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Acute Oral Toxicity Assessment of Ethanolic Seed and Fruit Pulp Extracts of *Syzygium cumini L.* IN Swiss Albino Mice

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Abstract
Folk medicine uses Syzygium cumini L. (Myrtaceae) extensively as an antidiabetic; yet, little is known regarding its toxicity, particularly with regard to the stem bark. In this work, Swiss albino mice were given acute oral doses of ethanolic extracts of Syzygium cumini seeds (ESE) and fruit pulp (EFPE). Mice were given oral dosages of Syzygium cumini seed(ESE) and fruit pulp (EFPE) ethanolic extracts at 100, 500, 1000, 2500, and 5000 mg/kg body weight for the acute toxicity testing. For 14 days following therapy, mortality, toxicity indicators, body weight, food intake, and gross results were tracked. The remaining animals were put to sleep and starved overnight at the conclusion of the research period in order to draw blood and remove a few critical organs for histology. Body and organ weights did not significantly differ between the treatment and control groups in either study. Furthermore, the toxicity study examined biochemical parameters such as blood glucose, creatinine, blood urea, alkaline phosphatase (ALP), bilirubin, total protein, and albumin, as well as haematological parameters such as red blood cell count (RBC), haemoglobin concentration (Hb), MCHC, platelets (PLT), and white blood cell differential count. These studies conclude that the ethanolic extracts of Syzygium cumini seeds (ESE) and fruit pulp (EFPE) administered acutely orally are safe, indicating that they can be used constantly without risk.
Keywords: *Syzygium cumini ; Jamun; acute toxicity; LD50, biochemical parameters*

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I. INTRODUCTION

The Myrtaceae family includes the jamun fruit, *Syzygium cumini* (L.) (also known as *Myrtus cumini*, *Calyptranthes jambolana*, *Syzygium jambolanum*, *Eugenia jambolana*, and *Eugenia cumini*). Other names for it include Indian blackberry, jiwat, salam, kerianduat, jambul, black plum, java plum, and jambolan. To ascertain the potential for value addition, researchers have examined the nutritional qualities and uses of jamun in a variety of food matrices. Since ancient times, Ayurveda has used jamun to treat digestive issues and diabetes. Many bioactive substances, including as phenolics, terpenoids, phloroglucinol derivatives, and saponins, have been discovered as the cause of *Syzygium cumini's* health-promoting qualities, which are now being validated.

The seed extracts, extract fractions, and isolated chemicals are being investigated for antidiabetic, antioxidant, anti-inflammatory, anticancer, antibacterial, cardioprotective, hepatoprotective, and neuroprotective qualities as part of a rigorous investigation into biological potential. Additionally, it has been discovered that the jamun contains nutrients (Priyanka et al., 2015; Singh et al., 2022). In addition to significant amounts of dietary fiber, the *Syzygium cumini* contains significant amounts of, amino acids, vitamin C, vitamin B complexes, essential minerals & trace elements, essential oil, albumin, and fats (Venu Gopal, et al, 2017; Qamar, et al-2022).

According to Dangour et al. (2008), fatty acid profile of *Syzygium cumini* indicates that the main fatty acids are lauric, myristic, palmitic, stearic, oleic, linolenic, malvalic, and sterculic acids, with - sitosterol serving as a phytosterol. These distinctive nutritional characteristics of *Syzygium cumini* point to its potential for use in the cosmetics and pharmaceutical sectors (Kannan et al., 2015; Ayenampudi et al., 2022). *Syzygium cumini's* high iron content promotes a rise in hemoglobin levels and serves as a blood purifying agent (Kalse et al., 2016; Singh et al., 2020). *Syzygium cumini's* iron aids in the fight against jaundice and anaemia (Kshirsagar et al., 2019; Tak et al., 2022).

Additionally, the calcium in the seed may assist fulfil the mineral's nutritional needs and may be utilized in dietary supplements for expectant and nursing women (Ghosh et al., 2017; Khadivi et al., 2022). Scientists have investigated *Syzygium cumini's* antioxidant capacity and demonstrated how it may be used to increase the oxidative stability of different food matrices (Kasthuri et al., 2017). However, no toxicity studies have been

published to far about this native *Syzygium cumini* , despite the growing health advantages and anecdotal evidence of long-term usage in rural areas.

In accordance with the OECD 425 up and down approach (OECD, 2008), Swiss albino mice were used to perform an acute oral toxicity test of *Syzygium cumini* seed and fruit pulp extract. This study specifically sought to identify the physiologic, behavioural, haematological, and blood chemical alterations, as well as clinical indicators of toxicity and death, as a precursor to the development of this berry as a high-value supplement or functional food component.

