Current Trends in Biotechnology and Pharmacy
Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

Syzygium cumini Protects Diabetic Wistar Rats Against Rosiglitazone-Induced Cardiotoxicity and Hepatotoxicity

Prashant L. Pingale, Rupali A. Patil*, Aishwarya S. Gadkari and Sunil. V. Amrutkar

Department of Pharmacology, Gokhale Education Society's Sir Dr. M. S. Gosavi College of Pharmaceutical Education & Research, Prin. T. A. Kulkarni Vidyanagar, Nashik 422005, Maharashtra

*Corresponding Author: E-mail Id; ruupalipatil@gmail.com

Abstract

Syzygium cumini (Family: Myrtaceae), is known to show antioxidant, antidiabetic, cardioprotective and hepatoprotective activities in ayurvedic system of medicine. This study aims at determining *in vitro* and *in vivo* antioxidant activity of ethanolic extract of *S. cumini* (ESC). Effect of ESC on rosiglitazone induced cardiotoxicity and hepatoxicity in alloxan-induced diabetic rats was studied. Results of study indicated that ESC exhibited significant protection against cardiac and hepatic damage caused by rosiglitazone in alloxan-induced diabetes in rats comparable to silymarin, used as reference standard. Cardioprotective and hepatoprotective activity may be attributed to presence of antioxidant activity.

Keywords: Alloxan, Antioxidant, Cardiotoxicity, Diabetes, Hepatotoxicity, Jambul**,** Rosiglitazone

Introduction

Plants based products have been in use for medicinal or other purpose right from the dawn of history. Ayurveda, the traditional Indian system of medicine, involves dispensing of plant products in various forms such as powders, extracts, decoction etc. Interest in medicinal plants has increased enormously over the last two decades. Herbal drugs constitute a major part in the traditional system of medicine of many countries. Large numbers of these herbal medicines have been incorporated into practice. Herbs have been used for easy accessibility and inexpensiveness. Several drugs of plant origin have been used in treatment of a variety of disorders. Many medicines and formulations have significant antioxidant properties and are beneficial in treating toxicity in animals. Phytoconstituents like alkaloid, flavonoids, tannins, gallic acid and β-sitosterol are known to possess antidiabetic, hepatoprotective and cardioprotective activity.

Syzygium Cumini (Family: Myrtaceae), commonly known as *jambul*, is native of India or East India and it's found in some other countries like Thailand, Philippines, Madagascar. Seed consist of alkaloid, flavonoid and tannins. *S. cumini* seeds are used in traditional medicinal system for diarrhea, dysentery, enlargement of spleen etc (1). Seed extract of *S. cumini*, the part most often used in Ayurvedic medicine, having good level of total phenolic activity. The patient's blood sugar and glycosuria were lowered by an oral dose of dry alcoholic extract of jamun seed. The seed powder is used as an antidote for strychnine poisoning in India (2). Oxidative stress can cause hepatotoxicity and cardiotoxicity due to free radicals. Hepatotoxicity and cardiotoxicity are treated with plants possessing free radical scavenging activities. Literature review revealed that *S. cumini* seeds having significant antioxidant activity (3). Present study was aimed to explore the potential of *S. cumini* through the use of drug induced hepatotoxicity and cardiotoxicity in experimental animals.

Materials and Method

***Animals*:** Wistar rats (150-200gm) were procured from Veterinary College, Mumbai. The

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

study was performed according to the CPCSEA guidelines and protocol was approved (Protocol number: MGV/PC/CPCSEA/XXXII/02/2016/06) by the IAEC.

***Drugs and Chemicals*:** Rutin and Gallic acid used for estimation for total phenol and flavonoid content. Rosiglitazone (Yarrow Pharmaceutical, Mumbai), Silymarin, Alloxan monohydrate (Sigma-Aldrich- USA) were used. Alanine Aminotransferase (ALT or SGPT), Aspartate Aminotransferase (AST or SGOT), Alkaline Phosphatase (ALP), Bilirubin, CK-MB, and LDH were all tested using biochemical kits.

***Preparation of Extract*:** The seed powder of *S. cumini* obtained from local market. The powdered material (190 gm) was extracted with ethanol using Soxhlet apparatus. Filtrate was obtained and evaporated to get solid ethanolic extract (Yield: 21.05%w/w).

***Preliminary Phytochemical Screening*:** Phytochemical screening of ethanolic extract of *S. cumini* (ESC) for presence of various phytoconstituents like carbohydrate, protein, alkaloids, flavonoids, phenolic content, glycosides, tannins, steroids was done using standard procedure(2,4).

Determination of I*n Vitro* Antioxidant Activity

Free Radical Scavenging Activity:

***DPPH Method*:** The ability to scavenge free radicals was tested against a stable free radical of DPPH (1, 1-diphenyl-2-picryl hydrazyl). Antioxidants react with DPPH to form 1,1-diphenyl-2-picryl-hydrazine (non-radical). The amount of discoloration reflects how effective the drug is at scavenging free radicals. ESC extract of varying concentration was used. Absorbance of solutions was recorded at 517nm. [3] Percentage inhibition was calculated. % inhibition versus concentration plot was used for determination of IC50 values.

***Reducing Power Assay*:** Due to presence of reductants (antioxidants) in the extracts, the Fe3+/Ferric cyanide complex is reduced to ferrous form (Fe2+). Depending on the reduction power of each extract, the yellow tint of the test solution changes to various shades of green and blue. Various concentrations of the plant extracts and Ascorbic acid were used. Increased absorbance of the reaction mixture indicates increase in reducing power (5). Reducing power was measured by measuring absorbance at 700 nm by varying the concentration of the extract and the contact time.

***Total Phenolic Contents*:** The total phenolics content of the plant extracts was determined using spectrophotometric method. A spectrophotometer set to 765 nm was used to determine the absorbance. For each analysis, the samples were produced in triplicate and the mean absorbance value was calculated. The calibration curve was generated using the same process for the standard gallic acid solution. The concentration of phenolics (mg/ml) was retrieved from the calibration curve based on the measured absorbance. In terms of gallic acid equivalent, the equivalent content of phenolics in extracts was calculated (mg of GA/g of extract) (6).]

***Total Flavonoids Contents*:** Estimation of Total flavonoid content in ESC seeds using spectrophotometer at 415nm is dependent on the development of a flavonoid-aluminium complex. Rutin was used as a reference compound. Calibration curve was prepared for rutin using same procedure as for sample. Calibration curve was used for measuring concentration of flavonoids (mg/ml). Flavonoid content in extracts was expressed in terms of rutin equivalent (mg of rutin/g of extract)(7).

***Experimental Induction of Hyperglycemia in Wistar Rats*:** Alloxan is the most well-known chemical substance utilized in diabetogenic research and type 1 diabetes induction. Alloxan is a urea derivative that promotes necrosis of pancreatic islet cells specifically(6). Alloxan monohydrate 120mg/kg will be administered by intraperitoneal route, blood was collected from tail

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

vein and glucose level was estimate by Glucometer. Rats with established hyperglycemia (blood glucose >300 mg/dl) were included for subsequent treatment.

