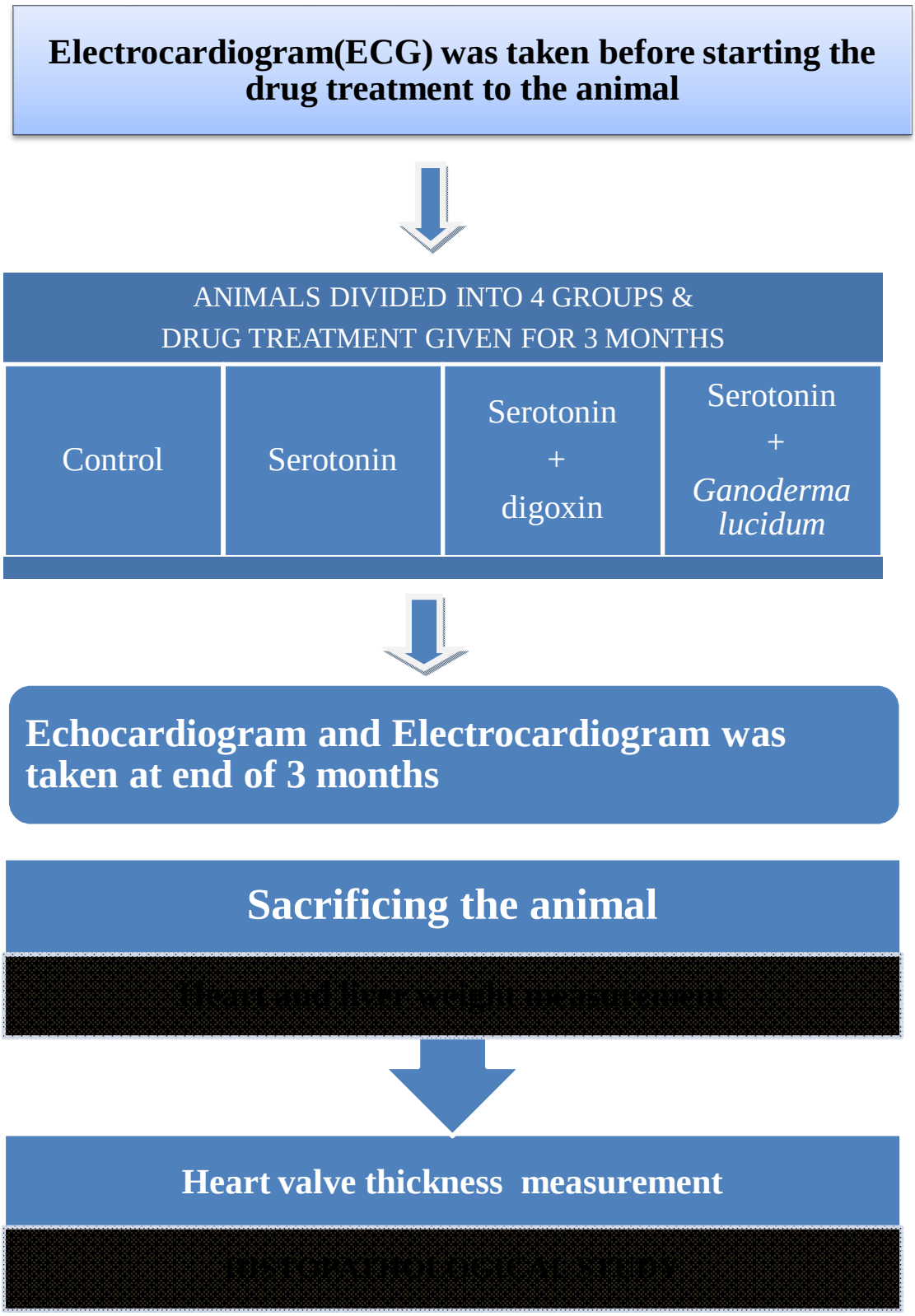
Digoxin purchased from Jayam medical in Chennai.



In vitro method



***In vivo* method**



VII. METHODOLOGY:

Reactive Oxygen Species Assay

SUPER OXIDE FREE RADICAL SCAVENGING ACTIVITY:

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences.

Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan. 100 µl of Riboflavin solution [20 µg], 200 µl EDTA solution [12mM], 200 µl methanol and 100 µl NBT (Nitro-blue tetrazolium) solution [0.1mg] were mixed in test tube and reaction mixture was diluted up to 3 ml with phosphate buffer [50mM].

The absorbance of solution was measured at 590nm using phosphate buffer as blank after illumination for 5 min. This is taken as control. 50 µl of different concentrations of coumarin compounds as well as standard preparation were taken and diluted up to 100 µl with methanol. To each of these, 100 µl Riboflavin, 200 µl EDTA, 200 µl methanol and 100 µl NBT was mixed in test tubes and further diluted up to 3 ml with phosphate buffer. Absorbance was measured after illumination for 5 min. at 590 nm on UV visible spectrometer . The IC50value for test and standard preparation were calculated.

% Super oxide Free Radical scavenging/Inhibition :

[Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

HYDROXYL RADICAL SCAVENGING ACTIVITY:

The scavenging activity of test sample on hydroxyl radical was measured according to the method of Klein et al (1992). Various concentrations of test sample were added with 1.0ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA solution (0.018%), and 1.0mL of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1M phosphate buffer, pH 7.4).The reaction was initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80–90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of1.0mL of ice-cold TCA (17.5% w/v). 3ml of Nash reagent was added and left at room temperature for 15min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm. The % hydroxyl radical scavenging activity is calculated by the following formula:

% Hydroxyl radical scavenging activity = [(Control OD-Sample OD)/Control OD] X100.

NITRIC OXIDE RADICAL SCAVENGING ACTIVITY:

2 mL of 10 mM sodium nitro prusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with sample. Various concentration of sample was taken and the mixture was incubated at 25degree for 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthyl ethylene diamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. (Garrat D C et al., 1964)

The nitric oxide radical scavenging activity was calculated as:

% Nitric oxide scavenging potential = [(Control OD-Sample OD)/Control OD] X100.

PHOSPHOMOLYBDENUM ASSAY:

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto et al (1999). Various concentration of the test sample was combined with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank.

The reduction activity was calculated as:

% Phosphomolybdenum reducing potential = [(Sample OD-Control OD)/Sample OD] X100.

IN VIVO METHOD

ANIMALS:

- The present study was conducted after obtaining approval from the Institutional Animal Ethics committee vide approval no: 11/243/CPCSEA and this protocol met the requirement of national guidelines of CPCSEA.
- All the animals used in this study were obtained from the animal house of Madras Medical College, Chennai-3.

Albino Wistar Rats: Young mature female Albino Wistar Rats weighing about100 to 200g were used which were obtained from the inbred colony of animal house of Madras Medical College, Chennai-3.

Appliances/Equipment:

Drug was administered by using the Rat tube for oral gavage made with stainless steel having blunt end. The rat oral gavage tube was connected to the 5ml/1ml syringe for the administration of required quantity of the drug. Tuberculin syringe with the capacity of 1ml has been used for the administration of the small quantity of the drugs.

Housing:

The animals were housed in well ventilated and air-conditioned animal house which was maintained at a constant temperature 23 ± 2°C with a relative humidity retained at 55-60%. Lighting was 12 hours light and 12 hours dark. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material.

Diet and water:

The animals were maintained on a diet of standard pellet & purified water. They were provided with food and water ad libitum

Animal Identification:

All the animal cages used in the study had proper identification i.e., labels bearing group name, weight of the animals, the drug and its volume to be administered according to their body weights. Animals in the cages marked with markers in tail, ear and head.

Serotonin Preparation And Administration:

Serotonin (5-HT Creatinin Sulphate Complex, Sigma-Aldrich) was dissolved in physiological saline at a concentration of 20 mg/mL. In order to avoid skin lesions like subcutaneous bleedings and traumatic wounds, the injection site was changed daily. An equal volume of physiological saline (1 mL/kg) was given to the placebo rats.

Red Reishi Mushroom Preparation:

The contents of the capsule of Reishi mushroom was dissolved in distilled water at a concentration of 50mg/ml.

Digoxin stock solution preparation:

Digoxin was dissolved in distilled water at a concentration of 0.25mg/100ml.

EXPERIMENTAL DESIGN:

S.NO	GROUPS	DRUG GIVEN	DOSE	NO OF ANIMALS	DURATION OF DOSE
1	Group 1	Saline	1ml/kg (p.o)	6	3 months
2	Group 2	Serotonin	20mg/kg (s.c)	6	3 months
3	Group 3	Serotonin + Digoxin	20mg/kg (s.c) + 1.25mcg/kg (p.o)	6	3 months
4	Group 4	Serotonin + Red reishi mushroom (*Ganoderma Lucidum)*	20mg/kg (s.c) + 500mg/kg (p.o)	6	3 months
Total no of animals					**24**

Table: 1 Rats were given daily serotonin injections subcutaneously (20 mg/kg); control were given saline. The serotonin was dissolved in saline just before it was injected. Digoxin was used as a standard in comparison to Red Reishi mushroom as it had been used to treat cardiac failure following carcinoid induced by serotonin .

The last injections were given 1 week before the rats were euthanized. At euthanization, the animals were weighed[30] .

ELECTROCARDIOGRAM:

ECG was taken before and after the drug treatment for all animals at Madras Veterinary College, Chennai .

ECHOCARDIOGRAPHY:

Ten minutes before imaging, the rat was anesthetized with 80 mg/kg ketamine intraperitoneally. Subsequently the anterior chest wall was shaved and the rat was placed in left lateral decubitus on a wooden bench in order to obtain optimal image quality.

A Vivid 7 Pro system with a 25 MHz neonatal probe (10S) was used. Images and loops were stored digitally for post-test analysis. The image sector was kept narrow in order to get a maximal frame rate. When necessary, cineloop speed was reduced for optimal jet evaluation. Regurgitant jets were assessed visually with 2D colour Doppler and continuous-wave Doppler in the parasternal short and long axis, apical three and four chamber views. If no retrograde flow was detectable with 2D colour Doppler or continuous-wave Doppler, regurgitation was considered absent. The pulmonary regurgitant flows were coded as present or absent in the short-axis view[29].