II. MATERIALS AND METHODS

Collection and preparation of samples

The totally ripe and mature fruits of *Syzygium cumini* were collected in the Patna region of Bihar. This fruit is seasonal and non-climacteric. When young, the colour intensity of these huge, oval-shaped *Syzygium cumini* fruits changes from green to purple, and when completely ripe, they turn dark-purple or black. Based on their consistency in size and colour, freshness, and absence of visible damage or infections, the fruits were selected and collected at the local market. The fruits were washed with deionized water. After that, the fruit seed was gently extracted using a peel from the pulp. The samples were separately packed in two polypropylene bags and stored at -20 °C until extraction.

Extraction procedure

The fruit pulp of *Syzygium cumini* was freeze-dried at -50 °C and 0.001 mbar at reduced pressure. The seeds were dried in the oven at 40 °C. When the pulp and seed were entirely dry, they were ground in a knife grinder. Ethanol was used to extract the bioactive components from fruit pulp and seeds. After dissolving 200 g of dried and powdered materials in 800 mL of solvent, they were continuously shaken for 48 hours using an orbital shaker. The supernatants were filtered using a Buchner funnel. The residue was again dissolved in the solvent. The samples were extracted three times using the same procedure for maximal extraction. As the pressure was reduced, the solvent (ethanol) was removed from the filtrates using a rotary evaporator. The filtrate was freeze-dried after evaporation and kept at -20 °C for further examination. On the day of the experiment, the powder was reconstituted in distilled water and vortexed for one minute to create control vehicles of distilled water and seed (ESE) and fruit pulp (EFPE) ethanolic extracts at different concentrations (100, 500, 1000, 2500, and 5000 mg/kg BW).

Experimental animals

In the laboratory animal room of the Mahavir Cancer Sansthan and Research Centre in Patna, Bihar, India, twenty-four eight-week-old Swiss albino mice were kept individually in commercial polycarbonate cages with stainless steel tops. They were kept at 22°C (±2°C) with a humidity of 30–60% and a 12-hour light–dark cycle (lights on at 7:00 A.M. and lights off at 7:00 P.M.). Distilled water and commercial maintenance mouse pellets were given freely. Before the trial, the mice were acclimated for a week.

Acute oral toxicity testing

The acute oral toxicity up-and-down-method was performed following the OECD Guideline 425 (OECD, 2008). Using the constant dose progression factor 100 mg/kg BW of set as an initial dose, mice were randomly allocated into five (10) treatment groups (n=2) per as follows:

Group 1 was given 100 mg/kg BW (ESE)
Group 2 was given 500 mg/kg BW (ESE)
Group 3 was given 1000 mg/kg BW (ESE)
Group 4 was given 2500 mg/kg BW(ESE)
Group 5 was given 5000 mg/kg BW(ESE)
Group 1 was given 100 mg/kg BW (EFPE)
Group 2 was given 500 mg/kg BW (EFPE)
Group 3 was given 1000 mg/kg BW ((EFPE)
Group 4 was given 2500 mg/kg BW(EFPE)
Group 5 was given 5000 mg/kg BW(EFPE)
Control group given distilled water(n=4)

To ensure there was no mortality, each medication was started one after the other, ranging from low to high dosage, after a 48-hour break. On the first day of the experiment, mice were fasted and weighed before receiving treatments. A 1-inch 22G stainless steel gavage needle (Thermo scientific, usa) and a 1 ml sterile disposable syringe were used.

Clinical sign of toxicity

Individual animals from each treatment group were observed for clinical symptoms of toxicity 30 minutes after the injection of the vehicle, EFPE, and ESE, 24 hours later, and every day after that until the fourteenth day of the experiment. Any unusual alterations to the skin, mucous membranes, eyes, hair, or somatoform activity were noted, along with any departures from the typical behavioural pattern. Clinical indications of diarrhoea, salivation, tremors, convulsions, lethargy, drowsiness, and coma were all noted and given particular consideration. All treatment groups' clinical indicators of morbidity and death were also taken into consideration.

Physiologic parameters

The body weight of each mouse per treatment group was measured weekly, on Days 1, 7, and 15, using a digital top loading balance (Shimadzu, Japan) prior to administration of vehicle or EFPE and ESE.

BLOOD CHEMISTRY ANALYSIS

Before the vehicle, EFPE, and ESE treatments were administered, blood was drawn on Days 1 and 14 of the trial. Before the blood was drawn, the mice were given a drop of tetracaine in their right eye and allowed to rest for two minutes. Using a heparinized capillary tube, 300µl of blood was drawn from the retro-orbital vein and transferred into a 0.5 mL microcentrifuge tube. In order to track the metabolic activity of the mice in each group, the relevant biochemical parameters were retrieved for the biochemical estimate. Alkaline picrate technique for serum creatinine, Nitro prussic method for serum urea, Reitman and Frankel method for alanine aminotransferase (ALT), and Modified IFCC method for aspartate aminotransferases (AST).

