Syzygium Cumini (L.) Seeds Extract Ameliorates Cisplatin Induced Hepatotoxicity in Male Wistar Rats

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Abstract

The discovery of cisplatin, *cis*-[Pt(II)(NH(3))(2)Cl(2)] ([$PtCl_2(NH_3)_2$] or CDDP), was a corner stone which triggered the interest in platinum(II)-and other metal-containing compounds as potential anticancer drugs. Cisplatin, is one of the most potent chemotherapy drugs widely used for cancer treatment. In our present study, an attempt has been made to study the effect of Cisplatin on biochemical and histopathological parameters and ameliorating effects of the *Syzygium cumini (L.)* aqueous seeds extract or *Eugena Jambolana* in male wistar rats. Adult male wistar rats were divided into four different groups. Group I Served as vehicle treated normal saline (Control), Group II Rats received single intra-peritoneal (Ip) injection of cisplatin (7mg/kg bw), Group III received *Syzygium cumini (L.)* aqueous seeds extract 400mg/kg/bw orally for 7 days beginning one day prior to cisplatin (CP) injection. Group IV Rats received alone *Syzygium cumini (L.)* aqueous seeds extract (400mg/kg bw) treated. Cisplatin exposure leads to adverse effects on hematological, hepatotoxic parameters including Erythrocytes (RBCs). Cisplatin induction leads to reduction in the levels of Enzymic and Non-Enzymic antioxidants levels. However, on treatment with *Syzygium cumini (L.)* aqueous seeds extract normalized the levels of all the biochemical and hematological parameters. These findings highlight the efficacy of *Syzygium cumini (L.)* aqueous seeds extract as protective effects Cisplatin induced hepatotoxicity.

Key Words: Cisplatin, *Syzygium cumini (L.)* seeds, Hepatotoxicity, Hepatoprotective, Free radicals, Antioxidants.

Introduction

Liver

The largest organ in human body is the Liver and the chief site for intense metabolism and excretion. Weighing in at around 3 pounds, the liver is the body's second largest organ. It has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. These platinum complexes react *in vivo*, binding to and causing cross linking of DNA, which ultimately triggers apoptosis (programmed cell death).

Cisplatin is the first member of a class of platinum containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. Carboplatin and oxiplatin are second- and third generation platinum drugs that have been introduced into clinical use because of their reduced toxicity. cisplatin has been widely used for chemotherapy [1]. It is potent, demonstrating one of the highest cure rates, for example over 90% in testicular cancers [1]. Cisplatin (CP) is an inorganic platinum compound with a broad spectrum anti-neoplastic activity against various types of tumors. It is used in the clinical treatment of several human cancers including those of the head, neck, testis, ovary, breast and bladder. Antitumor action of CP is attributed to its action on DNA molecules. Unfortunately, it has several side effects such as myelosuppression, nephrotoxicity, ototoxicity, neurotoxicity and bone marrow suppression, but its chief dose limiting side effect is cumulative nephrotoxicity. In vivo and in vitro evidences have suggested that enhanced oxidative/nitrosative stresses are involved in CP-induced nephrotoxicity. CP inhibits mitochondrial function and induces DNA damage and glutathione (GSH) depletion which are strongly associated with the renal toxicity of this compound. *Syzygium Cumini L.* (*Eugena Jambolana)* is a large evergreen tree up to 30 m high. Bark pale brown, slightly rough on old stems. Leaves opposite, simple, entire, elliptic to broadly oblong, smooth, glossy somewhat leathery, 7.5-15 cm long, short pointed at tips. Flowers white 7.5-13 mm across in branched clusters at stem tips, calyx cuplike, 4 petals, fused into a cap; many stamens. Fruit variable in size up to2.5 cm long, ellipsoid or oblong, crowned with truncate calyx-limb, black with pink juicy pulp. It is widely distributed throughout India, Ceylon-Malaya and Australia and known as Jamun, Jam, Jambul in India. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. Most of the plant parts of *Eugenia jambolana* are used in traditional system of medicine in India. According to Ayurveda, its bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst biliousness, dysentery and blood impurities

and to cure ulcers. The fruits are acrid and sweet, cooling, dry and astringent to bowels. They increase "Vata" and remove bad smell from the mouth. As per Unani system of medicine they acts as liver tonic, enriches blood, strengthens teeth and gums and forms good lotion for removing ringworm infection of the head.