Echocardiography was performed at Madras Veterinary College, Chennai.

HISTOPATHOLOGY:

The hearts were weighed and fixed in formalin for 2 h. Then they were embedded in paraffin, cut in an axial plane (from basis to apex) and stained with hematoxylin and eosin and alcian blue for glycosaminoglycans. Additional step sections, ranging from 1 to 3 (approximately 100 µg/ml between sections) were carried out for those heart sections without valves. Extreme care was taken in sectioning the heart so that the valves were mainly cut transversely (with the attachment sides of the leaflets visible on both ends of the valve).

The program was calibrated with a graduated slide. Microscopic images were used to evaluate blindly the cardiac valves and cardiomyocytes. The maximum thickness of every valve present was measured. The width of cardiomyocytes was measured on the left ventricle of each section[29].

VIII. RESULTS:

SUPER OXIDE ANION RADICAL SCAVENGING ACTIVITY:

S.No	Concentration (µg/ml)	% inhibition of Standard	% Inhibition of Test
1	200	39.18	34.26
2	400	46.71	38.23
3	600	51.81	45.29
4	800	67.98	55.73
5	1000	72.74	60.29
6	1200	87.41	73.23
7	IC 50 value	691.04 µg/ml	714.36 µg/ml

Table:2

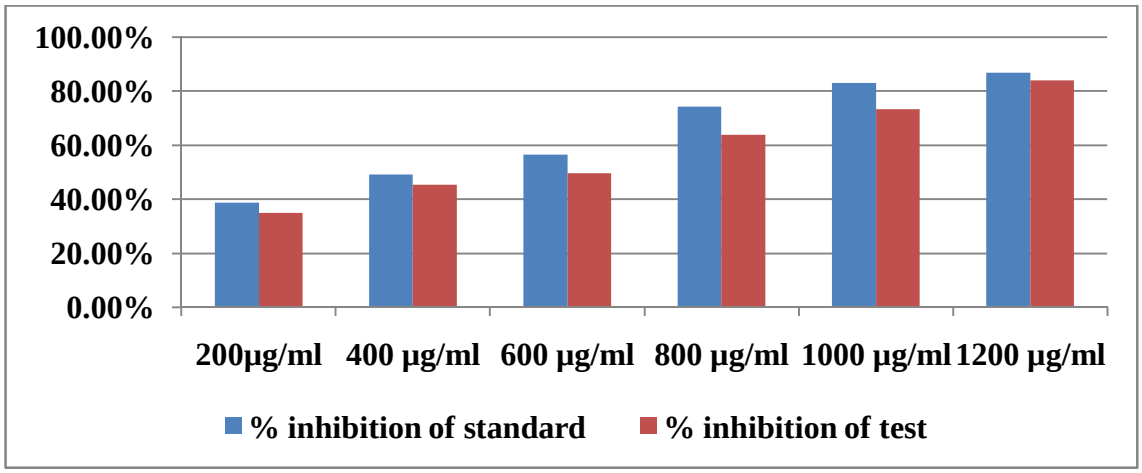


Fig:8 Graphical Representation Of Super Oxide Anion Radical Scavenging Activity.

IC 50 Value of Test (714.36µg/ml) was closely related to standard value (691.04 µg/ml).

HYDROXYL RADICAL ASSAY:

S.No	Concentration (µg/ml)	% Inhibition of standard	% Inhibition of test
1	200	29.72	30.42
2	400	39.61	40.39
3	600	49.99	45.35
4	800	58.41	49.88
5	1000	72.61	67.03
6	1200	87.18	75.74
	IC 50 value	669.04µg/ml	687.45 µg/ml

Table:3

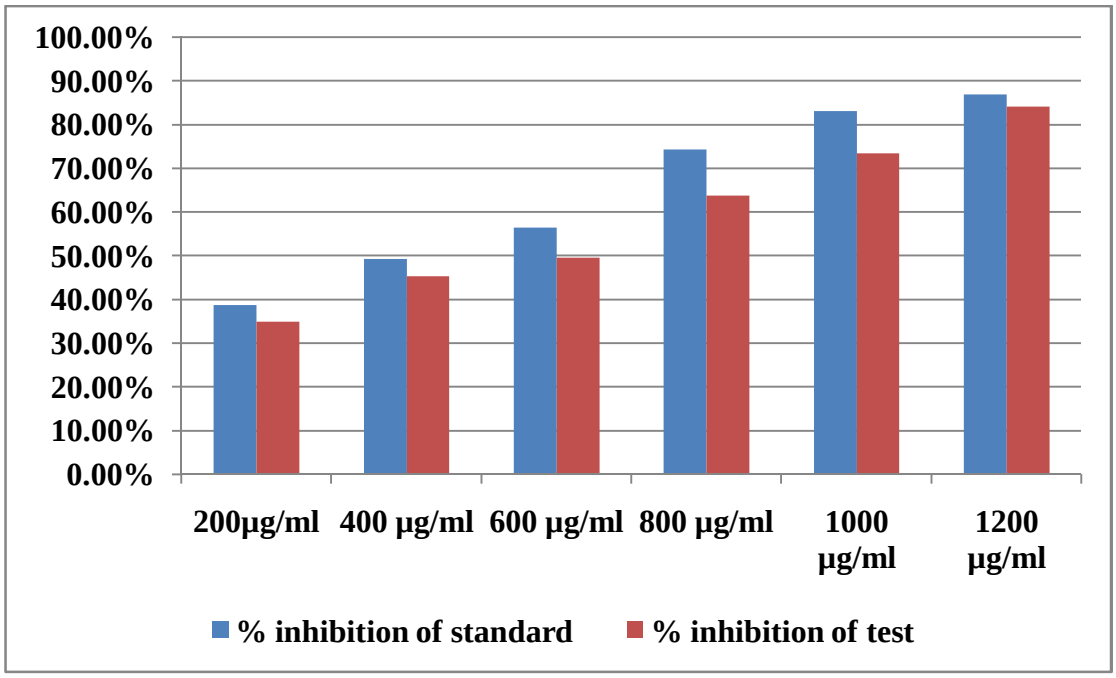


Fig: 9,Graphical representation of Hydroxyl Radical assay.

IC 50 Value of Test (687.45µg/ml) was closely related to standard value (691.04µg/ml).

NITRIC OXIDE ASSAY:

S.NO	CONCENTRATION (µg/ml)	% Inhibition of Standard	% Inhibition of Test
1	50	38.61	32.35
2	100	42.71	43.88
3	150	59.38	57.03
4	200	76.31	69.74
5	250	79.43	78.16
6	300	85.56	80.34
	IC50 value	496.58 µg/ml	459.23 µg/ml

Table:4

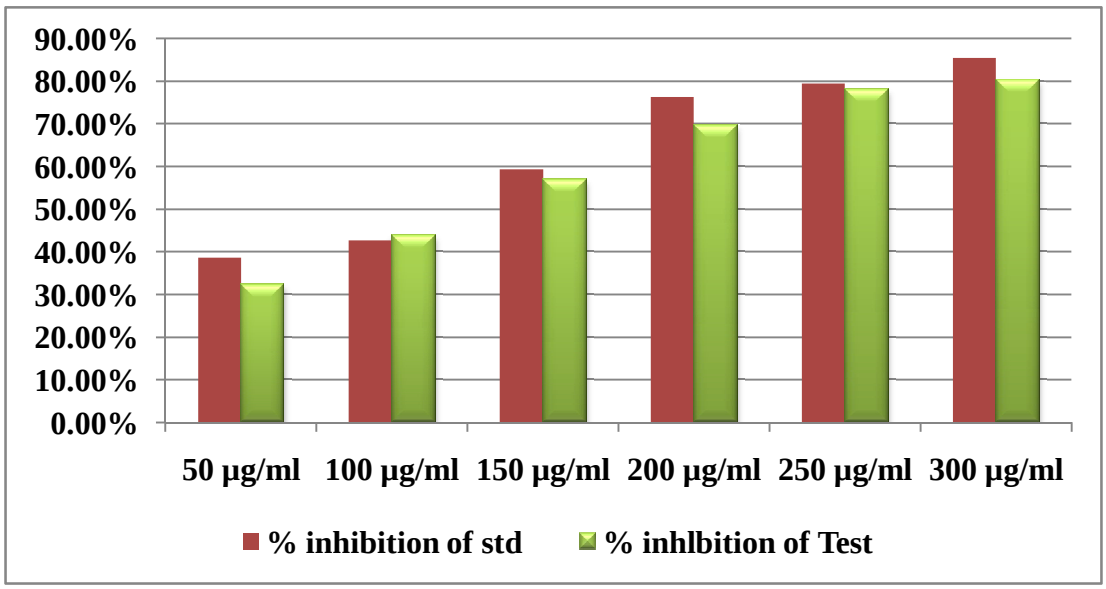


Fig: 10 Graphical representation of Nitric oxide assay.

IC 50 Value of Test (496.58µg/ml) was closed related to standard value (459.23 µg/ml).