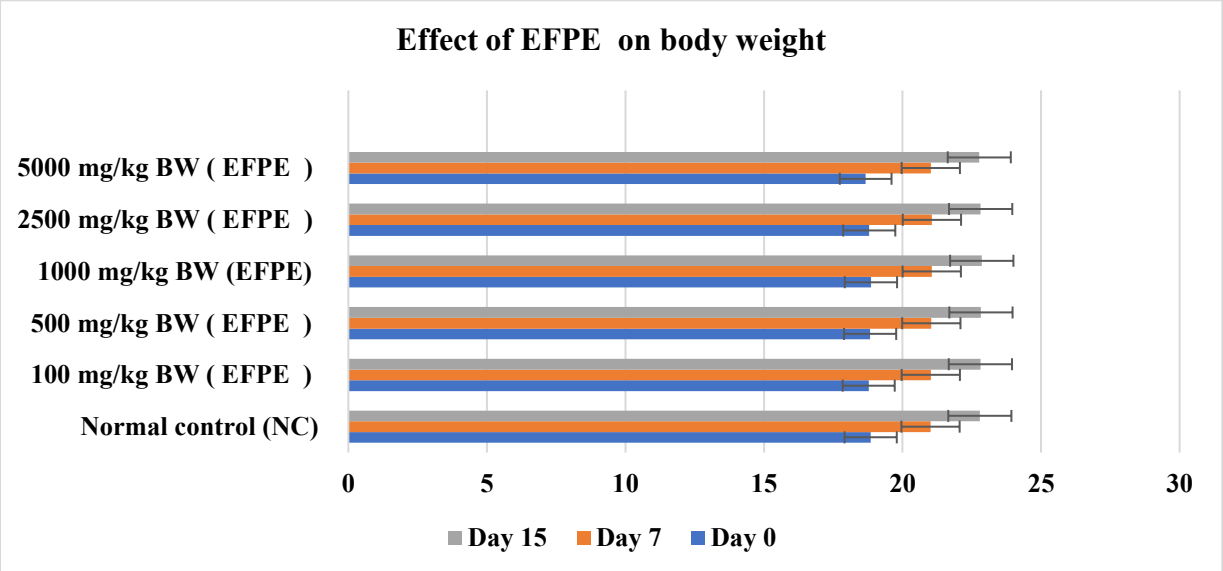
STATISTICAL ANALYSIS

The statistical analysis, data were expressed as the mean ± S.E.M. for statistical analysis of the data; group means were compared by one-way ANOVA (analysis of variance) with *Post Hoc* analysis. The Tukey Karmer Post Hoc test was applied to identify significance among groups. Graphs are plotted using MATLAB version 7.8.0 R2009a, Natick, Massachusetts: The Math works Inc.2009.

III. RESULTS AND DISCUSSION

Effect of EFPE and ESE on morbidity, mortality , clinical signs of toxicity and on body weight

Swiss albino mice given distilled water or increasing concentrations of EFPE and ESE did not exhibit any treatment-related morbidities or mortality during the trial period. Specifically, no unusual alterations in behaviour, physical characteristics, or indicators were seen. Swiss albino mice's mean body weight and mean body weight increase were unaffected by the administration of EFPE and ESE, with findings equivalent to those of the control group.