Experimental

Animals were divided into 6 groups (n=5). Group I: Distilled water (10 ml/kg, p.o.). Group II: Alloxan Monohydrate (120 ml/kg, i.p.). Group III: Alloxan (120 ml/kg, i.p.) with Rosiglitazone (10 ml/kg, p.o.) for 21 days. Group IV & V: ESC 100 mg/kg & 300 mg/kg respectively with Rosiglitazone (10 ml/kg) for 21 days. Group VI: Silymarin (60 mg/kg) with Rosiglitazone (10 ml/kg) for 21 days.

ESC, Rosiglitazone and Silymarin were administered through oral route.

***% Change in Body Weight, Relative Liver and Relative Heart Weight*:** Each animal's body weight was measured before treatment and sacrifice. Each animal's liver and heart samples were dissected and weighed.

***Preparation of Serum and Tissue Homogenate*:** After the 21-day therapy, the animals were sacrificed 24 hours later. Cardiac puncture was used to get blood samples. Centrifugation at 3000 rpm for 10 minutes separated the serum. The serum samples were kept at -20 °C to be used for liver and kidney function tests. For the determination of SOD, CAT, GSH, and LPO activity, a known amount of tissue (liver and heart) was weighed and homogenised in ice cold 0.1 M Tris-HCl buffer.

Determination of In Vivo Antioxidant Activity

Estimation of Superoxide Dismutase Activity (SOD): The ability of SOD to suppress the spontaneous oxidation of adrenaline to adrenochrome was measured compared to a reagent blank as the change in optical density every minute at 480 nm. The results were represented as units of SOD activity *per mg of wet tissue (8).*

Estimation of Catalase Activity (CAT): The assay of CAT is based on ability of CAT to initiate break down of hydrogen peroxide.

The absorbance was recorded at 240 nm every 10 seconds for 1minute. The results were represented as units of CAT activity per mg of wet tissue (9).

Estimation of Reduced Glutathione Activity (GSH): *It is based on the principle of* development of yellow colour when 5, 5' dithiobis (2-nitro-benzoic acid) (DTNB) is added to compound containing sulfhydryl groups. Absorbance was measured at 412 nm (nM/mg of wet tissue) (10).

Estimation of Lipid Peroxidative Indices (LPO): Plants and animals both experience lipid peroxidation, which is a complex process. Formation of thiobarbituric acid reactive substances (TBARS) was measured against reference blank at 535nm (nM/mg of wet tissue) (11).

Biochemical Assays

***Liver Function Tests Assessment*:**

***Aspartate Aminotransferase (AST or SGOT)*:** The amino group transfer between L-Aspartate and Ketoglutarate is catalysed by AST, resulting in Oxaloacetate and Glutamate. In the presence of Malate Dehydrogenase, the Oxaloacetate produced reacts with NADH to create NAD. Mean absorbance change per minute (∆A/ min.) was calculated (12).

Alanine Transaminase (ALT): L-Alanine and Ketoglutarate are transaminated by ALT to generate pyruvate and L-Glutamate. Lactate Dehydrogenase (LDH) then converts Pyruvate to Lactate while simultaneously oxidising NADH to NAD^+. At a wavelength of 340 nm, absorbance was measured after 60 seconds. Every 30 seconds, the reading was repeated. The average change in absorbance per minute (∆A/minute) was calculated (13,14,15).

Alkaline Phosphatase (ALP): Hydrolysis of colourless ρ-Nitro phenyl Phosphate to yellow coloured ρ-Nitrophenol and Phosphate occurs in presence of ALP at pH 10.3. Absorbance was recorded after every 30 seconds at 405 nm. The average change in absorbance per minute (∆A/minute) was determined (16).

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

***Bilirubin*:** Total bilirubin couples with diazotised sulphanillic acid in the presence of TBA to form pink coloured azobilirubin complex of both direct and indirect bilirubin and in the absence of TBA, only direct billirubin reacts with diazotised sulphanillic acid to form azobillirubin complex. The intensity of the colour formed is directly proportional to the bilirubin present in the sample**.** Absorbance was measured at 546 nm and 620 nm respectively(17).

Assessment of Cardiac Marker Enzyme

***Creatine Kinase (CK)-MB*:** An anti-CK-M antibody in the reagent completely inhibits the CK-M portion of the CK-MM and the CK-MB in the sample. Initial absorbance was measured after 10 minutes & absorbance was repeated after every 1, 2, and 3 minute at 340nm wavelength. Mean absorbance change per minute (ΔA/minute) was calculated (18).

Lactate Dehydrogenase (LDH) Activity: LDH catalyzes the conversion of pyruvate to NAD+ by reducing it with NADH. The rate of oxidation of NADH to NAD+ is assessed as a decline in absorbance proportional to the sample's LDH activity. Initial absorbance was read after 10 minutes and repeated after every 1, 2, and 3 minute at 340 nm. Average change in absorbance per minute was calculated (ΔA/minute) (19).

***Histopathological Examination*:** The liver and heart tissues were immediately removed after scarification of animals and preserved in 10% formalin solution before being sent for histopathological testing. These tissues were embedded in paraffin wax, cut into tiny thin slices of 3-5 µm thickness, stained with haematoxylin-eosin, and photographed under 40X magnification to observe for histological abnormalities.

***Statistical Analysis*:** The data was presented as a mean ± SEM. One-way ANOVA was used in the statistical analysis, followed by Dunnett's multiple comparison tests. Statistical significance was defined as p0.05.

Results

Phytochemical analysis of Ethanolic extract of *Syzygium cumini* seed (ESC) revealed presence of alkaloids, flavonoids, tannins and phenolic compounds in Figs 1-14.

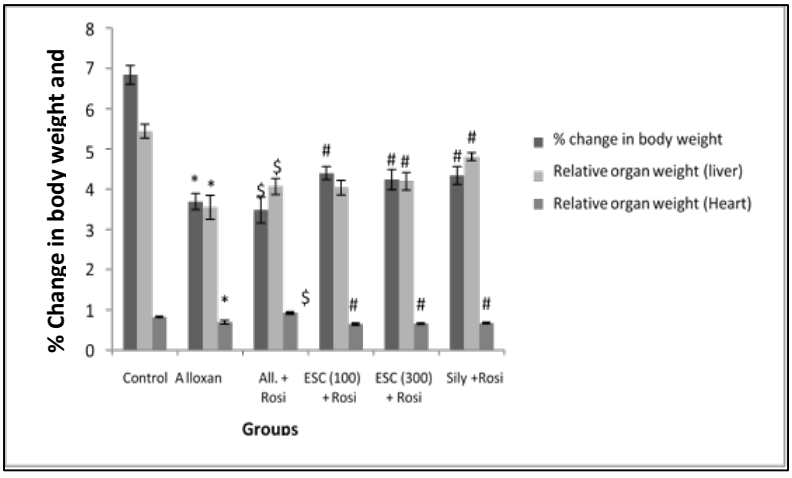

Fig 1. Effect of ethanolic extract of S*. Cumini* and RSG on percent body weight, relative organ weight (Liver and Heart) of rats. N=5, $^{*\$\#}$p<0.05 as compared to RSG treated group

