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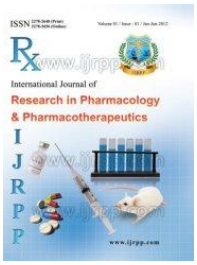

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Hepatotoxic effect of ethanolic extract of *syzygium cumini. Linn* leaves on experimental animals

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ABSTRACT

Syzygium cumini. Linn Leaves were widely used as good medication for several diseases like antimicrobial, cancer, antihypertensive etc.

Aim: The main goal of our study was to explore the acute and sub-chronic oral toxic effects of Ethanolic Extract of *Syzygium cumini. Linn* Leaves on experimental animals.

Materials and methods: Acute toxicity study was carried out by following OECD guideline no. 423. The different doses like 500, 1000, 2000, 3000, 4000 and 5000mg/Kg body weight were administered orally to the different groups of animals and observed for 24 hr after dosing and also observed for 14 days without giving drug. In sub-chronic oral toxicity study, the parameters were after administering daily oral doses of 1250, 2500 and 5000 mg/kg body weight for 28 days to the rats.

Results: Body weights of the rats observed weekly and Biochemical, hematological, histopathological assessments and relative organ weights of the rats were observed on 29th day.

Conclusion: By observing the hematological, biochemical parameters and the histopathological studies it is finally concluded that Ethanolic extract of *Syzygium cumini* leaves produces severe inflammation and fibrosis on Liver at oral doses of 1250, 2500 and 5000mg/kg body weight.

KEY WORDS: *Syzygium cumini. Linn* Leaves, Acute and Sub Chronic toxicity, EESCL, Hematology, Histopathology.

INTRODUCTION

Herbal plants play an input role in the human physical condition. About 80% of the world populations rely on traditional medicine which is based on medicinal plants [1]. Herbal drugs have gained significance in recent years because of their efficacy, low cost and effectiveness. These drugs are

used as either single plant extracts or fractions or mixtures of extracts from different plants. These plant extracts are standardized for their safety and efficacy [2]. Liver has a major role in guideline of physiological processes. Liver diseases are among the most serious ailment. The use of natural remedies for the management of liver diseases has a long history, starting with the ayurvedic treatment and extending to other systems of medicines. In nastiness of the availability of more than 300 preparations used in Indian system of medicine for the treatment of liver diseases only a small portion of hepatoprotective plants as well as formulations are pharmacologically evaluated for their efficacy. The 21st century has seen a paradigm shift towards evaluation of herbal products [3]. *Syzygium cumini* is a well acknowledged medicinal plant since ancient times [4]. The ethanolic leaf extract has been revealed to constitute phytochemical components like alkaloids, glycosides, triterpenoids, carbohydrates, saponins, tannins, flavnoids, phytosterols, amino acids and few phenolic components[5]. The various parts of the plant are pharmacologically proven to possess several properties like anti-inflammatory[6], chemo preventive[7], diuretic[8], anti-hyperglycemic[9], hepatoprotective [10] and anti-diabetic [11]. The present study investigated that the Hepatotoxic effect of the Ethanolic extract of *Syzygium cumini. Linn* leaves on experimental rats.

MATERIALS & METHODS

COLLECTION OF *SYZYGIUM CUMINI* LINN LEAVES

The leaves of *Syzygium Cumini* Linn were collected in October 2014, from the botanical garden of the Santhiram College of pharmacy and authenticated by Mr. P. Prasadarao M.Sc., Dept. Of Botany, PSC & KVSC government college, Nandyal. Then leaves were shade dried at room temperature and powdered, weighed and stored in a clean, dry and air tight container.

DRUGS AND CHEMICALS

N- Hexane -Moly chem. India pvt.Ltd, Mumbai,
Ethanol -Santhiram College of pharmacy, Kits (AST, ALT & Glucose) – EXCEL Diagnostics Pvt. Ltd, Hydrabad, Formaldehyde-Merck specialites pvt. Ltd, Mumbai.

PREPARATION OF ETHANOLIC EXTRACT OF *SYZYGIUM CUMINI LEAVES*

The dried powder was defatted by using n-hexane with maceration technique. The defatted powder is dried at room temperature. After that the dried defatted powder was extracted with ethanol at 70^0c by soxhlet apparatus. The solvent present in the extract removed by distillation and dried to get a solid mass[12].