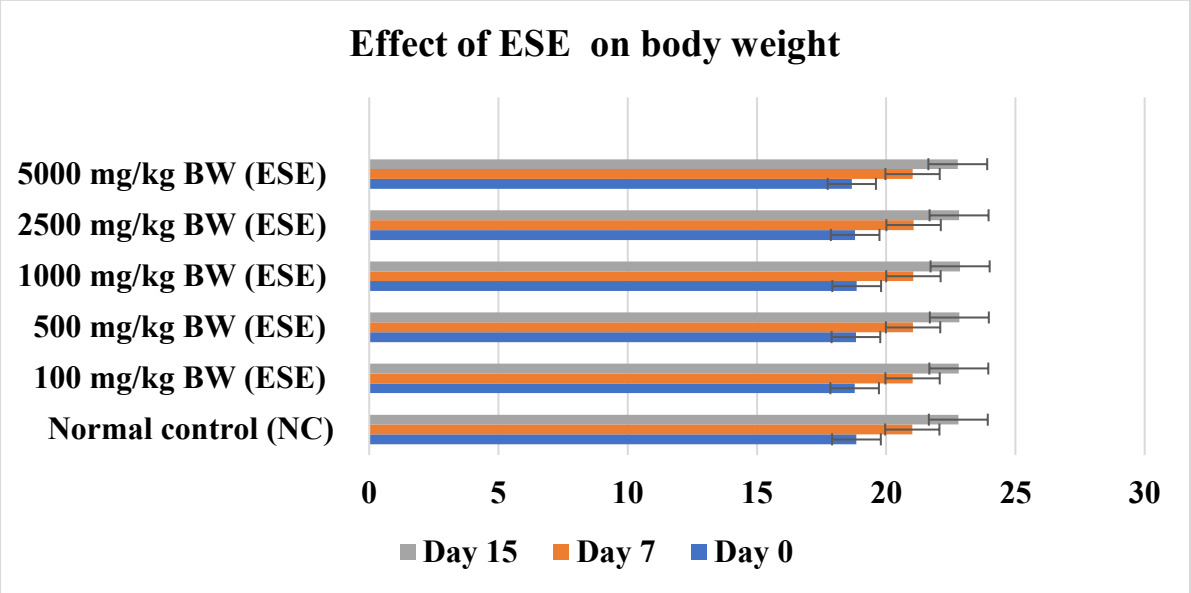


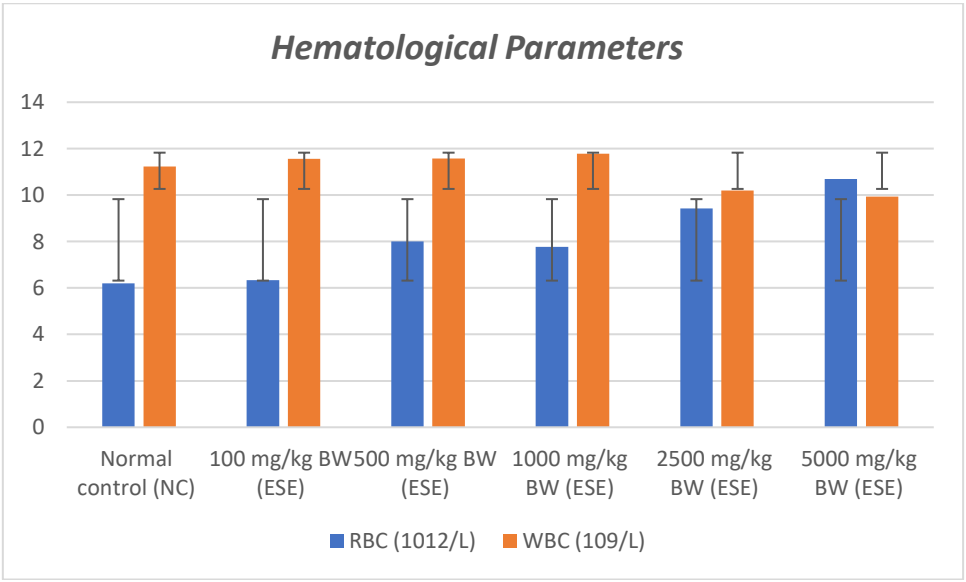
Figure 1: Mean body weight of EFPE (A) and ESE (B) mice given distilled water and varying doses of extract.

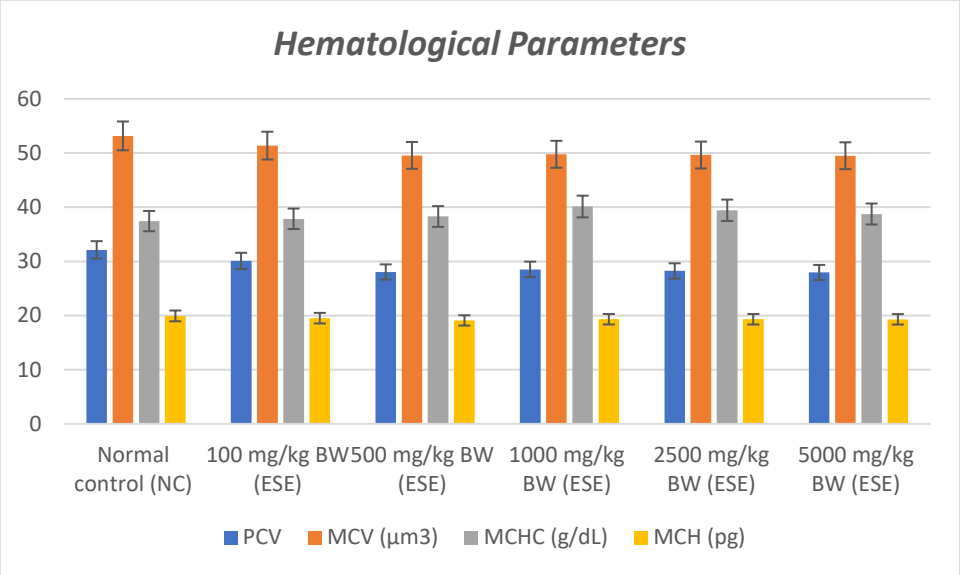
ACUTE TOXICITY AND LETHAL TEST (LD_{50}) ESTIMATION OF SEED (ESE) AND FRUIT PULP (EFPE) ETHANOLIC EXTRACTS OF *Syzygium cumini*