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

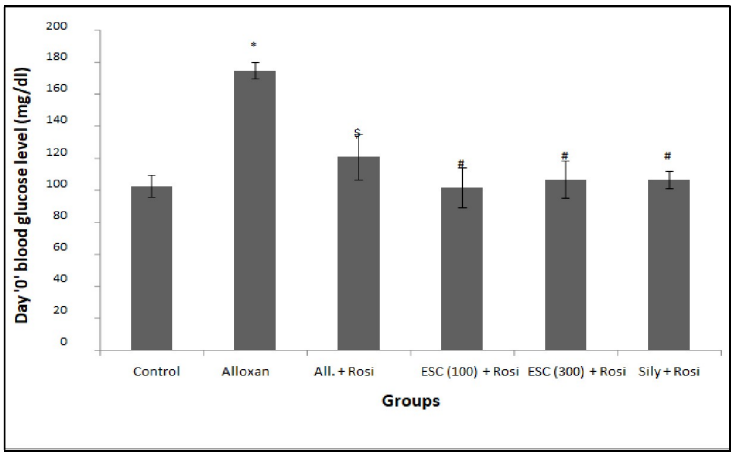

Fig 2. Effect of ethanolic extract of *S. Cumini* and RSG, alloxan on blood glucose level of rats

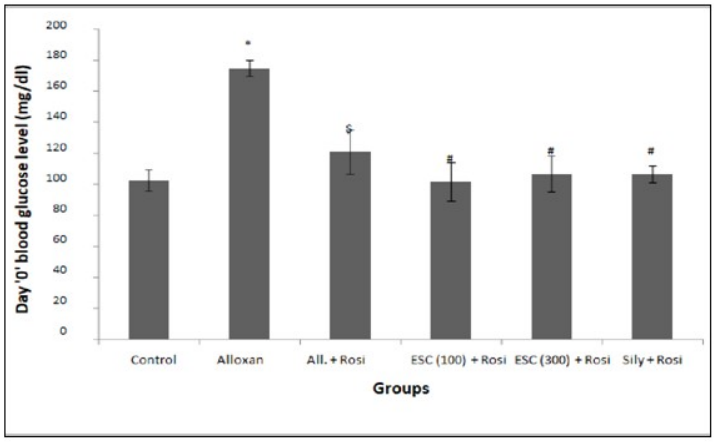

Fig 3. Effect of ethanolic extract of *S. Cumini* and RSG, alloxan on blood glucose level of rats

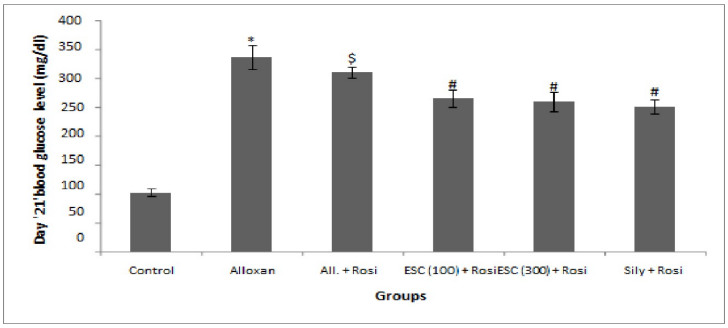

Fig 4. Effect of ethanolic extract of *S. Cumini* and RSG, alloxan on blood glucose level of rats

Cardiotoxicity and Hepatotoxicity

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

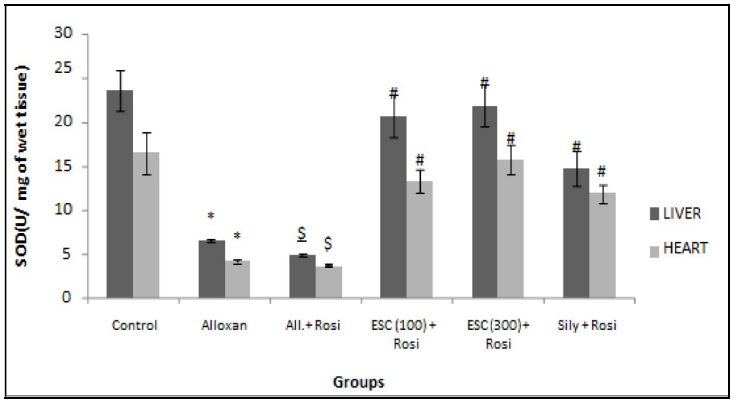

Fig 5. Effect of ethanolic extract of *S. Cumini* and RSG, alloxan on SOD level of rats

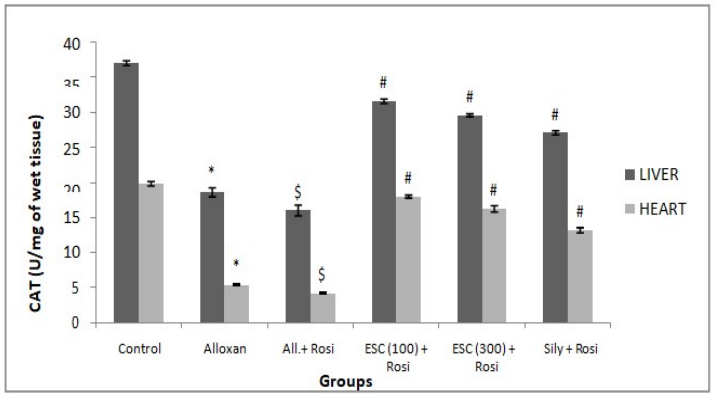

Fig 6. Effect of ethanolic extract of *S. Cumini* and RSG, alloxan on CAT level of rats

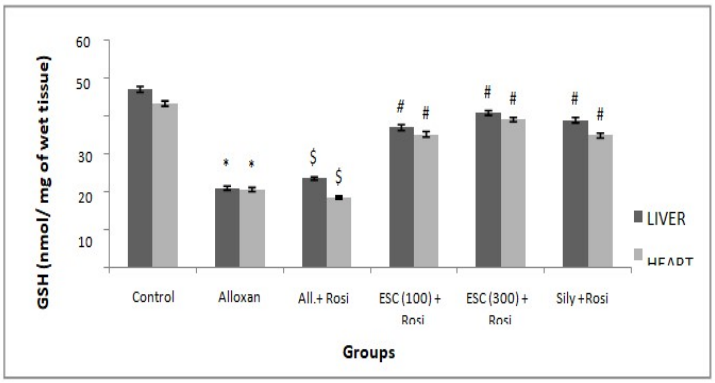

Fig 7. Effect of ethanolic extract of *S. Cumini* and RSG, alloxan on GSH level of rats