PHOSPHOMOLYBDENUM ASSAY:

S.No	Concentration (µg/ml)	% Inhibition of Std	% Inhibition of Test
1	200	34.83	34.83
2	400	45.28	45.28
3	600	49.56	49.56
4	800	63.84	63.84
5	1000	73.41	73.41
6	1200	84.2	84.2
	IC 50 value	567.41 µg/ml	567.41 µg/ml

Table: 5

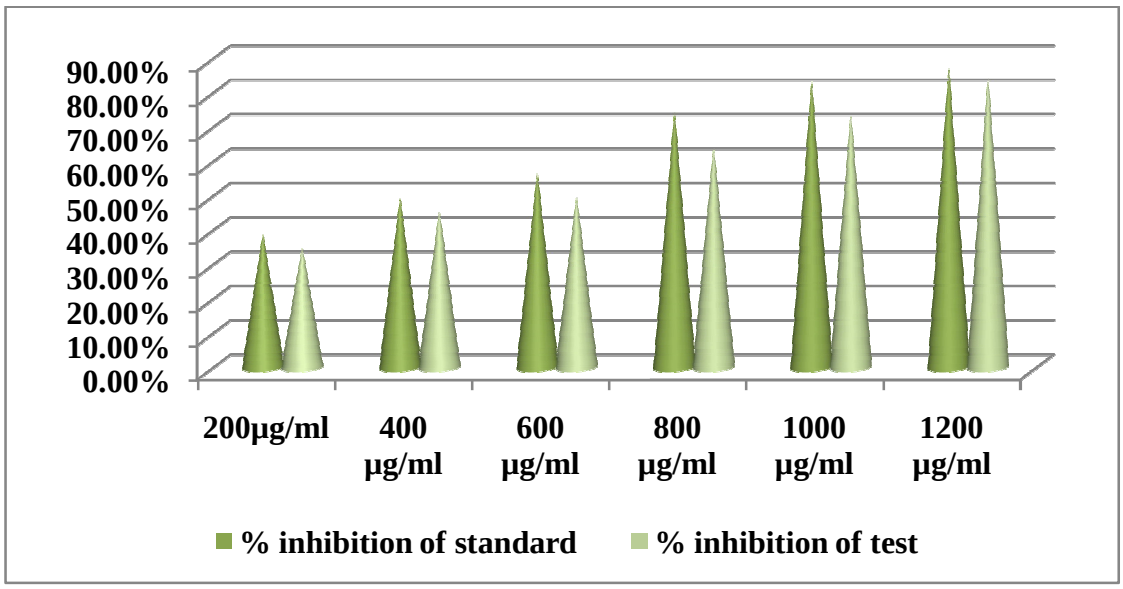


Fig:11 Graphical representation of Phosphomolybdenum Assay.

IC 50 Value of Test (596.54µg/ml) was closed related to standard value (567.41 µg/ml).

***In vivo* method**

Rats injected with serotonin alone developed macroscopic skin changes at the injection sites. The serotonin injections induced loose stools, dyspnea, drowsiness for several hours after injection in all animals at first week.

The signs of the serotonin injections were pronounced in the first four weeks of the study. It also caused hyperactivity and aggressive behaviour in serotonin treated animals. Their cardiac profile was evaluate by ECG and Echocardiography.

BODY WEIGHT :

GROUPS	BEFORE TREATMENT (gm)	AFTER TREATMENT (gm)
GROUP I	128.33±15.53	139.2±16.79
GROUPII	130.33±14.33	123±5.65[a]
GROUP III	132.83±10.36	136±4.6[b]
GROUP IV	129.66±9.89	134±10.08[b]

Table:6

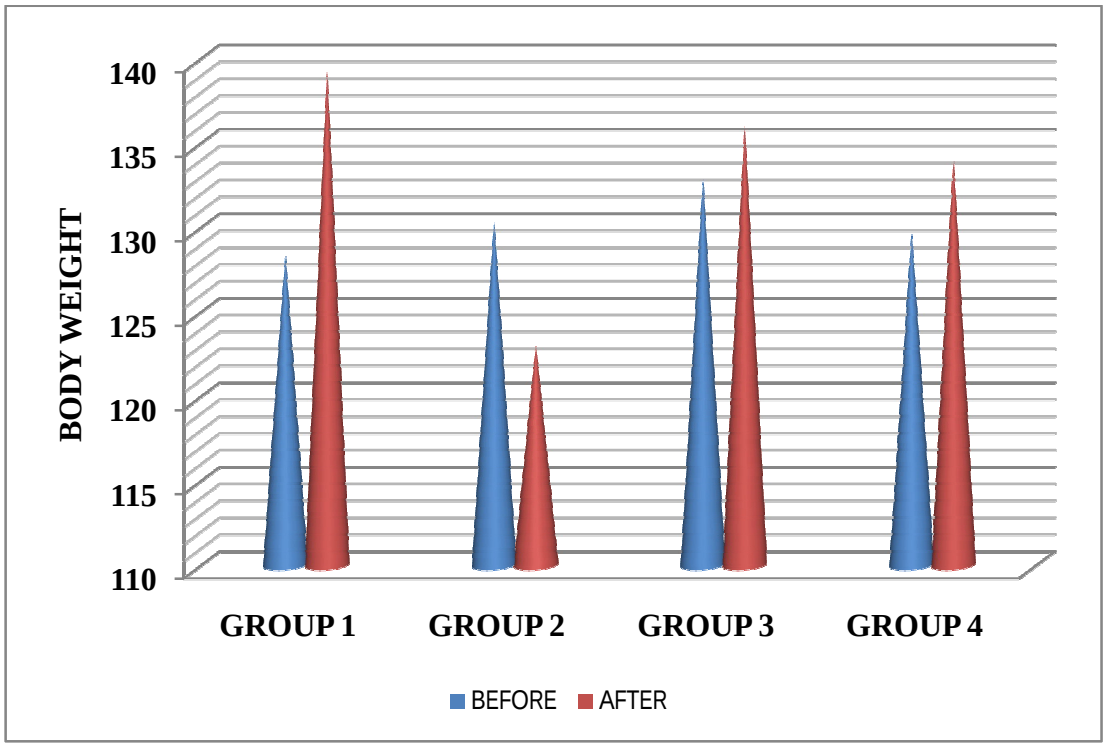
G I - Normal control G II – Disease control

G III Digoxin treated group G IV *Ganoderma Lucidum* treated group.

Values are expressed as mean ± SD, (n=6).

'a' => P < 0.01 compared to control group.

'b' => P < 0.01 compared to disease group.



Graphical representation of changes in body weight.

It was seen that the body weight decreased significantly in animals which were given serotonin as compared to control group. Treatment with digoxin showed a significant increase in body weight .Ganoderma lucidum treated group of animals also showed significant increase in body weight.

Electrocardiography recording in rats

HEART RATE: Normal heart rate (375 ± 20).

GROUPS	AT BASELINE	AFTER 3 MONTHS
GROUP I	366.66±12.16	383.83±13.19
GROUP II	368.83±6.91	**250±36.05**[a]
GROUP III	381.16±7.08	402±30.31[b]
GROUP IV	375.83±12.17	373±10.93[b]

Table:7

G I - Normal control G II – Disease control

G III Digoxin treated group G IV *Ganoderma Lucidum* treated group.

The values are expressed as mean ± SD, (n=6).

'a' => P values < 0.01 compared to control group.

'b' => P values < 0.01 compared to disease group.

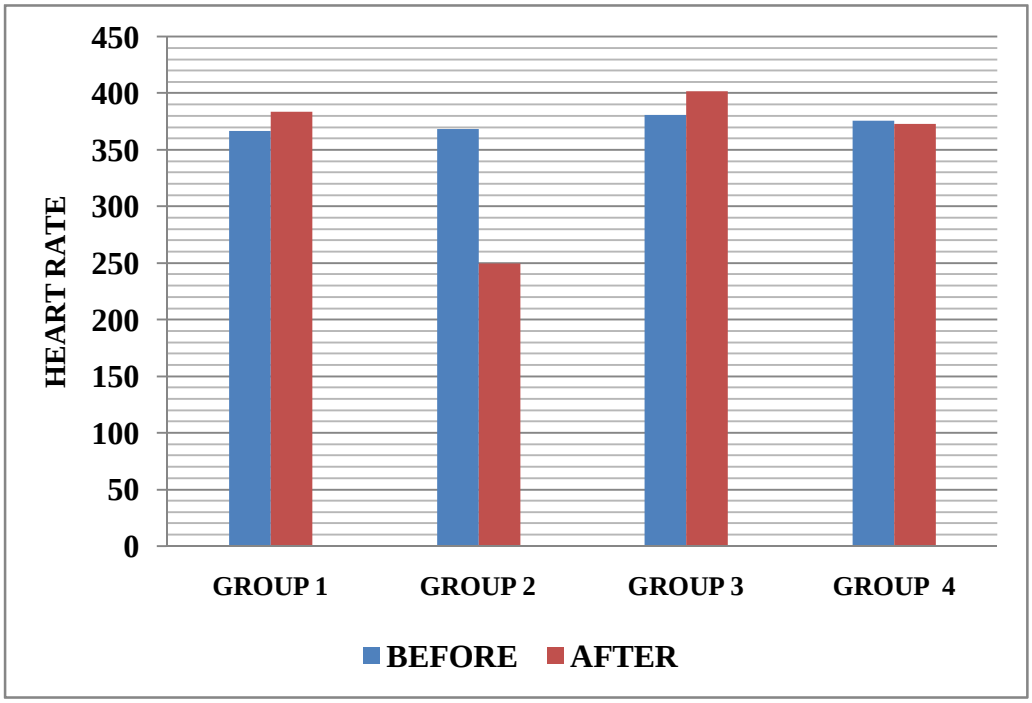


Fig:13 Graphical representation of changes in heart rate.

It was seen that the heart rate decreased significantly in animals which were given serotonin as compared to control group to the end of 3 months. Treatment with digoxin showed a significant increase in heart rate. Ganoderma lucidum treated group of animals showed no significant change in heart rate.