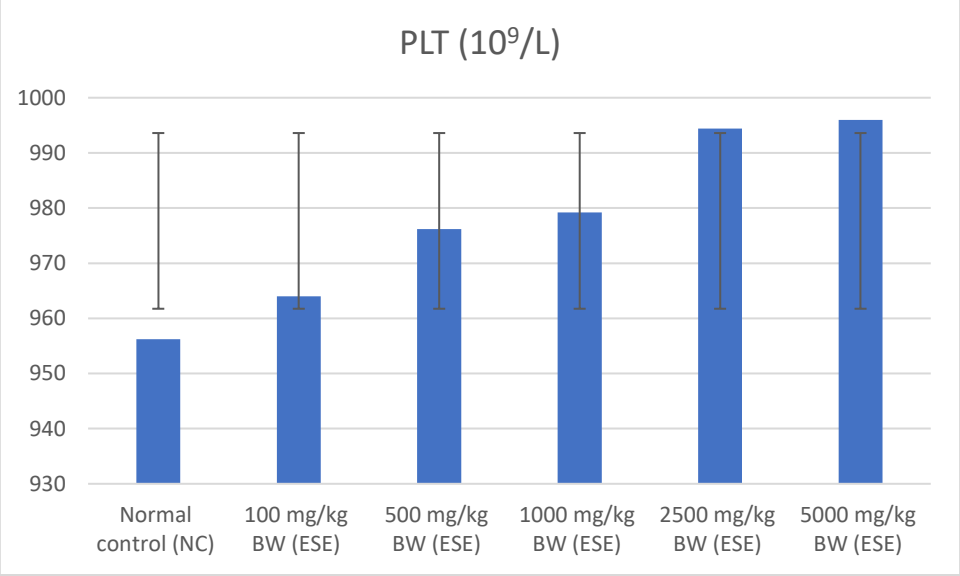
Oral Up to 5000 mg/kg of body weight of *Syzygium cumini* seed (ESE) and fruit pulp (EFPE) ethanolic extracts did not result in death or toxicological symptoms. An LD50 of 2000 mg/kg and above is designated as "unclassified" in accordance with the OECD criteria for acute oral toxicity (Organisation for Economic Co-operation and Development, 2001), meaning the medication is deemed safe. Therefore, further dosing to determine the LD50 of *Syzygium cumini* seed (ESE) and fruit pulp (EFPE) ethanolic extracts was not carried out. In their investigation of oral administration, Hilaly et al. (2004) showed that the maximum tolerated dosage (MTD) was equal to the no-observed-adverse-effect (NOAE) dose, which was 5000 mg/kg of body weight.

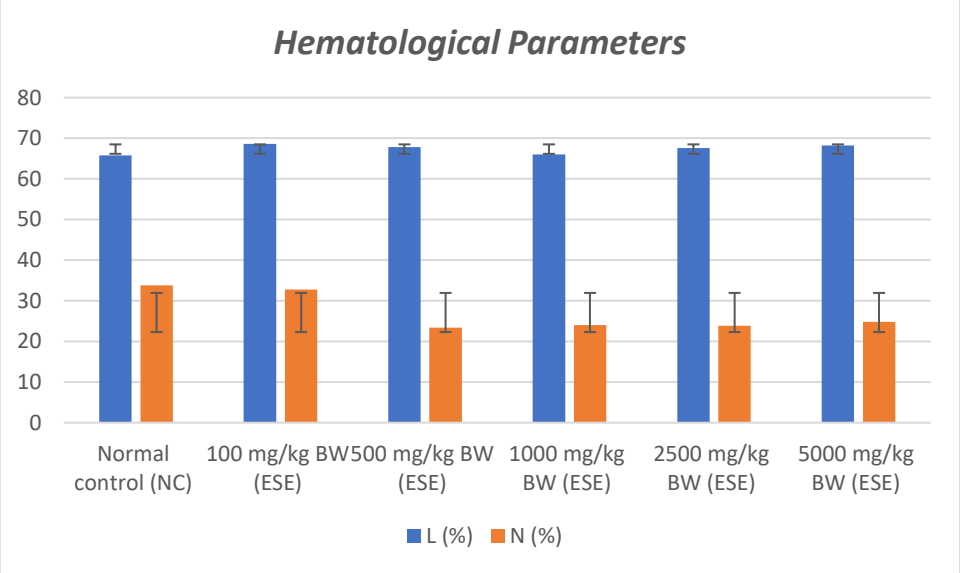
HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF MICE

Both the seed (ESE) and fruit pulp (EFPE) ethanolic extracts of *Syzygium cumini* did not significantly alter RBC, Hb, WBC, or platelets in the treatment group as compared to the control group, according to haematological analysis (Figures 2 and 3). There was no difference in the leukocyte differential count between the groups. There were not many notable variations in any of the parameters analysed between the control and treatment groups, according to the biochemical analysis (Figures 4 and 5).