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

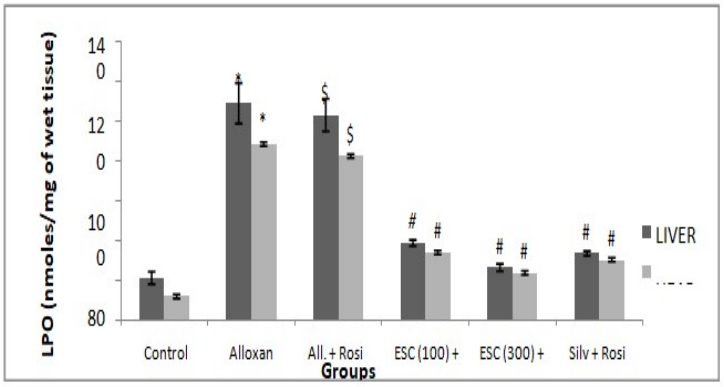

Fig 8. Effect of ethanolic extract of *Syzygium Cumini* and RSG, alloxan on LPO level of rats

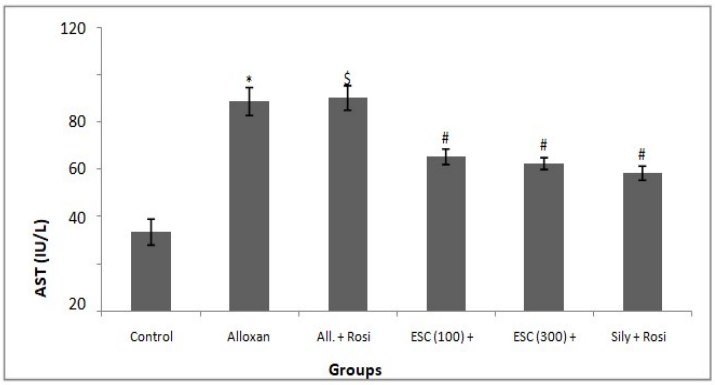

Fig 9. Effect of ethanolic extract of *Syzygium Cumini* and RSG, alloxan on AST level of rats

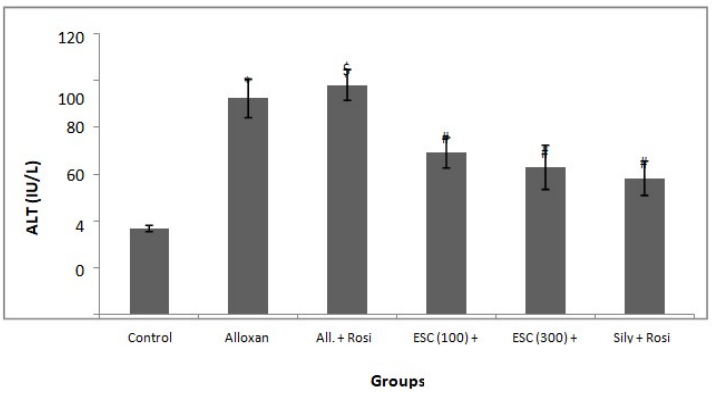

Fig 10. Effect of ethanolic extract of *Syzygium Cumini* and RSG, alloxan on ALT level of rats

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

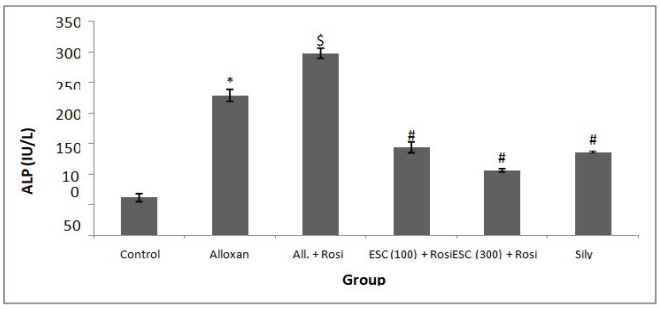

Fig 11. Effect of ethanolic extract of *Syzygium Cumini* and RSG, alloxan on ALP level of rats

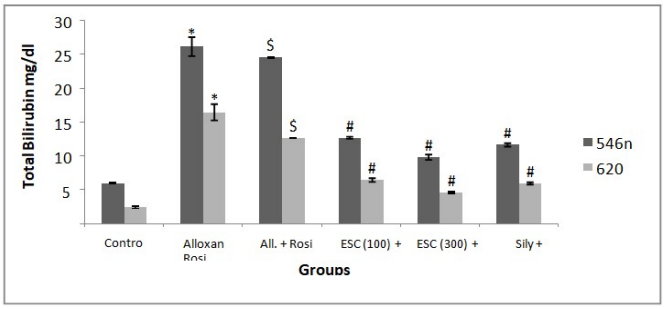

Fig 12: Effect of ethanolic extract of *Syzygium Cumini* and RSG, alloxan on bilirubin level of rats

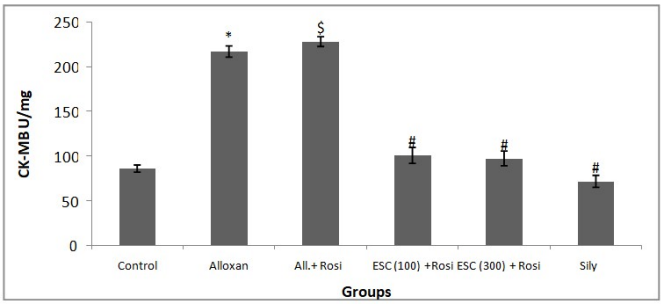

Fig 13. Effect of ethanolic extract of *Syzygium Cumini* and RSG, alloxan on CK- MB level of rats

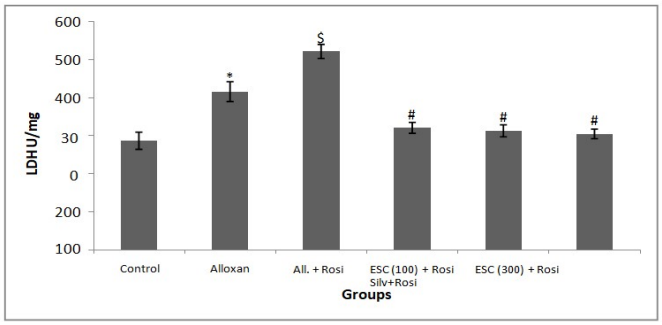

Fig 14. Effect of ethanolic extract of *S. Cumini* and RSG, alloxan on LDH level of rats

Cardiotoxicity and Hepatotoxicity

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

In Vitro Antioxidant Activity

Free Radical Scavenging Activity: The % Scavenging activity increased with the increase in concentration of the ESC. *S. cumini* extract shows good inhibition of DPPH radical and IC_{50} value was found to be 160µg/ml.

***Reducing Power Assay*:** The % Scavenging activity increased with the increase in concentration of the ESC. Ethanolic extract of *S. cumini* shows good reducing power as compared to Vit-C and IC_{50} value was found to be 300 µg/ml.