Fig:14

(GROUP I) No abnormality detected

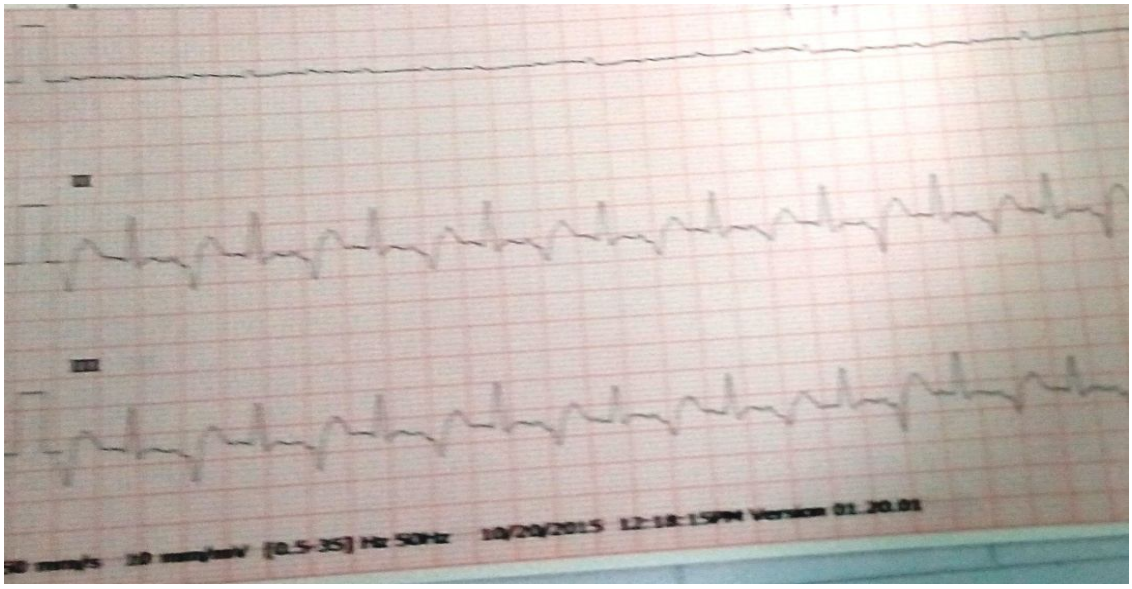


Fig: 15

AV BLOCK GROUP III

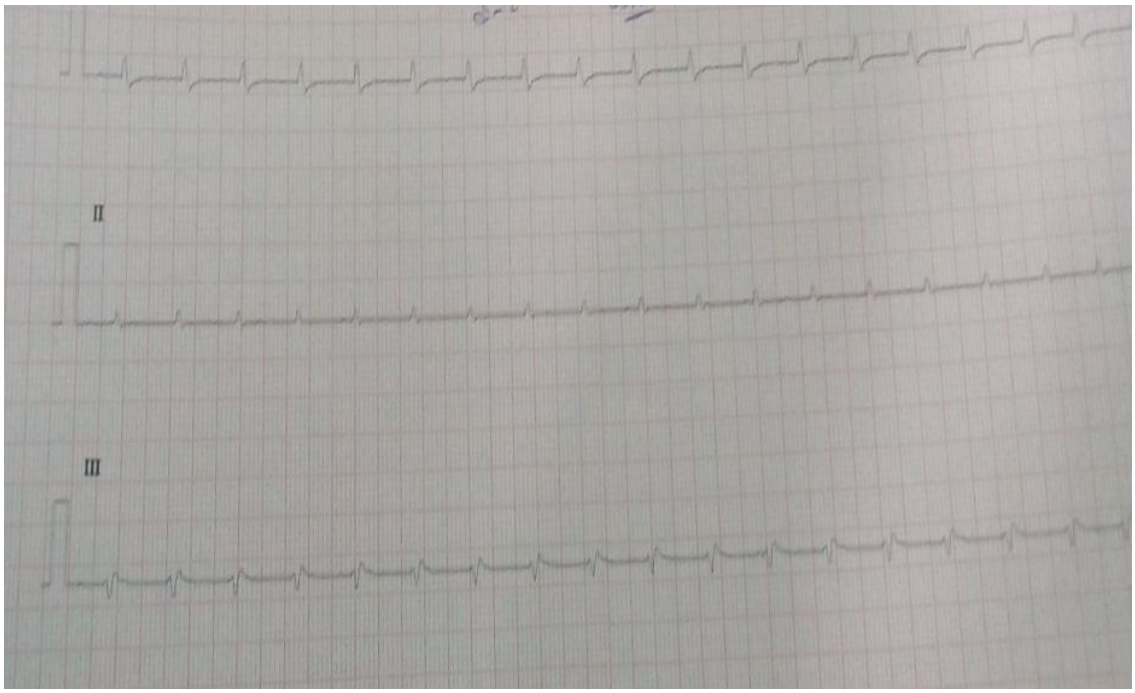


Fig:16

VENTRICULAR BI GEMINA GROUP II

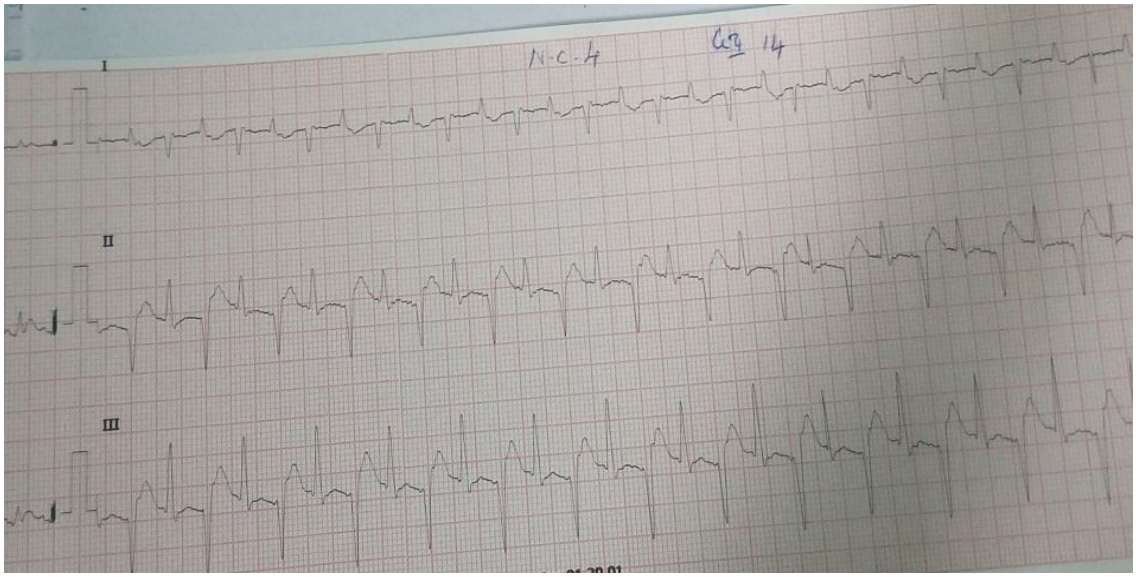


Fig:17

BRADYCARDIA GROUP II

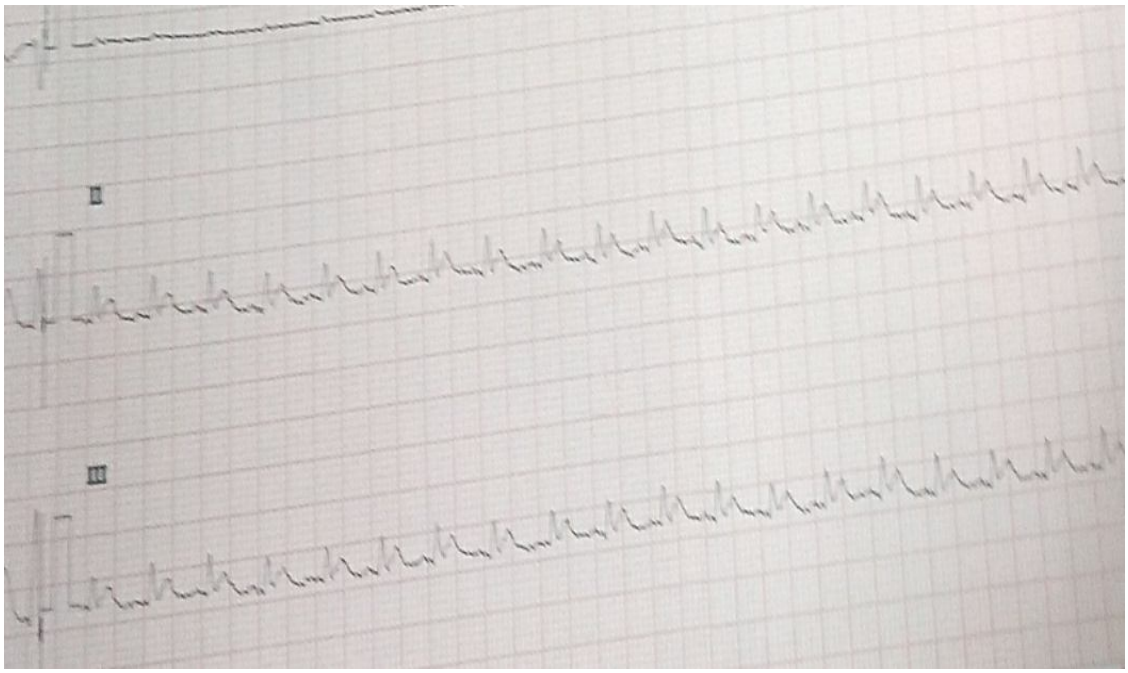


Fig:17

No Abnormality Detected GROUP III

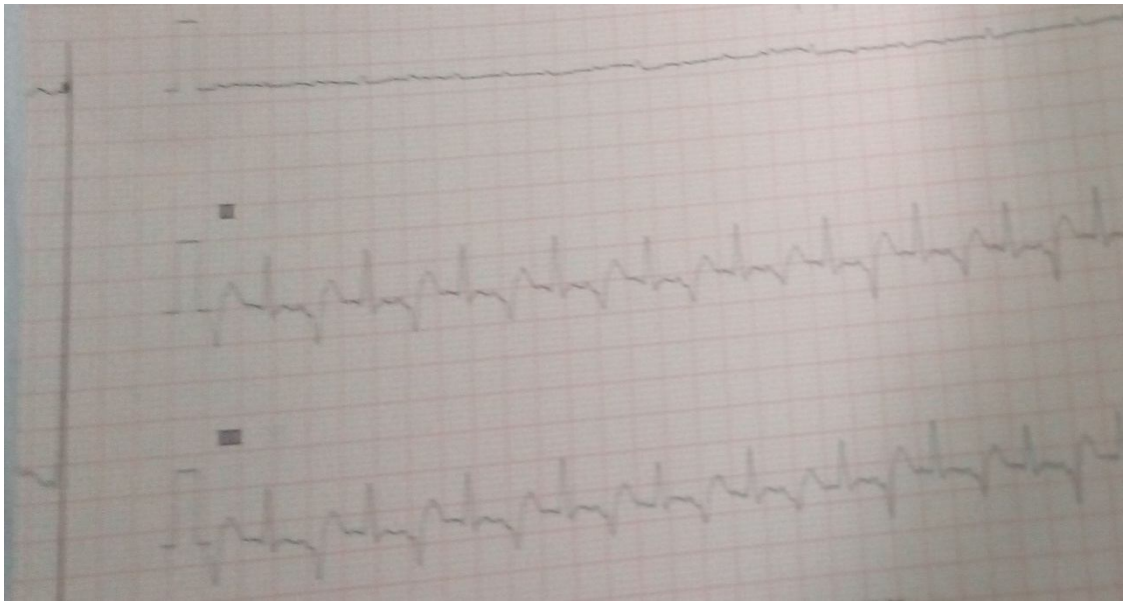


FIG: 18

SINUS TACHYCARDIA GROUP III

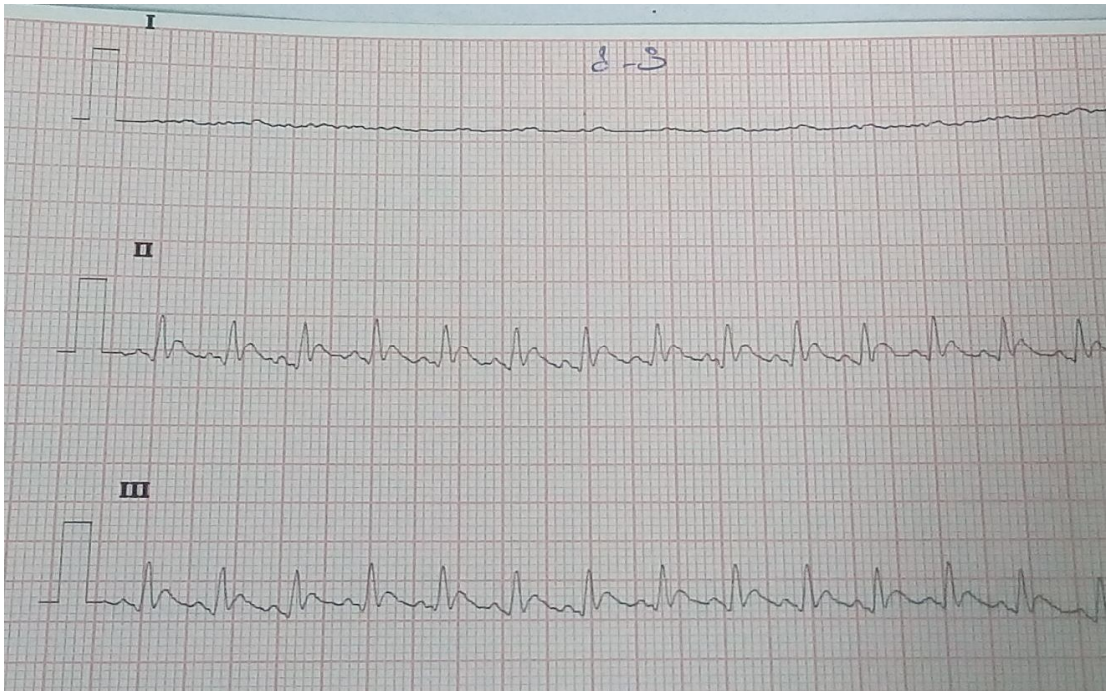


FIG:19

No Abnormality detected GROUP III

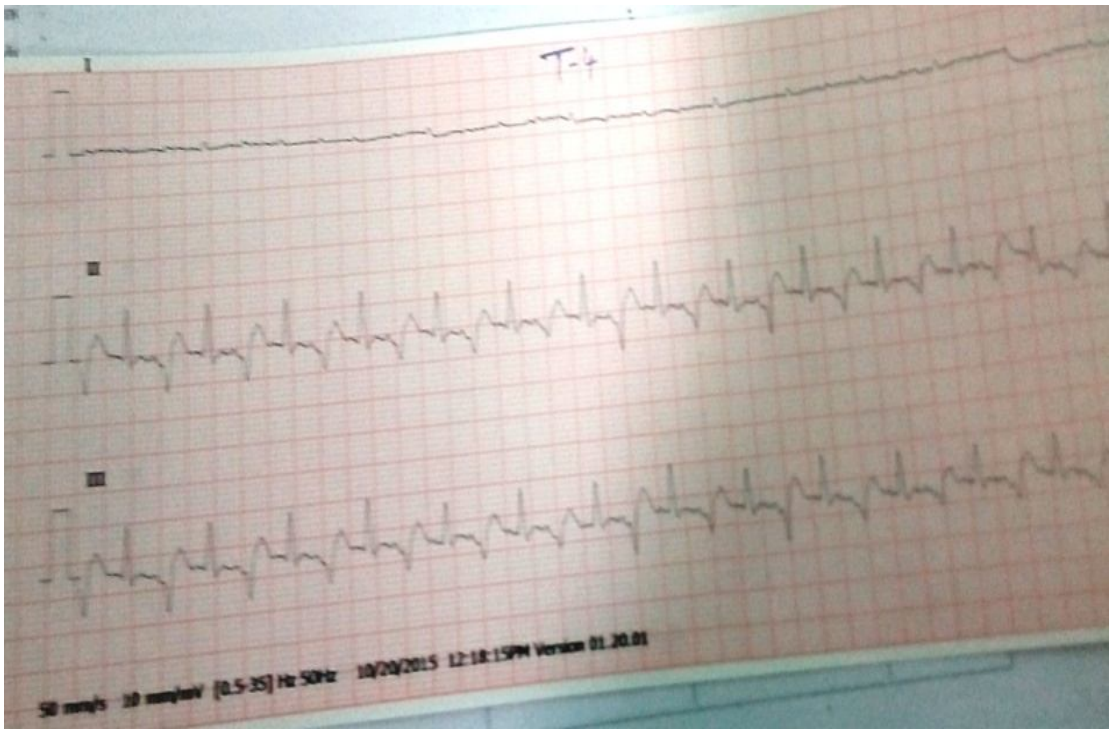


Fig:20

ECG REPORT :

At baseline

S.NO	GROUP I	GROUP II	GROUP III	GROUP IV
1	NAD	NAD	NAD	NAD
2	NAD	NAD	NAD	NAD
3	NAD	NAD	NAD	NAD
4	NAD	NAD	NAD	NAD
5	NAD	NAD	NAD	NAD
6	NAD	NAD	NAD	NAD

Table:8

ECG was normal at the beginning of the study in all the 4 groups.