Powdered seeds are used as a remedy in diabetes [2,3,4,5] and in metrorrhagia. Seed powdered in combination with mango kernels were administered with curd to overcome the problem of diarrohea and dysentery, enlargement of spleen and as diuretic in scanty or suppressed urine and oil of leaves is useful in skin diseases. Seeds of *Eugenia jambolana* contain glycosides, a trace of pale yellow essential oil, fat, resin, albumin, chlorophyll, an alkaloid- jambosine, gallic acid, ellagic acid, corilagin and related tannin, 3,6- hexa hydroxyl diphenoylglucose and its isomer 4,6- hexa hydroxyl diphenoylglucose, 1- galloyl glucose, 3- galloylglucose, quercetin and elements such as zinc, chromium, vanadium, potassium and sodium. Unsaponifiable matter of seed fat contains β-sitoterol. Dry seeds of *Eugenia jambolana* have been reported with 11.67% alcohol soluble extractive, 3.397% inorganic, 40% of water-soluble gummy fiber and 15% of water insoluble neutral detergent fibers.

Free Radicals

The term 'free radical' is used in a broad sense and also includes related reactive species such as 'excited states' that lead to free radical generation or those species that result from free radical reactions. In general, free radicals are very short lived, with half lives in milli-, micro-, or nanoseconds. Free radicals have been implicated in the etiology of several human diseases as well as ageing [6,7].

ROS and RNS are both produced in a well regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. Most cells can produce superoxide (O_2°-), hydrogen peroxide (H_2O_2) and nitric oxide (NO) on demand.

Antioxidants

Antioxidants are substances that neutralize free radicals or their actions [8]. Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radical, superoxide dismutase, (SOD), glutathione peroxidase glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems in every cell. α-Tocopherol (Vitamin E) is an essential nutrient which functions as a chain breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Antioxidants, capable of neutralizing free radicals or their actions, act at different stages. They act at the levels of prevention, interception and repair. Preventive antioxidants attempt to stop the formation of ROS. These include superoxide dismutase (SOD) that catalyses the dismutation of superoxide to H_2O_2 and catalase that breaks it down to water [8,9]. Interception of free radicals is mainly by radical scavenging, while at the secondary level scavenging of peroxyl radicals are effected. The effectors include various antioxidants like Vitamin C, Vitamin E glutathione, other thiol compounds, carotenoids flavonoids, etc., at the repair and reconstitution level, mainly repair enzymes are involved [8,9].

Materials and Methods

Chemicals

All the fine chemicals were purchased from Sigma Chemical Co., USA. Cisplatin (CP) was procured from Dabur Pharma Ltd., New Delhi, India. All other chemicals used were of good quality and analytical grade.

***Eugena Jambolana* Seeds Extract Preparation**

Fresh fruits of *Syzygium cumini (L.)* were collected from the local market. All *Syzygium cumini (L.)* fruits were washed with double distilled water (1/10 w/v). The ripe fruits of *S.cumini* are de-pulped and the obtained seeds are sun dried. These seeds are pulverized and kept in air tight glass container. 20g of seed powder is soaked in 1000ml of deionized water overnight and centrifuged at 4000g for 20min at 4°C the supernatant was collected. Aqueous extract was used because most of the antioxidant components are extracted in water. During the experiment *Syzygium cumini (L.)* aqueous seeds extract was daily prepared and administrated to rats.

Animal Model

Male albino rats of Wistar strain (200±10g) procured from Tamil Nadu University for Veterinary and Animal Sciences, (TANUVAS) Chennai, India were used for the study. Animals were fed with commercially available standard rat pelleted feed (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided ad libitum. The rats were housed under conditions of controlled temperature (25±2°C) and acclimatized to 12-h light, 12-h dark cycle. Animal experiments were conducted according to the guidelines of institutional animal ethical committee.

Experimental Design

Seggregation of Groups

Experimental animals were divided into four groups of six rats each as follows.

Group I : Served as vehicle treated normal saline (Control).

Group II : Rats received single intra-peritoneal (Ip) injection of cisplatin (7 mg/kg bw).

Group III : Rats received Cisplatin (CP) (7 mg/kg bw) as in group II and *Syzygium cumini (L.)* aqueous seeds extract (400 mg/kg bw) orally for 7 days beginning one day prior to cisplatin (CP) injection.