***Total Flavonoid Content*:** Total flavonoid content in *S. cumini* was found to be 48µg of rutin equiv/mg extract.

***Total Phenolics Content*:** Total phenolic content in *S. cumini* was found to be 125µg of Gallic acid equiv/mg extract.

Percent Body Weight, Relative Organ Weight (Liver and Heart)

Significant reduction in body weight was observed in Alloxan treated group as compared to normal group, while treatment group of *S. Cumini* and Silymarin shows significantly increased body weight as compared to RSG treated group. In RSG treated rats, significant increase in relative liver weight was observed as compared to Alloxan treated group, *S. Cumini* and Silymarin treated group shows significant increase in relative weight of liver as compared to RSG treated group. In RSG treated rats, significantly increase in relative heart weight was observed as compared to Alloxan treated group. *S. Cumini* and Silymarin shows significant decreased relative weight of heart as compared to RSG treated group.

Blood Glucose Level

Blood Glucose Level on Day '0': Blood glucose level on day '0' was significantly decreased in RSG treated rats as compared to Alloxan treated group. *S. Cumini* and Silymarin treatments shows significantly decreased body weight as compared to RSG treated group.

Blood Glucose Level on Day '3': Blood glucose level on day '3' was significant decreased in RSG treated rats as compared to Alloxan treated group. *S. Cumini* and Silymarin treatments shows significantly decreased blood glucose level as compared to RSG treated group.

Blood Glucose Level on Day '21': Blood glucose level on day '21' was significant decreased in RSG treated rats as compared to Alloxan treated group, *S. Cumini* and Silymarin treatments shows significantly decreased blood glucose level as compared to RSG treated group.

Antioxidant Studies

Effect of ESC on Superoxide Dismutase (SOD) Level in Rosiglitazone Induced Cardiotoxicity and Hepatotoxicity in Rats: Significant decreased SOD level in liver was observed in RSG treated rats as compared to Alloxan treated group.

Treatment of *S. Cumini* and Silymarin treated significant increase level of SOD in liver as compared to RSG treated group. Significant decreased SOD level in heart was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. Cumini* and Silymarin significant increase level of SOD in heart as compared to RSG treated group.

Effect of ESC on Catalase (CAT) Level in Rosiglitazone Induced Cardiotoxicity and Hepatotoxicity in Rats: Significant decreased CAT level in liver was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. Cumini* and Silymarin treated significant increase level of CAT in liver as compared to RSG treated group. Significant decreased CAT level in heart was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. Cumini* and Silymarin significant increase level of CAT in heart as compared to RSG treated group.

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

***Effect of ESC on Reduced Glutathione (GSH) Level in Rosiglitazone Induced Cardiotoxicity and Hepatotoxicity in Rats*:** Significant decreased GSH level in liver was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. Cumini* and Silymarin treated significant increase level of GSH in liver as compared to RSG treated group. Significant decreased GSH level in heart was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. Cumini* and Silymarin significant increase level of GSH in heart as compared to RSG treated group.

***Effect of ESC on Lipid Peroxidation (LPO) Level in Rosiglitazone Induced Cardiotoxicity and Hepatotoxicity in Rats*:** Significantly increased LPO level in liver was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. Cumini* and Silymarin treated significant decreased level of LPO in liver as compared to RSG treated group. Significantly increased LPO level in heart was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. Cumini* and Silymarin treated significant decreased level of LPO in heart as compared to RSG treated group.

Biochemical Assays

Effect of ESC on AST Level

Significant increase in AST level was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. cumini* significant decrease in AST level was observed as compared to RSG treated group. Silymarin treatment shows AST level was reduced as compared to RSG treated group

Effect of ESC on ALT Level

Significant increase in ALT level was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. cumini* significant decrease in ALT level was observed as compared to RSG treated group. Silymarin treatment shows ALT level was reduced as compared to RSG treated group.

Effect of ESC on ALP level

Significant increase in ALP level was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. cumini* significant decrease in ALP level was observed as compared to RSG treated group. Silymarin treatment shows ALP level was reduced as compared to RSG treated group.

Effect of ESC on Bilirubin Level

Significant increase in Bilirubin activity was observed in Alloxan treated rats as compared to RSG treated group. Treatment of *S. cumini* in significant decrease in Bilirubin activity was observed as compared to RSG treated group. Silymarin treatment show reduction in Bilirubin level was reduced as compared to RSG treated group.

Estimation of Cardiac Marker Enzyme

Effect of ESC on CK-MB Level

Significant increase in CK-MB level was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. cumini* significant decrease in CK-MB level was observed as compared to RSG treated group. Silymarin treatment shows reduction in CK-MB level compared to RSG treated group.

Effect of ESC on LDH Level

Significant increase in LDH level was observed in RSG treated rats as compared to Alloxan treated group. Treatment of *S. cumini* significant decrease in LDH level was observed as compared to RSG treated group. Silymarin treatment shows LDH level was reduced as compared to RSG treated group.

Histopathological Examination

Histopathological study of revealed disturbance in the normal liver and heart architecture due to hepatotoxin and cardiotoxin in Rosiglitazone treated animal, whereas animals treated with the ESC showed retention of the normal cellular architecture and it is comparable with the standard Silymarin group, hence confirming the significant Hepatoprotective and Cardioprotective effect of ethanolic extract of *S. cumini* seed in Figs 15 and 16.

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

Histopathological Examination of Liver

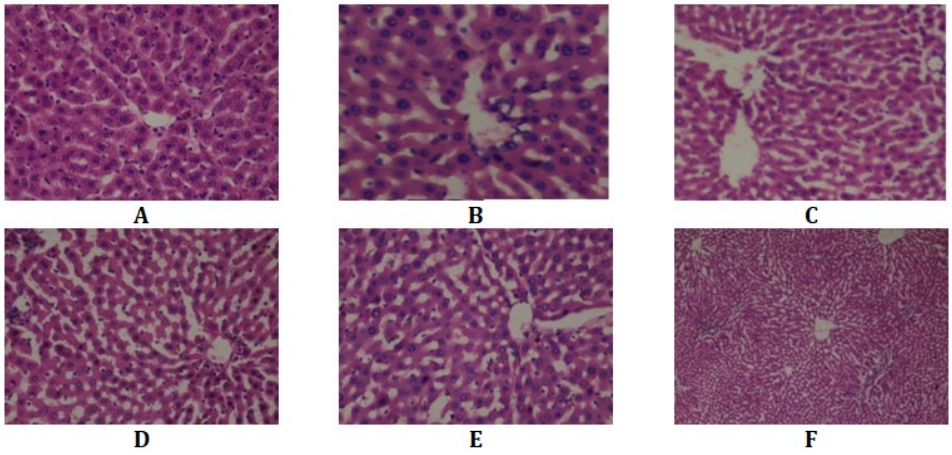

Fig 15. Histopathological studies of H & E stained section of liver showing 'A' vehicle treated animal with normal liver architecture with normal appearance of central vein, kuffer cell, and hepatocytes. RSG treated rat liver 'C' with mild fatty changes, focal necrosis and portal inflammation. ESC treated rat liver 'D' and 'E' with reversal of portal inflammation and destruction of central vein. Silymarin treated rat liver 'F' with normal liver architecture, central vein, and portal inflammation hepatocyte appearing normal