AFTER 3 MONTHS TREATMENT :

S NO	GROUP1	GROUP 2	GROUP 3	GROUP 4
1	NAD	VENTRICULAR BI GEMINA	SINUS TACHY CARDIA	NAD
2	NAD	BRADY CARDIA	TACHYCARDIA	NAD
3	NAD	1^{ST} DEGREE AV BLOCK	NORMAL SINUS RHYTHM	REDUCED QRS COMPLEX AMPLITUDE
4	NAD	BRADYCARDIA	NAD	REDUCED R AMPLITUDE
5	NAD	BRADYCARDIA	NAD	NAD
6	NAD	BRADYCARDIA	NAD	NAD

Table:9

G I - Normal control G II – Disease control

G III Digoxin treated group G IV *Ganoderma lucidum* group

IN GROUP I - Normal.

IN GROUP II - Bradycardia was present in four (66.6%) rats,

VBG waspresent in one (16.6%) rat,

1^{ST} degree AV Block was found in one (16.6%) rat.

IN GROUP III - (50%) of rats (three) did not show any abnormality.

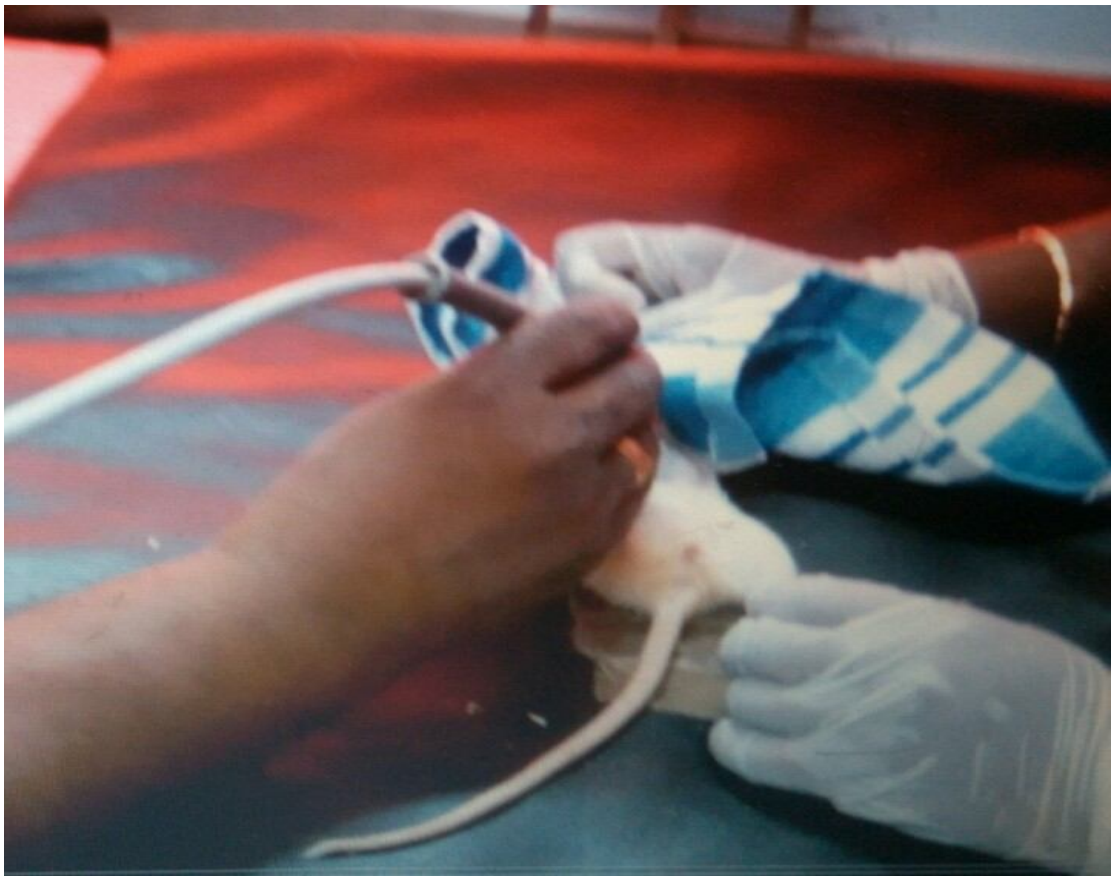
Tachycardia was present in two (33.3%) rats,

Normal sinus rhythm in one (16.6%) rat.