Hematological Parameters
60
50
40
30
20
10
0
Normal control (NC)
100 mg/kg BW (ESE)
500 mg/kg BW (ESE)
1000 mg/kg BW (ESE)
2500 mg/kg BW (ESE)
5000 mg/kg BW (ESE)
PCV
MCV (µm3)
MCHC (g/dL)
MCH (pg)


PLT (10^9/L)
1000
990
980
970
960
950
940
930
Normal control (NC)
100 mg/kg BW (ESE)
500 mg/kg BW (ESE)
1000 mg/kg BW (ESE)
2500 mg/kg BW (ESE)
5000 mg/kg BW (ESE)


Hematological Parameters
80
70
60
50
40
30
20
10
0
Normal control (NC)
100 mg/kg BW (ESE)
500 mg/kg BW (ESE)
1000 mg/kg BW (ESE)
2500 mg/kg BW (ESE)
5000 mg/kg BW (ESE)
L (%)
N (%)

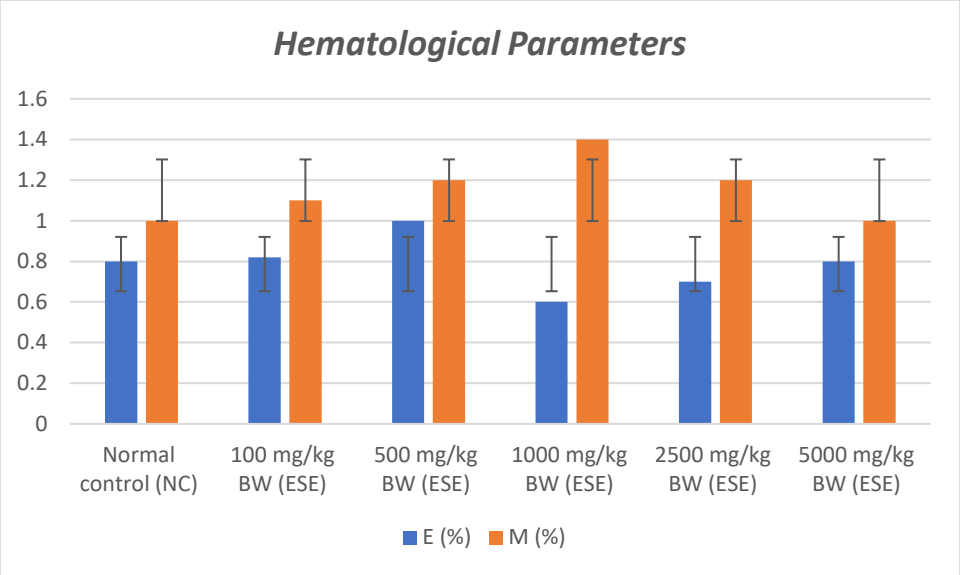
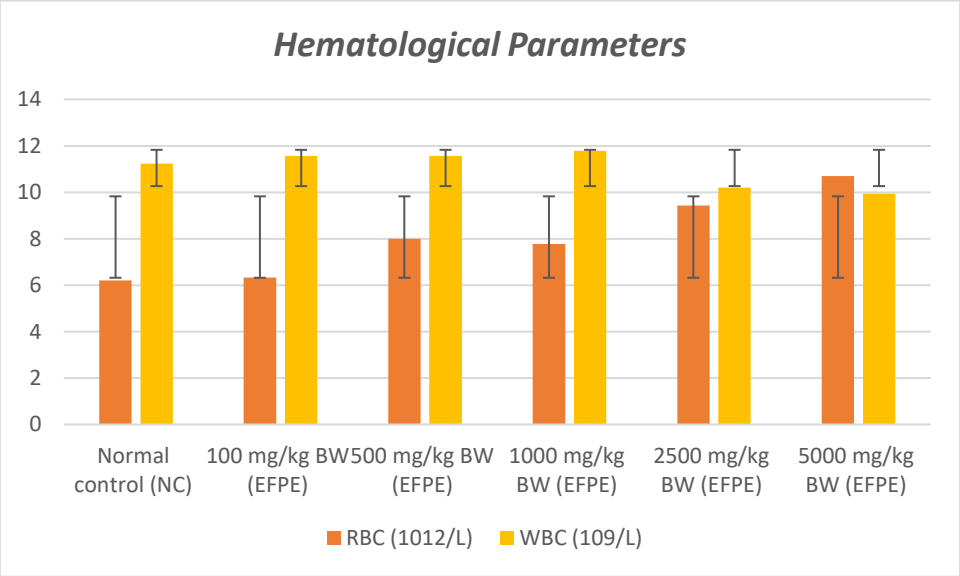
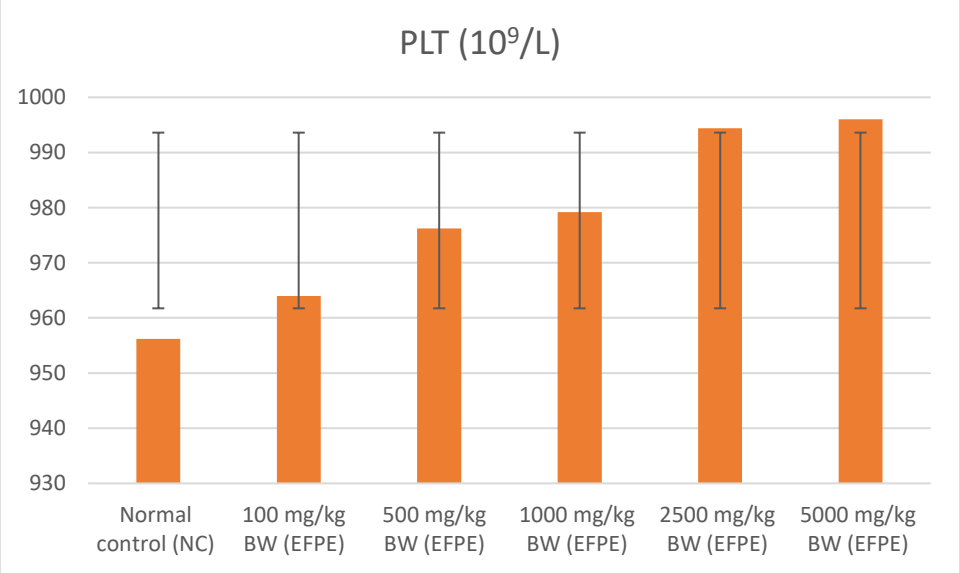