Histopathological Examination of Heart

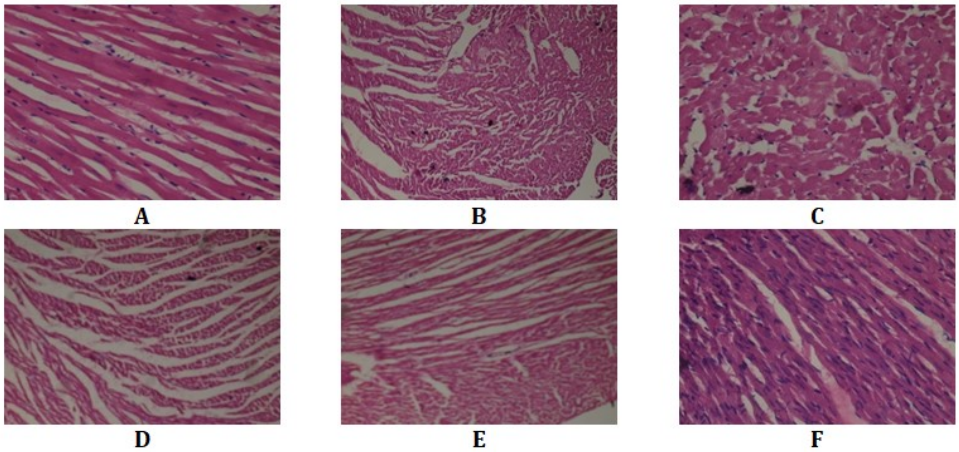

Fig 16. Histopathological studies of H & E stained section of heart showing 'A' vehicle treated animal with normal architecture of heart and to normal arrangement of the layer of myocardium, having a normal cellularity, congestion, fatty changes, atrophy and smaller nuclei. In RSG treated rat heart 'C' with myocardial inflammation, hypertrophy, and lipid accumulation and apoptosis. ESC treated rat heart 'D' and 'E' with reversal of lipid accumulation apoptosis, hypertrophy and myocardial inflammation. Silymarin treated rat heart 'F' with normal heart architecture, normal arrangement of the layer of myocardium, fatty changes and atrophy appearing normal

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

Discussion

The product of metabolic and physiological processes is reactive oxygen species (ROS). Environmental stress causes oxidative stress by disrupting the balance between free radical generation and antioxidant capabilities, resulting in oxidative stress due to excess ROS, antioxidant depletion, or both. When a cell's antioxidant capacity is depleted, damage to cellular macromolecules such as lipids, proteins, and DNA mutations occur, causing harm to target cells and tissues and, in some cases, cell death. This damage has been related to an elevated risk of diseases like diabetes, cardiovascular disease, cancer, and liver disease, among others [20]. Hepatotoxicity and cardiotoxicity has been reported as one of the damages caused by free radicals [21].

Rosiglitazone, an insulin sensitizer, is an antidiabetic compound. The use of RSG in diabetes, on the other hand, has been implicated in the development of cardiovascular disease [22]. In diabetic animals, RSG causes hepatotoxicity and mitochondrial dysfunction. Drugs having antioxidant activity are effective in treating RSG toxicity. RSG induced side effect such as an increased weight and fat gain upon treatment with 10mg/kg per day RSG [23]. The higher dose of ESC (300 mg/kg, p.o.) prevented the increased weight when compared to toxicity treated animals.

The ethanolic extract of *S. cumini* was subjected the presence of alkaloid, saponins, tannins Flavonoids and antioxidant compounds. This extract contains flavonoid and phenolics which have potential to contribute in management of diabetes and its complication. [4] *In vitro* antioxidant activity of seed extract of *S. cumini* was performed. The result of different assay using extract exhibited antioxidant activity and reducing power. Alloxan induced diabetic rats showed a significant reduction in body weight. Insulin deficiency occurs as alloxan affects protein and lipid tissues, glucose does not enter the cells. Instead of glucose, utilization of lipid and protein as energy sources increases and body weight losses due to severe damages in protein of tissues [24].

Toxicity group reflected a significantly increased liver and heart weight compared to other group because RSG leads lipid deposition in liver and heart [25, 26].

Furthermore, in the present study, treatment of Rosiglitazone in alloxan-induced hyperglycaemic rats shows significantly increased blood glucose level on day ‘0’ and increased glucose level on 3rd day compared to day ‘0’, but at the end of experiment on 21st day, the glucose level was significantly increased due to induction of alloxan monohydrate. RSG treated animals showed reduction of glucose level compared to alloxan treated animal. ESC treated animals shown significantly decreased blood glucose level compared to RSG treated animals. The continuous treatment with ESC for 21 days resulted in a considerable reduction in diabetic rats' blood glucose levels.

Alloxan causes diabetes by destroying the insulin-producing beta-islet in the pancreas. Alloxan treatment caused a multiphase glycemic response, with changes in plasma insulin concentration followed by alterations in beta cell ultrastructure, which eventually led to necrotic cell death [27].

Rosiglitazone decreases insulin resistance, an increase in glucose utilization, a decrease in hepatic glucose production, and an increase in β-cell function. The primary peripheral site of action is within adipose tissue, although other target tissues such as liver and skeletal muscle are also affected [28].

The most sensitive enzymatic indexes in liver and cardiac injury produced by ROS and oxidative stress are SOD and CAT. SOD is one of the most abundant internal antioxidant enzymes found in all aerobic cells, and it has an antitoxin impact against ROS. This dismutase superoxide anion produced during metabolism in cell. It reduces the toxicity of superoxide radicals by converting them to hydrogen peroxide [29]. CAT breaks down

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

hydrogen peroxide into water and oxygen. In normal homeostasis, the activity of both enzymes is sufficient to remove ROS [30]. For biological integrity to be maintained, there must be a balance between oxidation and antioxidant levels in the system. Oxidant such as superoxide anion (O^{2-}) hydroxyl radical (HO^{-}) may attack the membranes of the liver and heart cell causing oxidative stress. Results of this study shown significant decrease in anti-oxidants enzyme activity (SOD and CAT) in both liver and heart in RSG treated rats compared to alloxan treated group. Treatment of ESC and Silymarin treated group significantly increases level of SOD and CAT in both heart and liver as compared to RSG treated group. This may suggest that ESC can reduces ROS that may lessen the oxidative damage to the hepatocyte and myocardial tissue and improve the activities of the antioxidant enzyme, thus protecting liver and heart from Rosiglitazone damage.