IN GROUP IV - (66.6%) of rats did not show pathological change

Reduced QRS complex amplitude was shown in one (16.6%) rat

Reduced R amplitude was present in one (16.6%) rat.



ECHOCARDIOGRAM REPORT

S.No.	Group I	Group II	Group III	Group IV
1	NAD	Aortic Regurgitation	Mitral regurgitation	NAD
2	NAD	Mitral Regurgitation	Aortic Regurgitation	NAD
3	NAD	Tricuspid Regurgitation	NAD	NAD
4	NAD	Mitral Regurgitation	NAD	NAD
5	NAD	Aortic Regurgitation	NAD	NAD
6	NAD	Mitral Regurgitation	NAD	NAD

Table: 10

In GROUP I - No valvular abnormality was noted.

In GROUP II - Aortic regurgitation was present in two (33.3%) rats,

Mitral regurgitation was present in three (50%)of rats,

Tricuspid regurgitation was found in one (16.6%) rat.

In GROUP III - Aortic regurgitation was present in one (16.6%) rat,

Mitral regurgitation was present in (16.6%) of rats,

(66.6%) of rats did not show pathological changes.

In GROUP IV - No valvular abnormality was found.

GROUP I

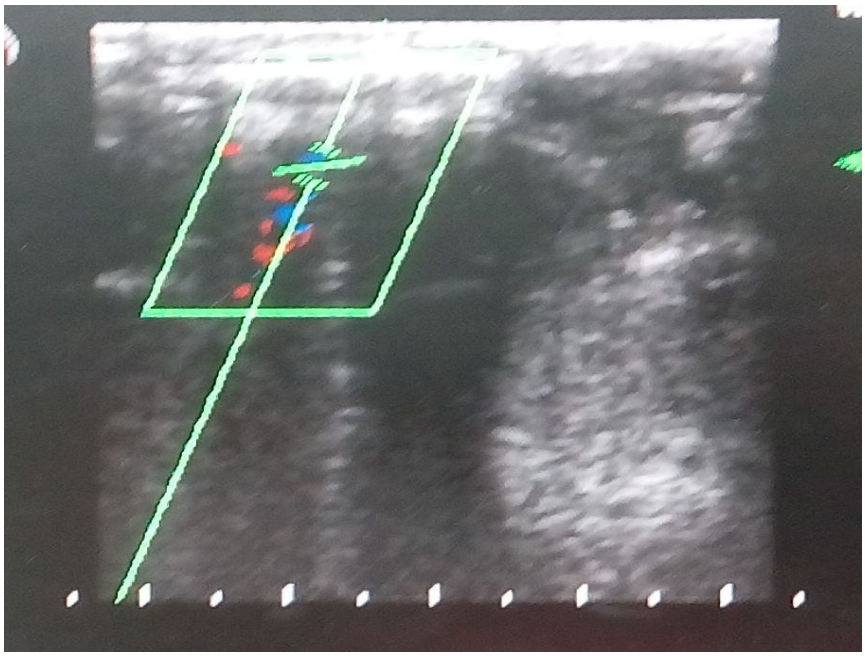


Fig:23 Echocardiographic examination did not show any pathological changes in control group.

GROUP IV

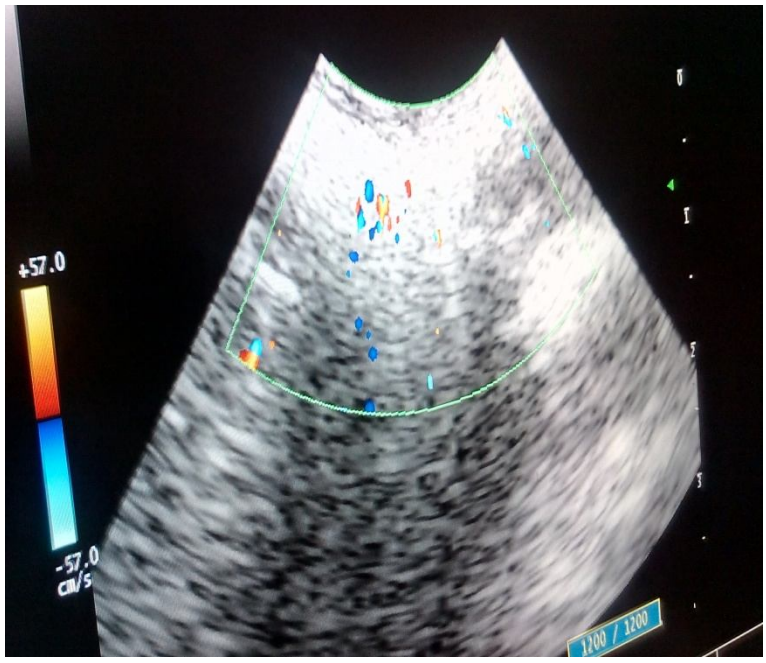


Fig:24 NORMAL STUDY

GROUP II :

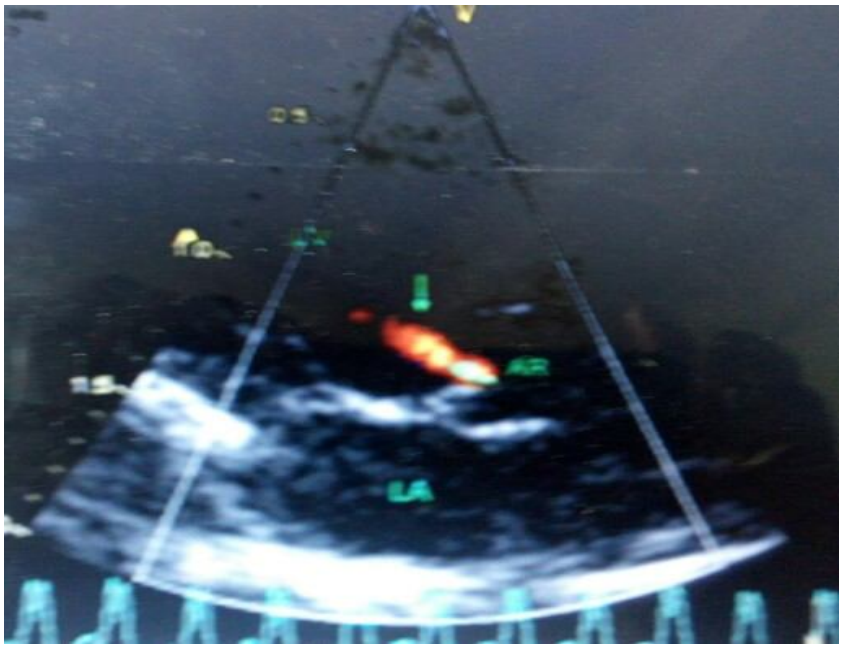


Fig:25 AORTIC VALVE REGURGITATION

GROUP II

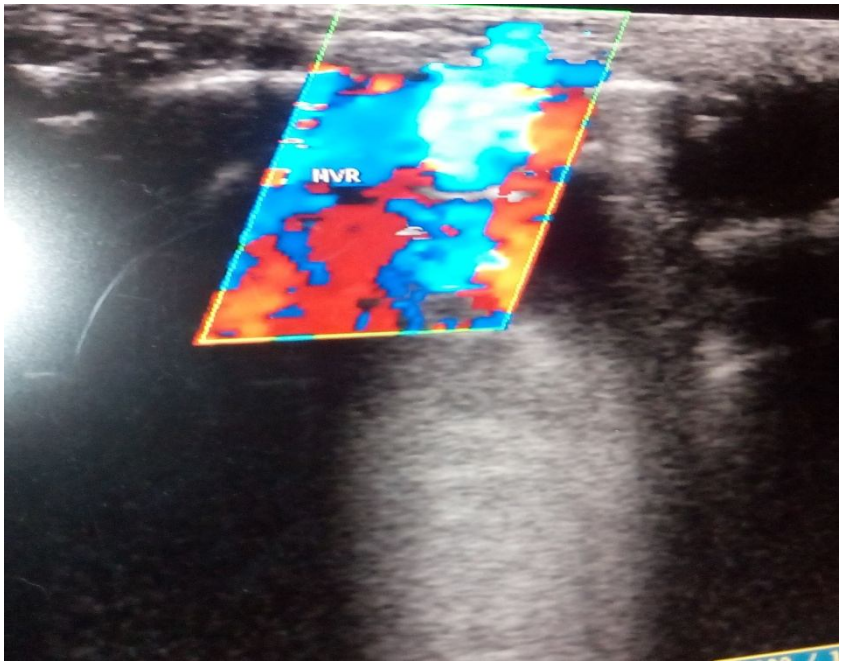


Fig:26 MITRAL VALVE REGURGITATION

ORGANS WEIGHT:

- Heart weight
- Liver weight

GROUP	HEART WEIGHT (gms)	LIVER WEIGHT (gms)
GROUP 1	0.7±0.025	4.89±0.13
GROUP 2	0.86±0.029[a]	6.2±0.2[a]
GROUP 3	0.77±0.016[b]	5.42±0.24[b]
GROUP 4	0.73±0.025[b]	5.15±0.14[b]

Table:11

G I - Normal control G II - Disease control

G II - Digoxin treated group G IV - *Ganoderma lucidum* treated group

Values are expressed as mean ± SD, (n=6).

'a' => P < 0.01 compared to control group.

'b' => P < 0.01 compared to disease group.