Group IV : Rats received alone *Syzygium cumini (L.)* aqueous seeds extract (400 mg/kg bw).

Collection of Samples for Biochemical Analysis

After the experimental period, the animals were anaesthetized by intra-peritoneal injection of phenobarbital sodium (30mg/kg body weight) and were sacrificed. Blood was collected in sterile tubes. Liver tissues were immediately excised and immersed in ice-cold physiological saline. A section of the liver ventricle was set aside for the microscopic studies.

Serum Separation

The blood samples collected in plain centrifuge tubes were kept in inclined position to allow complete clotting of blood and then centrifuged at 2500 rpm for 10 min. The resultant clear supernatant was pipetted out and preserved in small vials in the freezer for the purpose of biochemical investigations.

Preparation of Tissue Homogenate

The liver tissue was excised, rinsed in ice-cold physiological saline and homogenized in 0.1 M Tris-HCl buffer (pH 7.4) using a tissue homogenizer with a teflon pestle at 4°C. The resultant supernatant was kept under refrigeration until further biochemical analysis. All the assay procedures were carried out with in 48 hr of the sample collection.

Histopathological Study

Haematoxylin and Eosin Staining

A portion of hepatic (liver) tissue was fixed in 10 % formalin. The washed tissue was dehydrated in descending grades of isopropanol and cleared in xylene. The tissue was then embedded in molten paraffin wax. Sections were cut at 5-µm thickness and stained with haematoxylin and eosin (H&E).

Statistical Analysis

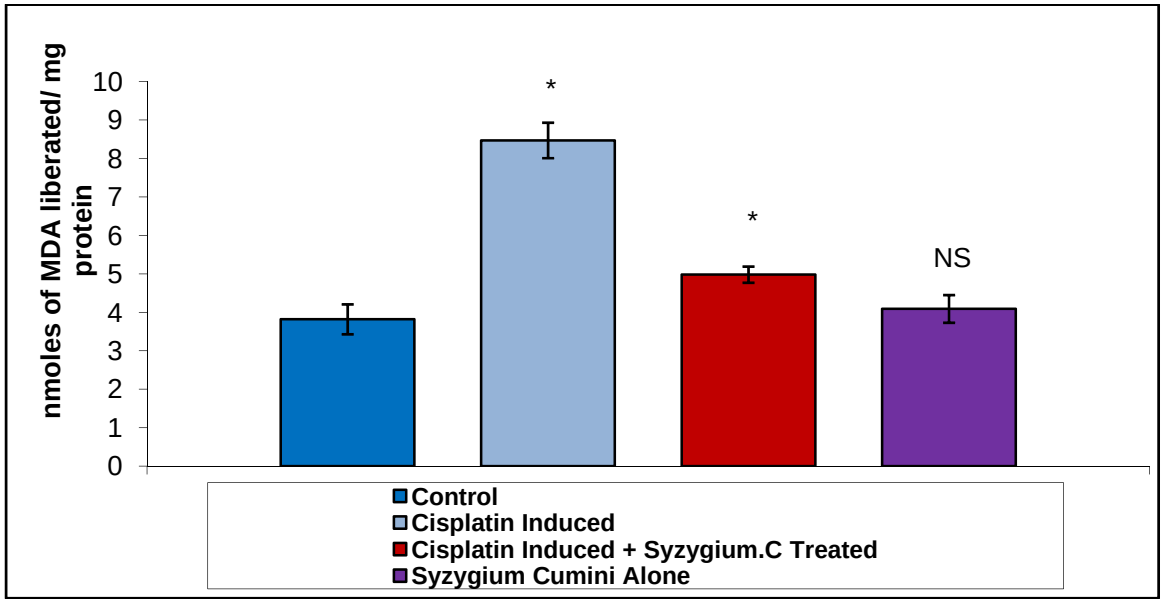
The values are expressed as mean ± SD for six rats in each group. All of the grouped data were analyzed with SPSS/13.0 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD) test. The '*p*' value of less than 0.05, 0.01, were considered to indicate statistical significance.