Through the action of GSH-peroxidase, glutathione (GSH) detoxifies hydrogen peroxide and lipid peroxides. Reduced glutathione levels were found to be significantly lower in RSG-treated rats' liver and heart tissue, indicating an imbalance between oxidant and defence mechanisms, whereas ESC treatment showed a considerable rise in tissue. The detoxifying enzymes may be indicated by ESC, and these enzymes may detoxify the toxicant's ROS delivery [31].

Lipid peroxidation has been suggested as the damaging mechanism in RSG-induced liver and cardiac injury. The levels of LPO in the liver and heart of animals in the toxicity group (RSG) were found to be higher in this investigation. Increased lipid peroxidation causes tissue damage, and antioxidant defence mechanisms fail to prevent the creation of too many free radicals. Lipid peroxidation was greatly reduced after pretreatment with ESC. As a result, it's probable that ESC's hepatoprotective and cardioprotective properties are attributable to its antioxidant capability [32].

To evaluate potential toxicity related with these organs, several hepatic and cardiac marker enzymes are employed. ALT, AST, ALP and serum bilirubin are hepatic marker enzymes, while CK-MB and LDH are cardiac marker enzymes. In addition, the antioxidant status of renal and hepatic tissue was evaluated to establish whether these organs were under any toxic stress.

In acute liver damage induced by RSG, in RSG treated animal was showed increase level of liver enzyme marker as compared to ESC treated animal ESC significantly reduced the elevated serum level of AST, ALT, ALP and total bilirubin as compared to toxicity treated animals. It was observed that ethanolic extract of seed extract of *S. cumini* (100 and 300 mg/kg) showed significant Hepatoprotective effect in diabetic rat as compared to other groups. This may be due to antioxidant property of seed extract; it showed nearly normal level of liver enzymes. Antioxidant neutralize the oxidants generate in the liver. Any changes between level of oxidants and antioxidants cause development of liver damage. Plants having antioxidant effect use to treat diseases due to oxidative stress. In this study showed *S. cumini* have liver protection effect in diabetic rats [33].

Increased level of hepatic enzyme markers indicator of liver injury. The disturbance in the transport function of the hepatocyte as a result of hepatic injury causes the leakage of enzyme from cell due to altered permeability of membrane [34]. A fatty liver is characterized by elevated circulating level of ALT and AST, markers of hepatocellular damage. ALP levels were, however, significantly increased, suggesting induction of liver injury [23].

RSG causes cardiotoxicity effect, cardiac biomarkers such as CK-MB and LDH has been used as indicator for cardiac disorder. Serum LDH and CK-MB activity have previously been demonstrated to be elevated in cardiomyopathy in diabetic patients, suggesting that they could be used as a marker for cardiovascular risk and cardiac muscular injury [35]. In our study, serum LDH

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

and CK-MB activities were found to be increased in diabetic rats, possibly due to myocardial dysfunction. Furthermore, a significant increase in serum LDH and CK-MB level was observed with RSG treatment, which was more marked in diabetic rats than normal rats, indicating that Rosiglitazone has a cardiotoxic effect.

RSG can induce myocardial infarction, cardiomyopathy in diabetes as shown by increasing cardiac marker. These effects may be associated to alterations of cardiac ion channels and other processes rather than oxidative stress [22]. Medicinal herbs have long been considered as a source of novel cardioprotective chemicals. Methanolic extract of S. cumini seed has efficiently protected the myocardium against isoprotrenol-induced myocardial infarction. [36]

Histopathological study of liver and heart revealed disturbance in the normal architecture of liver and heart due to toxicity in RSG treated animals, whereas animals treated with the ESC showed retention of the normal cellular architecture and it is comparable with the standard Silymarin group, hence confirming the significant hepatoprotective and Cardioprotective effect of ethanolic extract of *S. cumini* seeds. Aqueous seed extract of *S. cumini* (500 mg/kg) showed significant hepatoprotective effect in diabetic rat's compared to other group. This may due to antioxidant property of seed extract. Antioxidant neutralize the oxidant generated in the liver. Plant having antioxidant effect use to treat disease due to oxidative stress [33]. Hepatotoxicity caused by rosiglitazone can be reversed by co-administering Silymarin, a proven hepatoprotective drug, without affecting its hypoglycemic potential [34]. Methanolic extract of *S. cumini* seeds exhibited cardioprotective effect on isoprotrenol-induced myocardial infarction of rat [36].

Silymarin administration causes the organ's morphological structure to resemble its physiological appearance. Cardiomyocytes are cylindrical and regular, with no evidence of degeneration or necrosis observable. Silymarin has been shown to protect heart and liver tissue against doxorubicin-induced damage [37]. Study reflects good antidiabetic, cardioprotective and hepatoprotective activity of ethanolic extract of *S. cumini* seed at 100 mg/kg and 300 mg/kg orally and effect produced by the higher dose of ESC was similar to that produced by Silymarin, having hepatoprotective cardioprotective activity.

Conclusion

Our finding highlights the efficacy of ESC as protective effect against RSG induced oxidative damage to the myocardial and hepatic cell which induced toxicity. RSG induction leads to reduction in level of enzymic and non-enzymic antioxidant. However, the treatment of ESC normalised the level of all biochemical and antioxidant parameter. It can be concluded that ethanolic seed extract of *S. cumini* provide a protective and antidiabetic effect in RSG induced cardiotoxicity and hepatotoxicity in diabetic Wistar rats.

References

1. Jadhav, V. M., Kamble, S. S., & Kadam, V. J. (2009). Herbal medicine: Syzygium cumini: a review. Journal of Pharmacy Research, 2(8), 1212-1219.

2. Shrikant Baslingappa, S., Nayan Singh J, T., Meghatai M, P., & Parag M, H. (2012). Jamun (*Syzygium cumini* (L.)): a review of its food and medicinal uses. Food and Nutrition Sciences.

3. Nair LK, Begum M, Geetha S. (2013). In vitro-antioxidant activity of the seed and leaf extracts of Syzygium cumini. J Envir Sci Toxicol Food Technol. 7(1):54-62.

4. Kamtekar, S., Keer, V., & Patil, V. (2014). Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. Journal of Applied Pharmaceutical Science, *4*(9), 61.

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

5. Waghulde, H., Kamble, S., Patankar, P., Jaiswal, B., Pattanayak, S., Bhagat, C., & Mohan, M. (2011). Antioxidant activity, phenol and flavonoid contents of seeds of Punica granatum (Punicaceae) and *Solanum torvum* (Solanaceae). Pharmacologyonline, 1, 193-202.

6. Tripathi, V., & Verma, J. (2014). Different models used to induce diabetes: a comprehensive review. International Journal of Pharmacy and Pharmaceutical Sciences, 6(6), 29-32., 2014; 6:30-32.

7. Raza, A., Saif-ul-Malook, N. S., Qasrani, S. A., Sharif, M. N., Akram, M. N., & Ali, M. U. (2015). Extraction of bioactive components from the fruit and seed of jamun (Syzygium cumini) through conventional solvent extraction method. American-Eurasian Journal of Agricultural and Environmental Sciences, 15(6), 991-996.

8. Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry, 47(3), 469-474.

9. Beers, R. F., and Sizer, I. W. (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem., 195, 133-140.

10. Ellman, G. L. (1959). Determination of sulfhydryl group. *Arch. Biochem. Biophys*, *82*(1), 70-77.

11. Latha S., Vijaykumar R., Shrikumar R. (2016) In vivo anti oxidative effect of Polyherbal formulation of flax seed, fenugreek and jamun seed on streptozotocin Nicotinamide Induced Diabetic Rats. International Journal of Pharma and Bio Sciences; 7(4):607-611.

12. Clin J., (1986) IFCC method for the measurment of catalytic concentration of enzymes. J. Clin Chem Clin Biochem. 24:497

13. Schumann, G., Bonora, R., Ceriotti, F., Clerc-Renaud, P., Ferrero, C.A., Férard, G., Franck, P.F., Gella, F.J., Hoelzel, W., Jørgensen, P.J. and Kanno, T., 2002. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 C. Part 2. Reference procedure for the measurement of catalytic concentration of creatine kinase. 635-642.

14. Young D., (1997) In Effect of Preanalytical Variables on Clinical Laboratory Tests 2nd. AACCpress, Washington;4-489.

15. Serap T., Burcu T., Ibrhim G., Tulay G., Zeynep, Anjumana O., Teoman A (2011). Serum Alkalime Phosphatase Levels in Healthy Children and Evaluations of Alkaline Phosphatase z-scores in Different Types of Rickets, Journal Clinical Research Pediatric Endocrinology; 3(1): 7–11

16. Johnsan A.M., Rohlfs E.M., Silverman L.M. Protein, (1999) In TIETZ Textbook of clinical chemistry, Burtis C.A., AND Ashwood E.R.,Eds. W.B.Saunders, Philadelphia, 3: 477-540

17. Billing, B., Haslam, R., & Wald, N. (1971). Bilirubin Standards and the Determination of Bilirubin by Manual and Technicon AutoAnalyzer Methods: Prepared for the Scientific and Technical Committee of the Association of Clinical Biochemists. Annals of Clinical Biochemistry, 8(1-6), 21-30.

18. Panteghini, M., Falsetti, F., Chiari, E., & Malchiodi, A. (1983). Determination of aspartate aminotransferase isoenzymes in hepatic diseases—preliminary findings. Clinica Chimica Acta, 128(1), 133-140.

19. Green, R. M., & Flamm, S. (2002). AGA technical review on the evaluation of liver chemistry tests. Gastroenterology, 123(4), 1367-1384.

20. Atale, N., & Rani, V. (2016). Syzygium cumini: an effective cardioprotective via its antiglycoxidation potential. International Journal of Pharmaceutical Science Review and Research, *37*(1), 42-51.

21. Maheswari, R., & Manohari, S. (2015). Syzygium cumini (L.) seeds extract ameliorates

Vol. 16 (Supplementy Issue 2) 139 - 154, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.72

cisplatin-induced hepatotoxicity in male Wistar rats. International Journal of Pharma Sciences and Research, *6*(2), 444-50.

22. Manar AN., Dina SL., Hamdy AG., Mohammed SE. (2015) Role of oxidative stress in cardiac hypertrophy induced by Rosiglitazone in diabetic rats. International Journal of Pharmaceutical Research and Bio-science;4(1):238-250.

23. Hemmeryckx, B., Gaekens, M., Gallacher, D. J., Lu, H. R., & Lijnen, H. R. (2013). Effect of rosiglitazone on liver structure and function in genetically diabetic Akita mice. Basic & clinical pharmacology & toxicology, 113(5), 353-360.

24. Winarsi, H., Sasongko, N. D., Purwanto, A., & Nuraeni, I. (2014). Effect of cardamom leaves extract as antidiabetic, weight lost and hypocholesterolemic to alloxan-induced Sprague Dawley diabetic rats. International Food Research Journal, 21(6), 2253.

25. Hemmeryckx, B., Hoylaerts, M. F., Gallacher, D. J., Lu, H. R., Himmelreich, U., D'hooge, J., ... & Lijnen, H. R. (2013). Does rosiglitazone affect adiposity and cardiac function in genetic diabetic mice? European journal of pharmacology, 700(1-3), 23-31.

26. Wu, L., Wang, R., Champlain, J. D., & Wilson, T. W. (2004). Beneficial and deleterious effects of rosiglitazone on hypertension development in spontaneously hypertensive rats. *American journal of hypertension*, *17*(9), 749-756.

27. Rohilla, A., & Ali, S. (2012). Alloxan induced diabetes: mechanisms and effects. *International journal of research in pharmaceutical and biomedical sciences*, *3*(2), 819-823.

28. Papoushek, C. (2003). The "Glitazones": rosiglitazone and pioglitazone. Journal of Obstetrics and Gynaecology Canada, *25*(10), 853-857.

29. Scandalios, J. G. (1993). Oxygen stress and superoxide dismutases. Plant physiology, 101(1), 7.

30. Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease. Journal of clinical pathology, 54(3), 176-186.

31. Jorg BS., Jorg L., Jan S., Johannes D., (2000) Glutathione, oxidative stress and neurodegeneration. European Journal of Biochemistry. 267:4904- 4911.

32. Thiffault, C., Aumont, N., Quirion, R., & Poirier, J. (1995). Effect of MPTP and L-deprenyl on antioxidant enzymes and lipid peroxidation levels in mouse brain. Journal of neurochemistry, 65(6), 2725-2733.

33. Behera, S. R., Sekkizhar, M., & Sarath Babu, K. (2014). Hepatoprotactive activity of aqueous extract of Syzygium cumini seed on streptozotocin induced diabetes in rats. International journal of ayurvedic and herbal medicine, 4(2), 1470-1477.

34. Swamy, S., Krishna, K. L., & Nidavani, R. B. (2013). Reversal of rosiglitazone hepatotoxicity by silymarin on rats. International Journal of Pharmaceutical Sciences and Research, 4(6), 2301.

35. Huang, E.J., Kuo, W.W., Chen, Y.J., Chen, T.H., Chang, M.H., Lu, M.C., Tzang, B.S., Hsu, H.H., Huang, C.Y. and Lee, S.D. (2006). Homocysteine and other biochemical parameters in type 2 diabetes mellitus with different diabetic duration or diabetic retinopathy. Clinica Chimica Acta, *366*(1-2), 293-298.

36. Mastan, S. K., Chaitanya, G., Latha, T. B., Srikanth, A., Sumalatha, G., & Kumar, K. E. (2009). Cardioprotective effect of methanolic extract of Syzygium cumini seeds on isoproterenol-induced myocardial infarction in rats. Der Pharmacia Lettre, *1*(1), 143-149.

37. Rašković, A., Stilinović, N., Kolarović, J., Vasović, V., Vukmirović, S., & Mikov, M. (2011). The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. Molecules, *16*(10), 8601-8613.