ACUTE TOXICITY STUDY

The acute oral toxicity study was carried out for ethanolic extract of *Syzygium cumini* leaves using the fixed dose method according to OECD guideline no. 423. Healthy adult albino rats weighing between 150 to 180 g were used for the study. Animals were divided into five groups of six animals each and kept fasted overnight. The different doses like 500, 1000, 2000, 3000, 4000and 5000mg/Kg body weight were administered to the group I, II, III, IV, V, VI, respectively. After administering the ethanolic extract of *Syzygium cumini* leaves in different groups the behavioral changes, Eyes, Salivation , Diarrhea , Mortality etc. were observed for 24 hr (OECD guidelines) and also observed for 14 days without giving drug[13].

SUB CHRONIC TOXICITY STUDY

EXPERIMENTAL ANIMALS

Either sex Wister rats weighing about 110 to 130 g were used in the study. The study protocol was reviewed and approved by the institutional animal ethical committee of Santhiram College of pharmacy (1519/PO/a/11/CPCSEA). Animals were obtained from Sainath enterprises, Hyderabad. Rats were housed in polyacrylic cages (38x23x10 cm). They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 12 h light/ 12 h dark) and maintained humidity 60±5% and an ambient temperature of 25±2%. All experiments were performed between 9:00 am to 4:00 pm. The animals were free access to standard diet and water *ad libitium* and allowed to acclimatize for one week before the experiments. The commercial pellet diet contained 22% protein, 4% fat, 4% Fiber, 36% carbohydrates and 10% ash w/w[14].

PREPARATION OF DRUG AND MODE OF ADMINISTRATION

Ethanolic extract of *Syzygium cumini* leaves (EESCL) was formulated as aqueous suspension with 2% acacia as suspending agent. The suspension of the ethanolic extract of Syzygium cumini leaves was administered orally consecutively for 28 days. The suspension was freshly prepared immediately before use.

EXPERIMENTAL DESIGN

The animals were divided into 4 groups of 6 rats in each group.

Group I Served as control group provided standard diet and water *ad libitium*

Group II Administered with EESCL (1250mg/kg, p.o)

Group III Administered with EESCL (2500mg/kg, p.o)

Group IV Administered with EESCL (5000mg/kg, p.o)

The doses were given once a day for at morning for 28 days. Animals were provided with food and water as usual. All the animals were observed twice daily for mortality and any behavioral changes. On 29thday the animals were sacrificed and isolate the kidneys and sent for histopathological studies[15].

PARAMETERS TO BE EVALUATED

On 29th day hemoglobin (Hb) Red blood cell (RBC) count, WBC count, Glucose levels, Aspartate Aminotransferase (AST), serum Alanine Aminotransferase (ALT) were estimated. Animals were then sacrificed and kidney was isolated and kept in 10% formalin solution for histopathological work. The organs were observed for any gross changes with eye and the histopathological changes were observed under microscope.

STATISTICAL ANALYSIS

Data were expressed as Mean ± SEM (n=6). The results were analyzed using one way ANOVA in graph pad prism ver.5.03. To determine the level of significant difference between each treatment and the control group using Tukey test.

RESULTS

ACUTE TOXICITY STUDY

Acute toxicity study performed as per the OECD Guidelines 423, the results reveal that the Ethanolic extract of *Syzygium cumini* leaves have been found to be non toxic up to dose level 5000mg/kg body weight of experimental animals. No mortality was observed during either on first day and up to 14 days of observation.

SUB CHRONIC TOXICITY STUDY

BODY WEIGHT

During the experimental period all rats showed a significant increase in body weight compared to their initial values. No mortality was observed during the whole experiment period (Table 1).

LIVER WEIGHT

Repeated oral dose of Ethanolic extract of *Syzygium cumini* leaves for 28 days induced remarkable gross morphological changes in test groups. Weights of isolated organ such as liver showed significant decrease in group-II, III, IV Compared to normal. In group- IV animals showed drastically decrease in liver weight (Table 2).

HAEMATOLOGICAL PARAMETERS

Haematological parameters rendered significant changes in Ethanolic extract of *Syzygium cumini* leaves treated animals compared to normal group. In group-III&IV animals RBC, WBC, Hb levels are significantly increased compared to Group-I (Table 3).

BIOCHEMICAL PARAMETERS

Repeated oral dose of Ethanolic extract of *Syzygium cumini* leaves for 28 days caused significant increase in hepatic transaminase i.e. SGOT& SGPT levels significantly decrease in glucose level in group-II, III & IV animals when compared to normal group (Table 4).

HISTOPATHOLOGICAL STUDIES

Histopathological examination of isolated liver was determined under microscope. In normal animals liver showed that Portal, periportal region was appeared normal, Hepatocytes, portal triad with bile duct, Hepatocytes and sinusoidal space also appeared normal and the results were showed in Fig: 1. group-II animal's liver showed that Multifocal, moderate Periportal inflammation along with fibrosis noticed in the portal region but the group-III & IV animals liver

showed large sized parasitic cyst surrounded by chronic inflammation in which proliferation of connective tissue [fibrosis] with infiltration of inflammatory cells noticed and moderate to severe granuloma formation noticed in the liver due to parasitic infestation and the results were showed in Fig: 2, 3 & 4.