Results and Discussions

In our present study, an attempt has been made to study the effect of Cisplatin on biochemical and histopathological parameters and ameliorating effects of the *Syzygium cumini (L.)* aqueous seeds extract in male wistar rats. In view of our findings, it is possible to conclude that *Syzygium cumini (L.)* aqueous seeds extract administration results in pronounced oxidative stress and hepatic damage. *Syzygium cumini (L.)* aqueous seeds extract treatment would protect hepato toxicity against cisplatin induced toxicity through decreasing ROS, hydroxyl radicals, lipid peroxidation, preventing protein degradation, DNA fragmentation because *Syzygium cumini (L.)* aqueous seeds extract are instrumental for the bioactive compounds, antioxidant properties, above results may be important mechanisms underlying the protective effects of *Syzygium cumini (L.)* aqueous seeds extract observed in cisplatin hepato toxicity. Thereby, we provide the mechanistic basis of *Syzygium cumini (L.)* aqueous seeds extract clinical application to protect patients from cisplatin-induced hepato toxicity. *Syzygium cumini (L.)* aqueous seeds extract increased the hepato and serum levels of oxidative stress markers such as lipid peroxidation etc., However, *Syzygium cumini (L.)* aqueous seeds extract treatment significantly inhibited the formation of lipid peroxides in cisplatin exposed rats. This could be due to potential antioxidant effect of *Syzygium cumini (L.)* aqueous seeds extract. Treatment with *Syzygium cumini (L.)* aqueous seeds extract significantly decreased the serum and hepatic levels of proteins, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S- transferases, glutathione reductase, AST, ALT, ALP and Vitamin C & E. *Syzygium cumini (L.)* aqueous seeds extract treatment improved the antioxidant status by increasing the activities/levels of these enzymic and non-enzymic antioxidants. Exposure to cisplatin showed significant increase in the activities of hepatic injury marker enzymes and a subsequent decrease in these enzyme activities in liver. Supplementation of *Syzygium cumini (L.)* aqueous seeds extract reverted these hepatic injury markers enzyme activities to near normal. The electrophoretic pattern of proteins by SDS PAGE also showed the protective role of *Syzygium cumini (L.)* aqueous seeds extract. The native gel electrophoretic pattern of SOD and CAT also

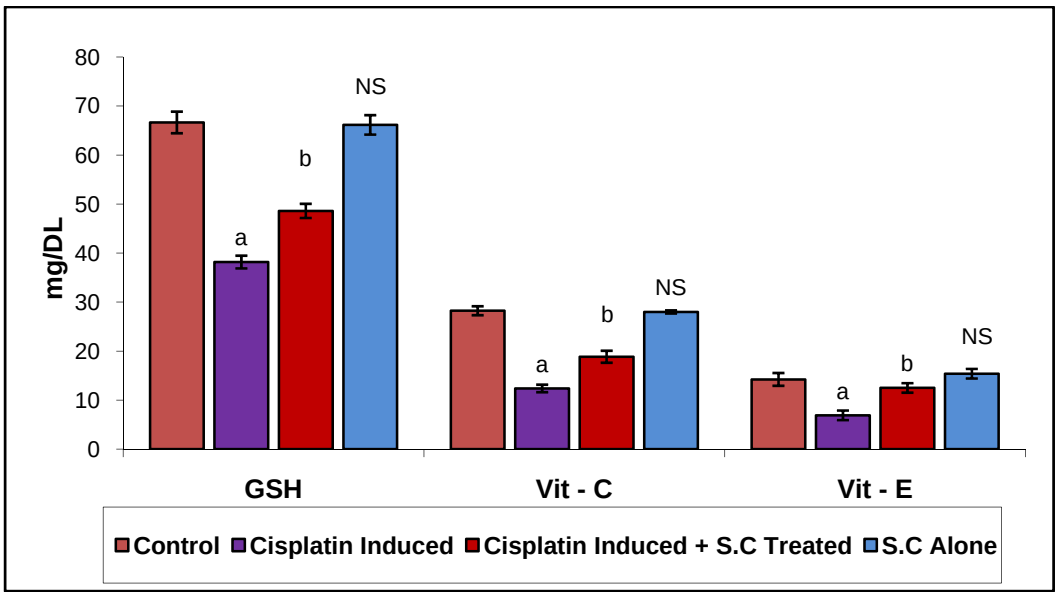
shown the protective efficacy of *Syzygium cumini (L.)* aqueous seeds extract on cisplatin induced hepato toxicity. Along with biochemical and molecular alterations we have also observed histological changes in hepatic tissues. Histopathological studies revealed alterations of the hepatic tissues caused by cisplatin exposure were prevented by *Syzygium cumini (L.)* aqueous seeds extract administration. Epidemiological evidence links high antioxidant status with low risk of degenerative disease including tumor promotion and cancer in humans. The increased consumption of fresh vegetables and fruits is usually associated with the decreased use of fish, meats and fats. Furthermore, supplementation of bioavailable and safe antioxidants are essential because we do not get enough antioxidant vitamins and minerals from foods and beverages we consume daily. These research studies demonstrate *Syzygium cumini (L.)* aqueous seeds extract as a safe, novel, highly potent and bioavailable free radical scavenger and antioxidant possessing a broad spectrum of health benefits. *Syzygium cumini (L.)* aqueous seeds extract functions at the genetic level and promotes therapeutic efficacy. Further mechanistic and clinical studies are in progress to unveil the mechanism of this novel natural antioxidant.