ORGAN WEIGHT:

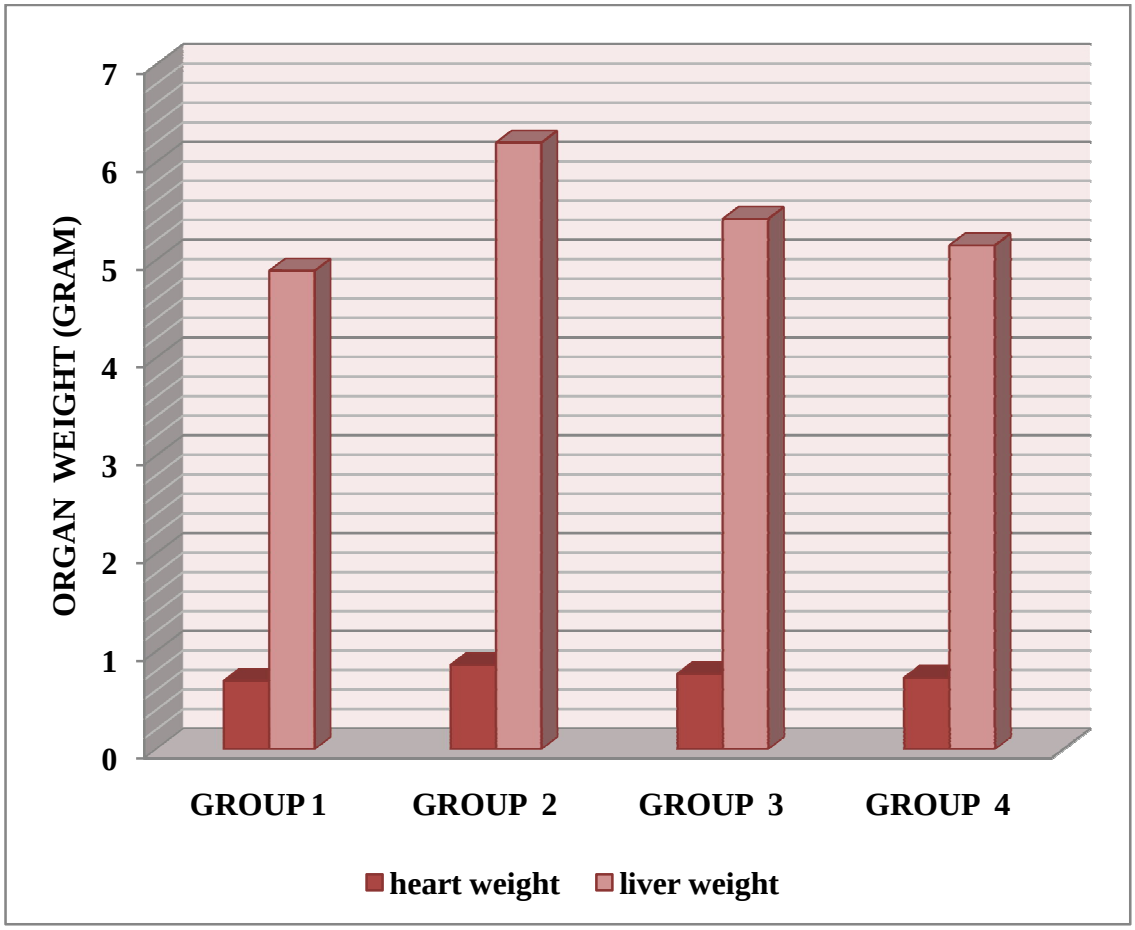


Fig:20 Graphical representation of organ weight

It was seen that the organ weight increased significantly in animals which were given serotonin as compared to normal group. Treatment with digoxin showed a significant decrease in organ weight. *Ganoderma lucidum* treated group showed significant decrease in organ weight.

LEFT VENTRICULAR WALL THICKNESS

GROUPS	LEFT VENTRICULAR WIDTH (µm)
GROUP I	0.26±0.07
GROUP II	0.5±0.06[a]
GROUP III	0.33±0.08[b]
GROUP IV	0.31±0.07[b]

Tab:12, The values are expressed a mean ± SD, (n=6).

G I - Normal control G II–Disease control

G II–Digoxin treated group G IV - *Ganoderma lucidum* treated group

'a' => P values < 0.01 compared to control group.

'b' => P values < 0.01 compared to disease group.

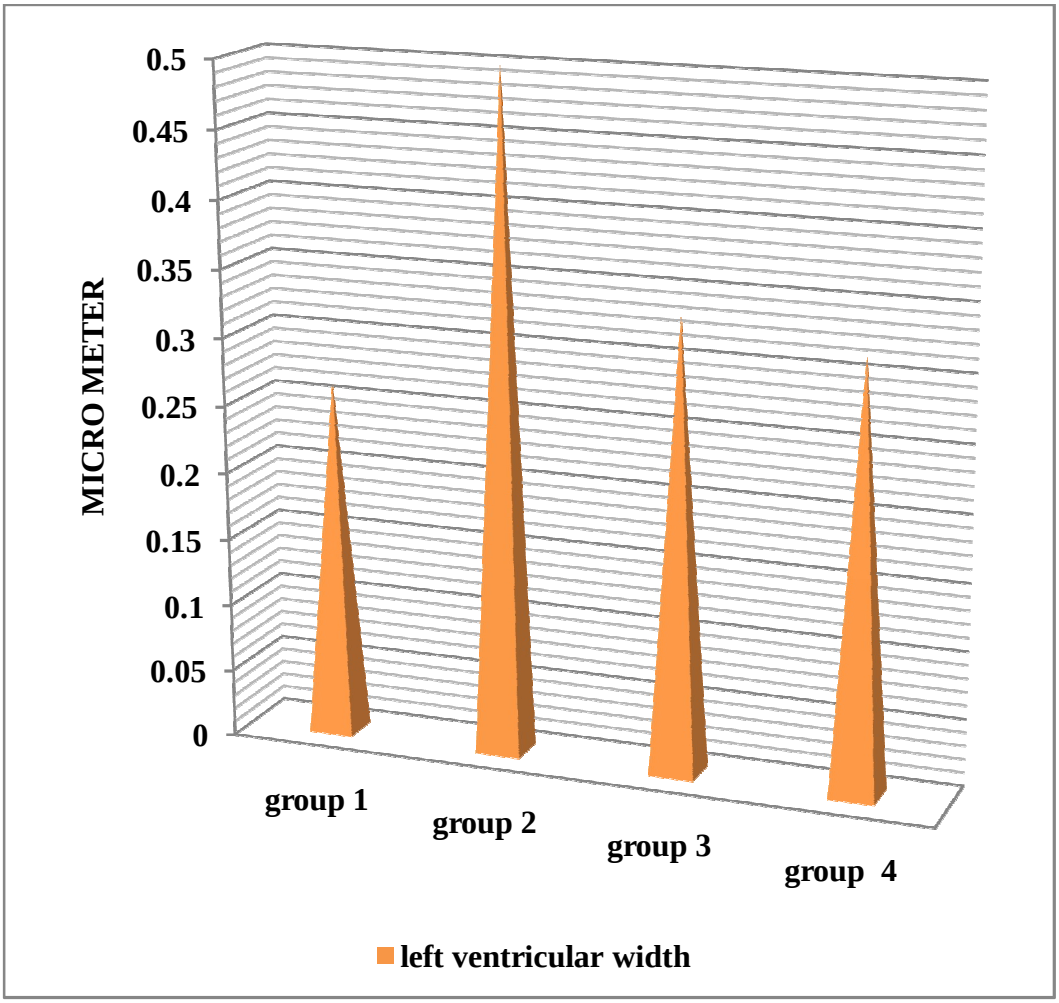


Fig:27 Graphical representation of left ventricular thickness.

It was seen that the left ventricular wall thickness increased significantly in animals which were given serotonin as compared to control group. Treatment with digoxin showed a significant decrease in left ventricular wall thickness. *Ganoderma lucidum* treated group also showed significant decrease in the left ventricular wall thickness.

HEART VALVE THICKNESS MEASUREMENT :

Groups	AORTIC VALVE(µm)	MITRAL VALVE (µm)	PULMONARY VALVE (µm)	TRICUSPID VALVE (µm)
I	0.0433±0.01	0.11±0.005	0.053±0.01	0.0310.004
II	0.0766±0.01[a]	0.13±0.008[a]	0.085±0.01[a]	0.0560.005[a]
III	0.0516±0.02[b]	0.14±0.011[b]	0.063±0.01[b]	0.038±0.007[b]
IV	0.0466±0.01[b]	0.135±0.008[b]	0.05±0.01[b]	0.041±0.004[b]

Tab:14 The values are expressed a mean ± SD.

'a' => P values < 0.01 compared to control group.

'b' => P values < 0.01 compared to disease group.

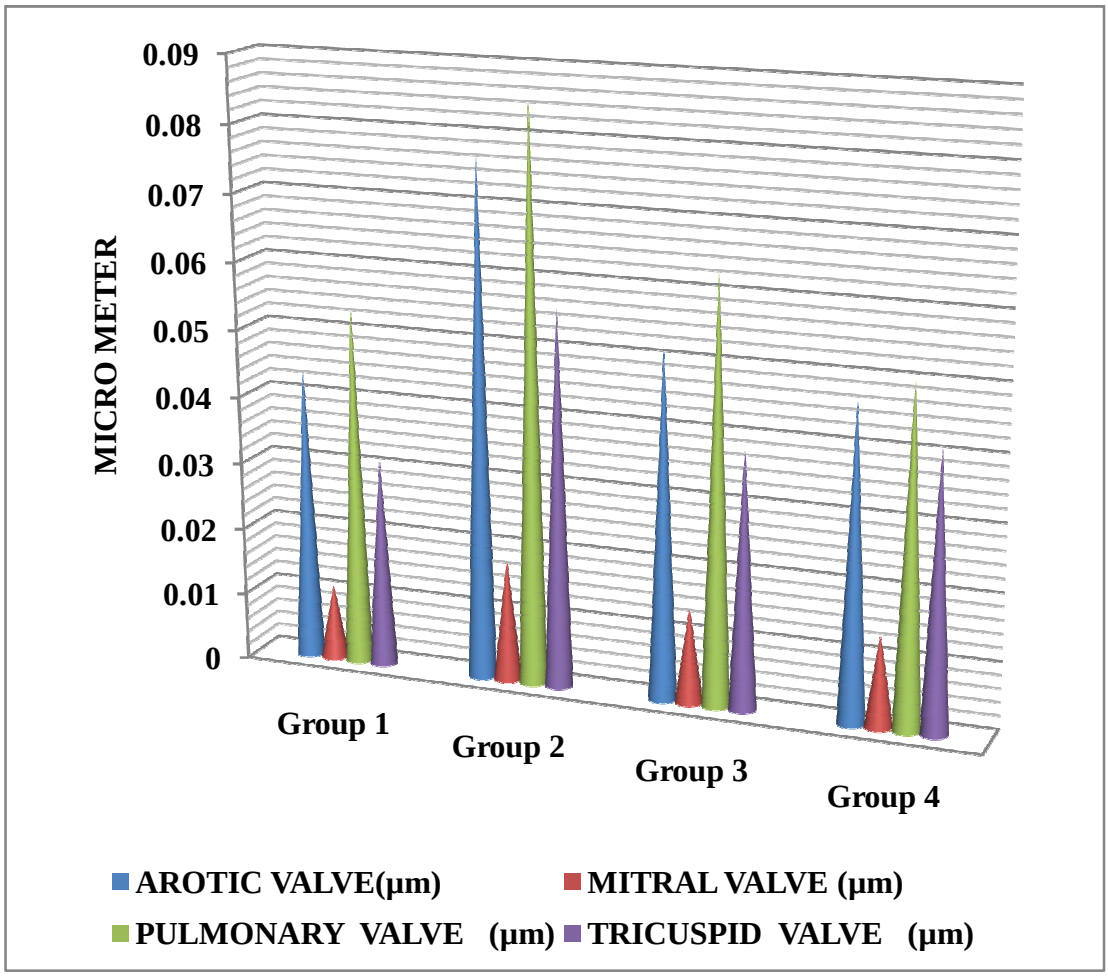


Fig:28 Graphical representation of valve thickness.