Table 1: Changes in body weight of rats during the administered with different doses of Ethanolic extract of *Syzygium cumini leaves*

Dose	1st week	2nd week	3rd week	4th week
Group I	109.3±0.76	125.4±0.34***	138.26±0.56***	147.51±0.87***
Group II	127.2±0.58	128.2±0.65***	137.22±0.22***	148.43±0.73***
Group III	131.1±0.26	133.4±0.73***	14.341±0.24***	149.87±0.36***
Group IV	133.7±0.43	134.2±0.41***	149.92±0.33***	161.72±0.44***

Values are Mean± S.E.M of 6 animals in each group. One way ANOVA used.
*= P<0.05; **= P<0.01;***=P<0.001 Compared to Group-I

Table 2: Effects of Ethanolic extract of *Syzygium cumini leaves* to change in liver weight of control and experimental rats (sub chronic toxicity studies)

S.no	Groups	Liver weight (g)
1	Group I	6.918±0.05062
2	Group II	4.322±0.04799***
3	Group III	4.232±0.2027***
4	Group IV	3.78±0.1731***

Values are Mean± S.E.M of 6 animals in each group. One way ANOVA used.
*= P<0.05; **= P<0.01;***=P<0.001 Compared to Group-I

Table 3: Effects of Ethanolic extract of *Syzygium cumini leaves* on hematological parameters of the control and experimental rats (sub chronic toxicity studies)

Haematological Parameters	Group I	Group II	Group III	Group IV
Hb (g %)	9.1 ± 0.23	9.9±0.64	10.12 ± 0.32***	9.78 ± 0.42***
RBC (Cumm)	4.7±0.09	5.00±0.18	5.83±0.29***	5.5±0.54***
WBC (mil./Cumm)	8567±135.89	9186 ± 76.57	9986±143.7***	9865±202.2***

Values are Mean± S.E.M of 6 animals in each group. One way ANOVA used.
*= P<0.05; **= P<0.01;***=P<0.001 Compared to Group-I

Table 4: Effect of Ethanolic extract of *Syzygium cumini leaves* on serum biochemical markers in control and experimental rats (sub chronic toxicity studies)

Parameters	Group I	Group II	Group III	Group IV
SGOT	24.83±0.6009	25.50±0.7638***	31±0.9661***	35.17±0.6009***
SGPT	56.33±0.667	62.83±0.6009***	65±0.5774***	72.83±0.7032***
Glucose	73.51±0.9455	70.88±1.867	46.14±1.005***	36.15±1.428***

Values are Mean± S.E.M of 6 animals in each group. One way ANOVA used.
*= P<0.05; **= P<0.01;***=P<0.001 Compared to Group-I

HISTOPATHOLOGICAL STUDIES

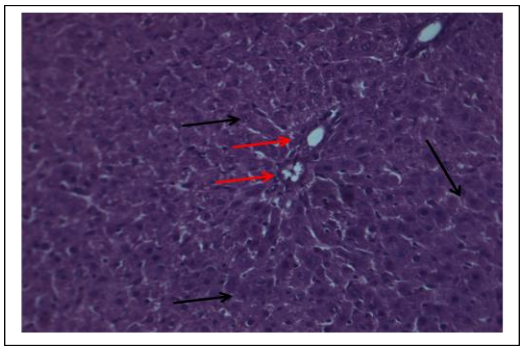

Fig 1: Group-I (2% acasia, PO)

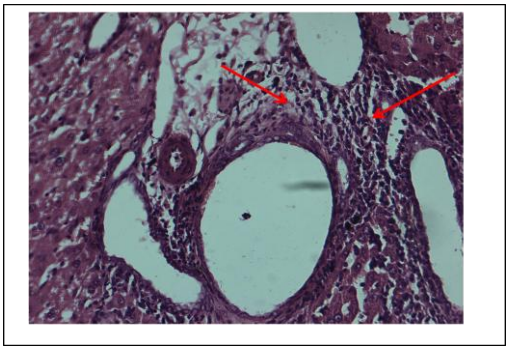

Fig 2: Group-II (EESCL-1250mg/kg, PO)