Graph 1. Effect of cisplatin and *Syzygium cumini (L.)* seeds on the activities of lipid peroxidation in the serum of control and experimental group of rats.



Results are expressed as mean ± SD for 6 different sets of experiments. Values are considered significantly different at $P < 0.05$ with post-hoc LSD test.

Graph 2. Effect of cisplatin and *Syzygium cumini (L.)* seeds on the level of GSH, Vitamin C and E in plasma of control and experimental group of rats.



Results are expressed as mean ± SD for 6 different sets of experiments. Values are considered significantly different at P < 0.05 with post-hoc LSD test. Statistically significant variations are compared as follows [a]Cisplatin-induced vs control. [b]Cisplatin- induced + *Syzygium Cumini* treated vs Cisplatin-induced. : [a,b]indicates P < 0.05 and NS indicates non-significant.

Table 1. Effect of cisplatin and *Syzygium cumini (L.)* aqueous seeds extract on the activities of SOD, CAT, GPx, GST and GR in the serum of control and experimental rats.

Particulars	**Control**	**Cisplatin induced**	**Cispatin induced + *Syzygium cumini (L.)* aqueous seeds extract treated**	***Syzygium cumini (L.)* aqueous seeds extract alone**
SOD	3.47±0.07	1.65±0.04	2.96±0.05	3.58±0.03
CAT	155.63±6.77	96.65±3.54	124.63±4.534	159.35±5.85
GPx	4.67±0.24	2.74±0.36	3.64±0.35	4.26±0.63
GST	0.98±0.012	0.39±0.014	0.74±0.02	0.97±0.019
GR	1.75±0.06	1.15±0.03	1.54±0.04	1.78±0.06

Results are expressed as mean ± SD for 6 different sets of experiments. Values are considered significantly different at P < 0.05 with post-hoc LSD test.

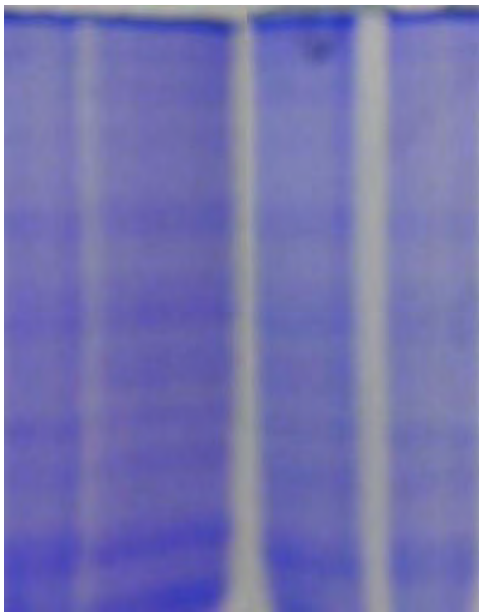
Table 2. Effect of cisplatin and *Syzygium cumini (L.)* aqueous seeds extract on the hepatic marker enzymes in control and experimental group of rats.

Particulars	**Control**	**Cisplatin induced**	**Cisplatin induced + *Syzygium cumini (L.)* aqueous seeds extract treated**	***Syzygium cumini (L.)* aqueous seeds extract alone**
ALP	4.63±0.12	9.36±0.08	3.43±0.09	4.35±0.42
SGOT	167.83±6.86	278.34±8.65	196.65±3.86	173.63±6.54
SGPT	88.75±4.63	156.65±6.55	108.45±3.64	90.45±4.34

Results are expressed as mean ± SD for 6 different sets of experiments. Values are considered significantly different at P < 0.05 with post-hoc LSD test.