It was seen that the heart valve thickness increased significantly in animals which were given serotonin as compared to control group. Treatment with digoxin showed a significant decrease in heart valve thickness. *Ganoderma lucidum* treated group showed significant decrease in the heart valve thickness.

HISTOPATHOLOGY OF HEART:

Fig : 29 GROUP I

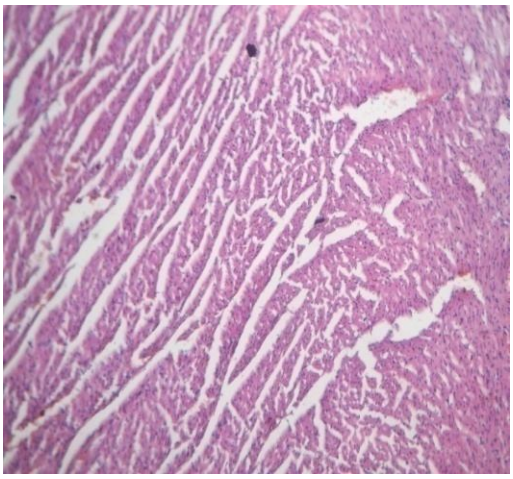


Fig : 30 GROUP II

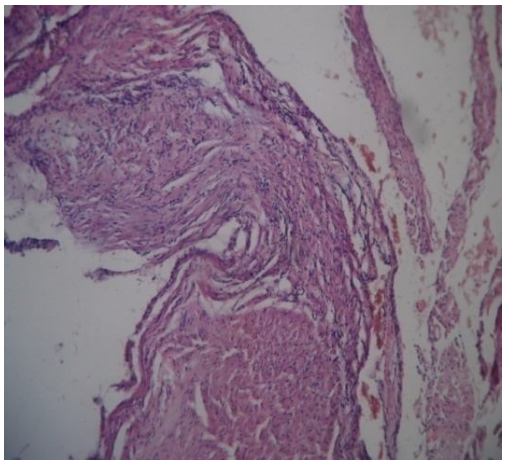


Fig 31 : GROUP III

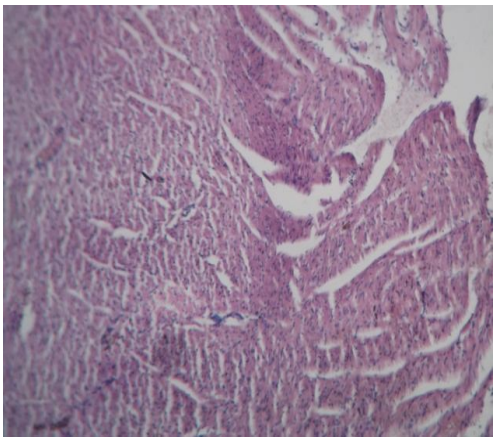
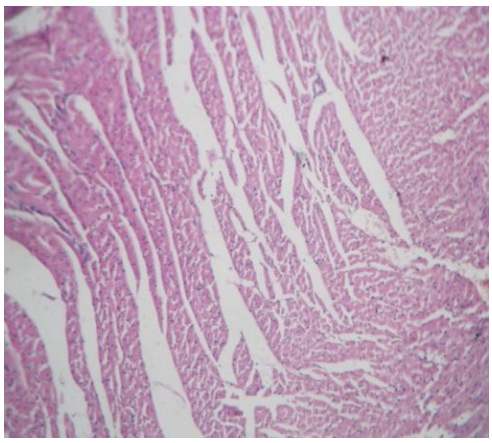


Fig 32 : GROUP IV



DISCUSSION:

Carcinoid heart disease occurs in over 65% of patients with the carcinoid syndrome and is characterized by thickening of cardiac valves, leading to heart failure. Serotonin plays a major role in the development of carcinoid heart disease[32]. Serotonin induces oxidative stress in human heart by means of increased reactive oxygen species especially superoxide ($O_2^{\cdot}$).

ROS appears to participate in serotonin induced mitogenesis in carcinoid heart disease. Antioxidants have been found to prevent the mitogenic effects of serotonin[28].

Ganoderma lucidum is a traditional Chinese medicine, which consists of triterpenes called ganoderic acids, polysaccharides, mannitol, canthaxanthin, lanostan and alkaloids. Sterols isolated from the mushroom include, ganoderol, ganoderenic acid[20].

It has been known to have numerous pharmacological effects including immune modulating, anti-inflammatory, anti cancer , anti diabetic effect. It inhibits mitogen protein kinase enzyme, increases anti- oxidative enzyme activity and exhibits direct free radical scavenging activity. Reishi mushroom prevents the cardiotoxicity by its antioxidant effect. Hence this study was undertaken to evaluate the effect of *Ganoderma lucidum* on inhibiting serotonin induced carcinoid heart disease by its antioxidant property[16,19].

In vitro antioxidant activity was carried out Super oxide radical scavenging assay method, Hydroxyl radical scavenging assay method, Nitric oxide method and Phosphomolybdenum assay method. It was observed that as the concentration increases, the % scavenging also increases linearly for *ganoderma lucidum* and standard preparation.

In case of super oxide free radical scavenging assay method, the IC50 value (714.36µg/ml) of *Ganoderma lucidum* was almost equal to standard value(691.04µg/ml) which indicates *Ganoderma lucidum* has potent antioxidant activity.

In Hydroxyl radical scavenging assay method, IC50 value of red reishi mushroom (687.45µg/ml) was showing only much lesser difference on comparison with the standard value (679.04µg/ml).

IC 50 values (496.51µg/ml),(596.54 µg/ml) of reishi closely related to standard values (459.23 µg/ml) and (537.41 µg/ml) respectively by nitric oxide scavenging method Phosphomolybdenum assay method. It was found that red reishi mushroom displayed antioxidant potential aganist reactive oxygen species.

In *in vivo* study, serotonin alone treated group of rats showed significant reduction in the body weight which was prevented by the *Ganoderma lucidum* and this correlates with the study done by Bjorn.I.Gustafsson et al (2005)[30]

At baseline ECG were normal in all groups. After 3 months of serotonin induction, serotonin alone treated rats showed bradycardia (66.6%), ventricular bi gemina (16.6%) and 1st degree AV block (16.6%). Heart rate reduced significantly ($P<0.01$) which was found to be reversed in the Reishi mushroom treated group upto (66.6%) where as the Digoxin treated group showed normal heart rate in 66.6% and sinus tachycardia in 16.6%.

In Echocardiography serotonin alone treated animals, showed Mitral regurgitation(50%), Aortic regurgitation(33.3%) and Tricuspid regurgitation(16.6%) and this finding correlated with the study done by Bjorn.I.Gustafsson et al (2005).[30]

Among the rats treated with Digoxin no valvular abnormality was found in 66.6% where as (16.6%) showed mitral regurgitation and 16.6% showed aortic regurgitation.

No valvular abnormality was found in *Ganoderma lucidum* treated group depicting its excellent protective effect in preventing serotonin induced valvular changes.

Ganoderma lucidum showed significant decrease in organ mass induced by serotonin. It is proved that this mushroom may have the anti proliferative property on cells which was in accordance with the study done by Hauso, Loennechen JP et al (2007)[29] .

Macroscopically, left ventricular wall thickness is more in serotonin alone treated group (0.5±0.06µm) compared to mushroom treated group(0.31±0.01µm) which correlates with the study done by Steven Droogmans et al(2007)[31].

In valvular pathology, the thickness of aortic (0.0766±0.01µm), pulmonary (0.085± 0.01µm) ,tricuspid (0.056± 0.005µm), mitral valves (0.13± 0.008µm) in serotonin alone treated group was significantly increased due to the valvular lesion which was decreased by *ganoderma lucidum* treated group. More over regurgitant aortic, mitral and tricuspid valves were thicker than non regurgitant valves.

In histopathological examination, the serotonin treated animals were found to have increased cellularity of myofibrobasts in collagenous matrix with extensive hypertrophy of cardiac smooth muscle. Aortic valves showed myxoid degeneration.

Ganoderma lucidum treated group showed normal histology of cardiac muscle fiber sections studied from aortic, mitral, tricuspid and pulmonary valves in 84% and mild hypertrophy of cardiac smooth muscles in 16.6%.

Ganoderma lucidum treated rats exhibited a reduction of incidence of aortic, tricuspid and mitral regurgitation on echocardiography and normal aortic, tricuspid and mitral thickness in histopathological evaluation which correlates with the study done by Steven Droogmans et al(2007)[31].

In this study *Ganoderma lucidum* had a preventive effect against the development of serotonin induced carcinoid heart disease in rat model.

IX. CONCLUSION:

- The antioxidant property of *ganoderma lucidum* was confirmed by *in vitro* studies namely Super oxide radical scavenging assay method, Hydroxyl radical scavenging assay method, Nitric oxide method, Phosphomolybdenum assay method.

- *Ganoderma lucidum* inhibited effects of serotonin induced carcinoid heart disease probably by reducing oxidative damage by scavenging free radicals in the cells.

REFERENCE:

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