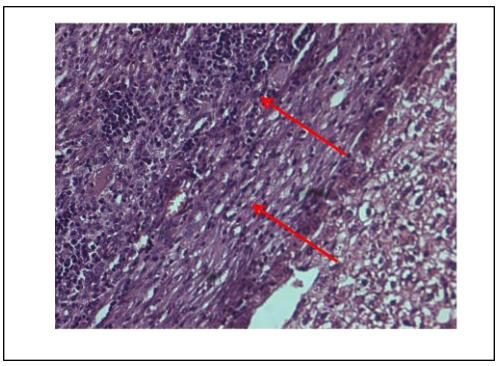

Fig 3: Group-III (EESCL-2500mg/kg, PO)

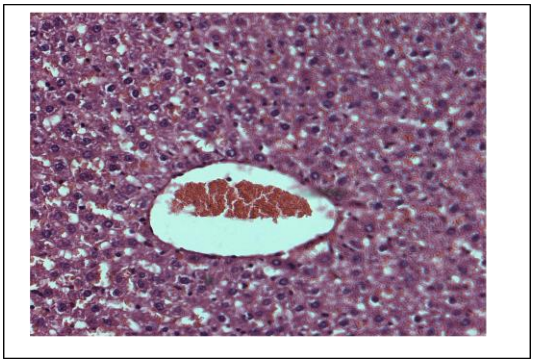

Fig 4: Group-IV (EESCL-5000mg/kg, PO)

DISCUSSION

The Ethanolic extract of *Syzygium cumini* leaves have been reported to possess significant anti-inflammatory[6], diuretic[8], anti-hyperglycemic[9], hepatoprotective[10], chemopreventive [7] and anti-diabetic[11]. It is however an established fact that some plant extracts could be inherently dangerous, containing naturally occurring toxins which may be cytotoxic [16]. Accordingly most of the herbal preparations do not have drug regulatory approval to demonstrate their safety and efficacy[17]. It is therefore pertinent to establish the safety of medicinal

plant preparations through toxicological assessments. Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds. If any injury to it or impairment to its function may leads to complications on one's health[18]. In the present study, acute oral administration of Ethanolic extract of *Syzygium cumini* leaves to rats at a dose level of up to 5000mg/kg did not cause any mortality or toxic symptoms up to 14 days of observation. Similar kind of results was reported at different LD50 values for different plant extracts. The oral LD50 of ethanol extract of *Vitex leucoxylon* leaf (>3000mg/kg), cold water infusion extract of the same plant (1050mg/kg), ethanolic extracts of *Ailanthus excelsa* (1000mg/kg), *Toddalia asiatica* (350mg/kg) and *Araucaria bidwilli* (250 mg/kg) have been reported[19]. Sub-chronic doses of EESCL (1250, 2500 and 5000 mg/kg) caused significant increase in the body weights of the experimental rats. This may be a warning that the drug does not influence the feed utilization ratio of the animals. EESCL causes significant decrease in liver weight of group- II, III and IV compare to Group-I. It can be done by may be the formation of fibrosis and granuloma of liver cells. The Hematogical parameters like Hb, RBC, total WBC count of the treated rats were not significantly different compared to group-I. The enzymes, Aspartate Aminotranferase (AST) and Alanine Aminotransferase (ALT) showed progressive increase in activities at all doses administered, with most significant effect at the 5000mg/kg. These findings imply that the extract, may at these doses, affect the liver. Serum ALT and AST are useful indices for identifying inflammation and necrosis of the liver [20]. EESCL causes a significant decrease in serum glucose level in EESCL treated rats compare to group-I Histopathalogical examination of livers from all group animals. The repeated oral administration of EESCL showed that the group-II animal's liver showed that Multifocal, moderate periportal inflammation along with fibrosis noticed in the portal region but the group-III & IV animals liver showed large sized parasitic cyst surrounded by chronic inflammation in which proliferation of connective tissue [fibrosis] with infiltration of inflammatory cells noticed and moderate to severe granuloma formation noticed in the liver due to parasitic infestation.

CONCLUSION

Ethanolic extract of *Syzygium cumini* have been reported to possess significant anti-inflammatory[6], diuretic[8], anti-hyperglycemic[9], hepatoprotective[10], chemopreventive [7] and anti-diabetic[11]. The present study reports hepatotoxicity of ethanolic extract of *Syzygium cumini* leaves on experimental rats at dose levels of 1250, 2500 and 5000mg/kg body weight for 28 days. The extract has reduced glucose levels considerably but the levels of body weights, RBC, WBC, Hemoglobin, AST and ALT were found to be elevated. Histopathologically multifocal moderate tubular nephritis, multifocal moderate tubular degeneration in the kidney was observed. The results of the subchronic studies on the liver function parameters indicated that the Ethanolic extract of *Syzygium cumini* be used with caution especially at higher doses and prolonged treatment.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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