Fig 1. Effect of cisplatin and *Syzygium cumini (L.)* aqueous seeds extract on the SDS-PAGE pattern of the hepatic tissue of control and experimental groups.

1 2 3 4



Protein fragmentation analysis by SDS-PAGE electrophoresis in rat liver tissue homogenate.

Lane 1: Control

Lane 2: Cisplatin– Induced

Lane 3: Cisplatin + *Syzygium cumini (L.)* aqueous seeds extract

Lane 4: *Syzygium cumini (L.)* aqueous seeds extract.

Summary

The protective effect of *Syzygium cumini (L.)* aqueous seeds extract on Cisplatin induced hepatotoxicity poisoning in male wistar albino rats model was studied. Oxidative stress was proposed to be an important/vital reason for the hepatotoxic activity. Hence this work was designed to biochemically evaluate the protective effect of *Syzygium cumini (L.)* aqueous seeds extract against Cisplatin induced hepatotoxicity by assessing the Biochemical parameters, Antioxidant Enzymes status, Non-Enzymatic antioxidant Enzymes, Lipid peroxidation [10], proteins etc.,

Exposure to cisplatin significantly decreased the serum and hepatic antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase [11], glutathione-S-transferases [12] & glutathione reductase (Table 1) and non enzymic antioxidants like reduced glutathione, vitamin C & vitamin E (Graph 2) and the activities of *Syzygium cumini (L.)* aqueous seeds extract treatment improved the antioxidant status by increasing the activities/levels of these enzymic and non-enzymic antioxidants.

Exposure to cisplatin showed significant increase in the activities of hepatic marker enzymes such as SGOT, SGPT, alkaline phosphatase (Table 2) in serum and a subsequent decrease in these enzyme activities in liver after supplementation of *Syzygium cumini (L.)* aqueous seeds extract.

The electrophoretic pattern of proteins by SDS-PAGE (Fig 1) also showed the protective role of *Syzygium cumini (L.)* aqueous seeds extract on cisplatin induced protein fragmentation. Along with biochemical and molecular alterations we have also observed histological changes in hepatic tissue. Histopathological studies revealed alterations of the hepatic tissue caused by cisplatin exposure were prevented by *Syzygium cumini (L.)* aqueous seeds extract administration.

Conclusion

The liver in normal physiological conditions is resistance to oxidative damage because of their efficient protective mechanisms. However, under oxidative stress, the liver (hepatocytes) and their mechanisms are very sensitive to oxidative damage due to their content of enzymes which are continuously exposed to high concentration of oxygen. The work was designed to evaluate the protective effect of *Syzygium cumini (L.)* aqueous seeds extract against cisplatin induced oxidative damage/injury in hepatocytes.

In our present study, Cisplatin induced shows the decrease in the levels of antioxidants and Non-Enzymic antioxidant Enzymes levels, increase in the levels of lipid peroxidations (Graph 1). Cisplatin exposure alters the biochemical parameters viz., liver antioxidant and Non-Enzymatic antioxidants. However, *Syzygium cumini (L.)* aqueous seeds extract normalized the levels of antioxidant and non enzymatic antioxidant Enzymes.

Our findings highlight the efficacy of *Syzygium cumini (L.)* aqueous seeds extract as protective effects against cisplatin induced oxidative damage to the hepatic cells which induced toxicity. Thus it is concluded that *Syzygium cumini (L.)* aqueous seeds extract provide a protective effect in the cisplatin induced hepatotoxicity.

Cisplatin exposure leads to adverse effects on hematological, hepatotoxic parameters including Erythrocytes (RBCs). Cisplatin induction leads to reduction in the levels of Enzymic and Non-Enzymic antioxidants levels. However, on treatment with *Syzygium cumini (L.)* aqueous seeds extract normalized the levels of all the biochemical and hematological parameters. These findings highlight the efficacy of *Syzygium cumini (L.)* aqueous seeds extract as protective effects Cisplatin induced hepatotoxicity and further molecular studies should be done in near future and some studies are under progress.

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