Figure 2: Haematological analysis of control mice given distilled water and ESE varying doses of extract





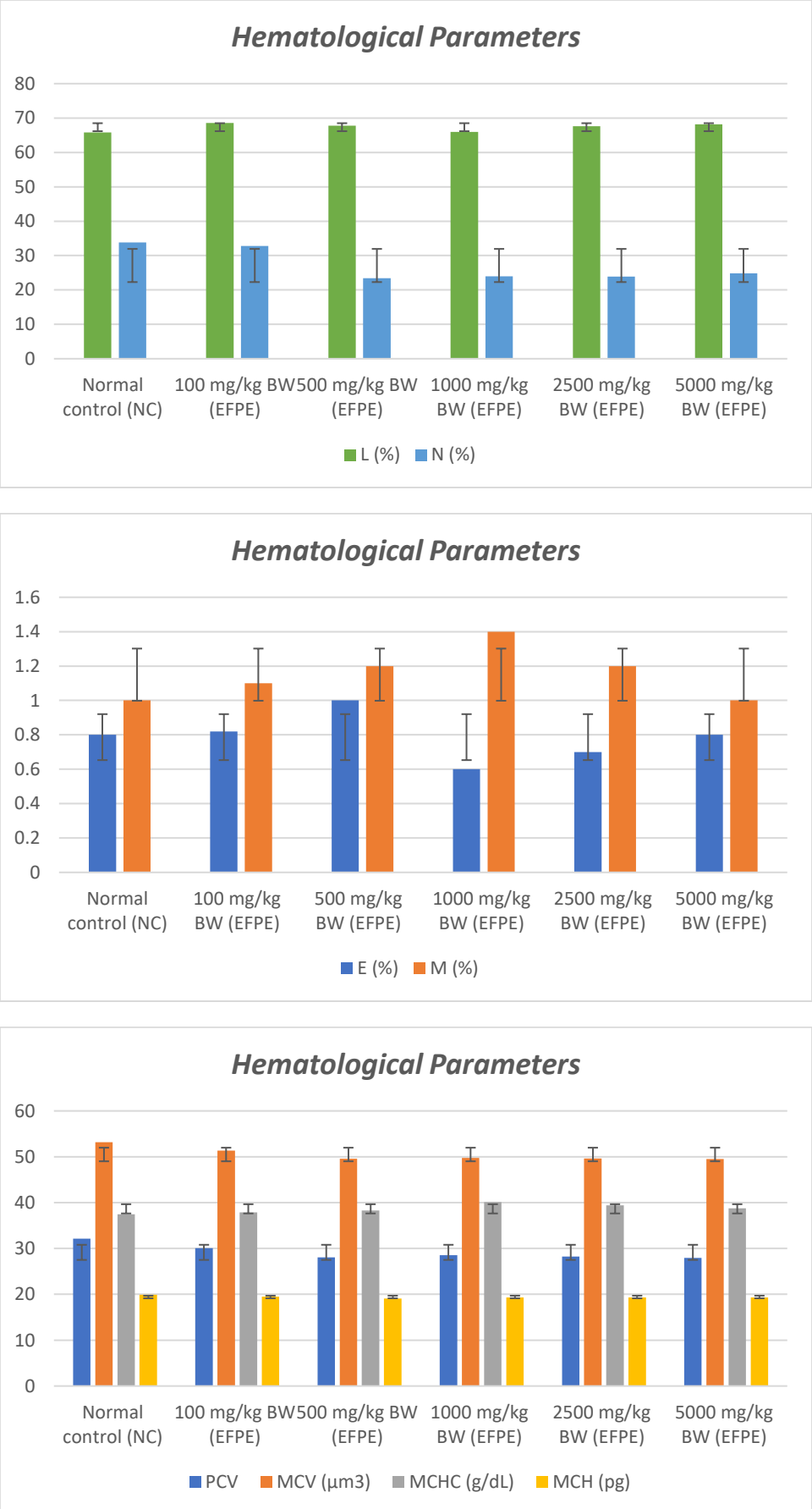


Figure 3: Haematological analysis of control mice given distilled water and EFPE varying doses of extract.

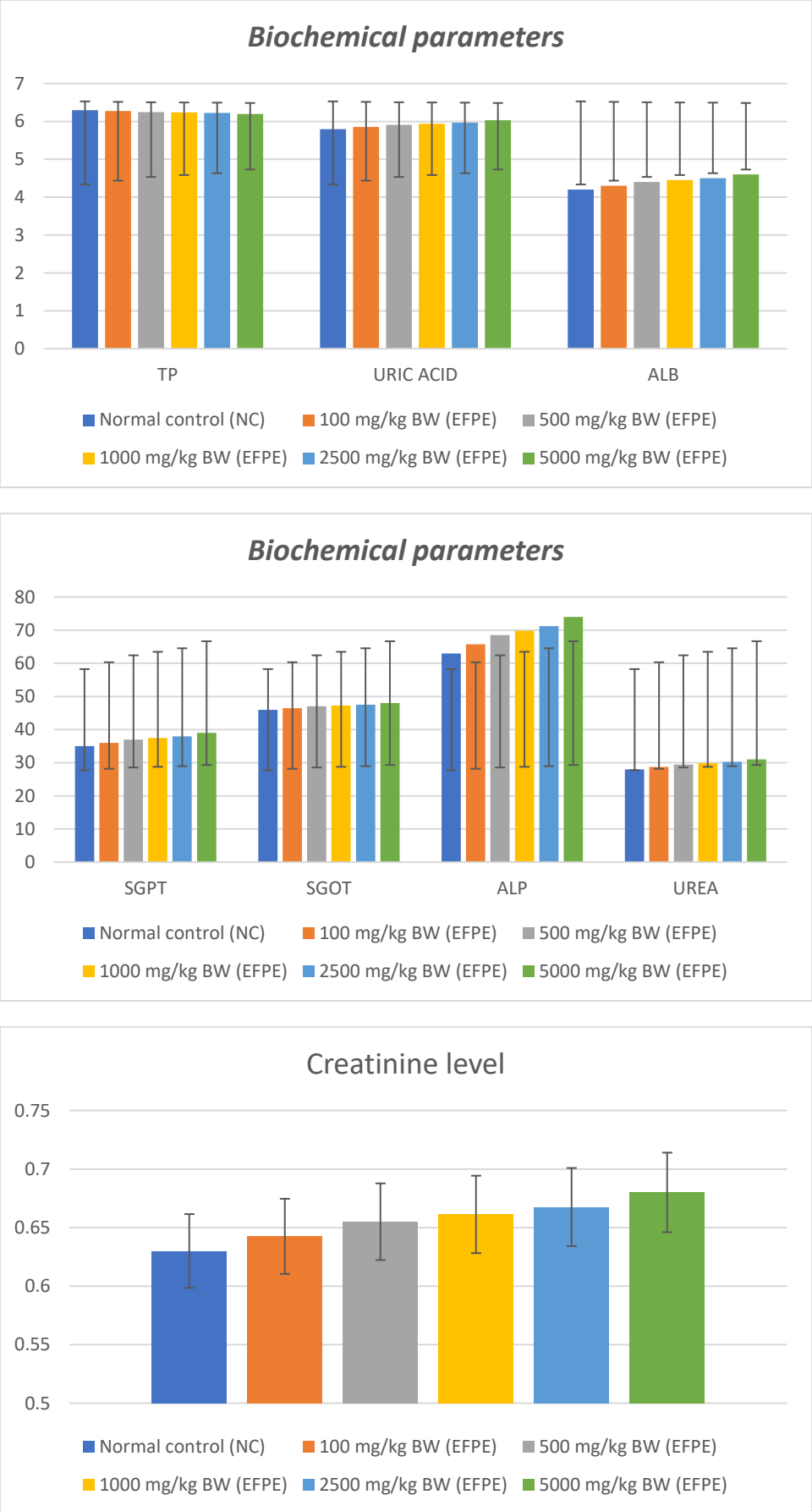


Figure 4: Biochemical analysis of control mice given distilled water and EFPE varying doses of extract.

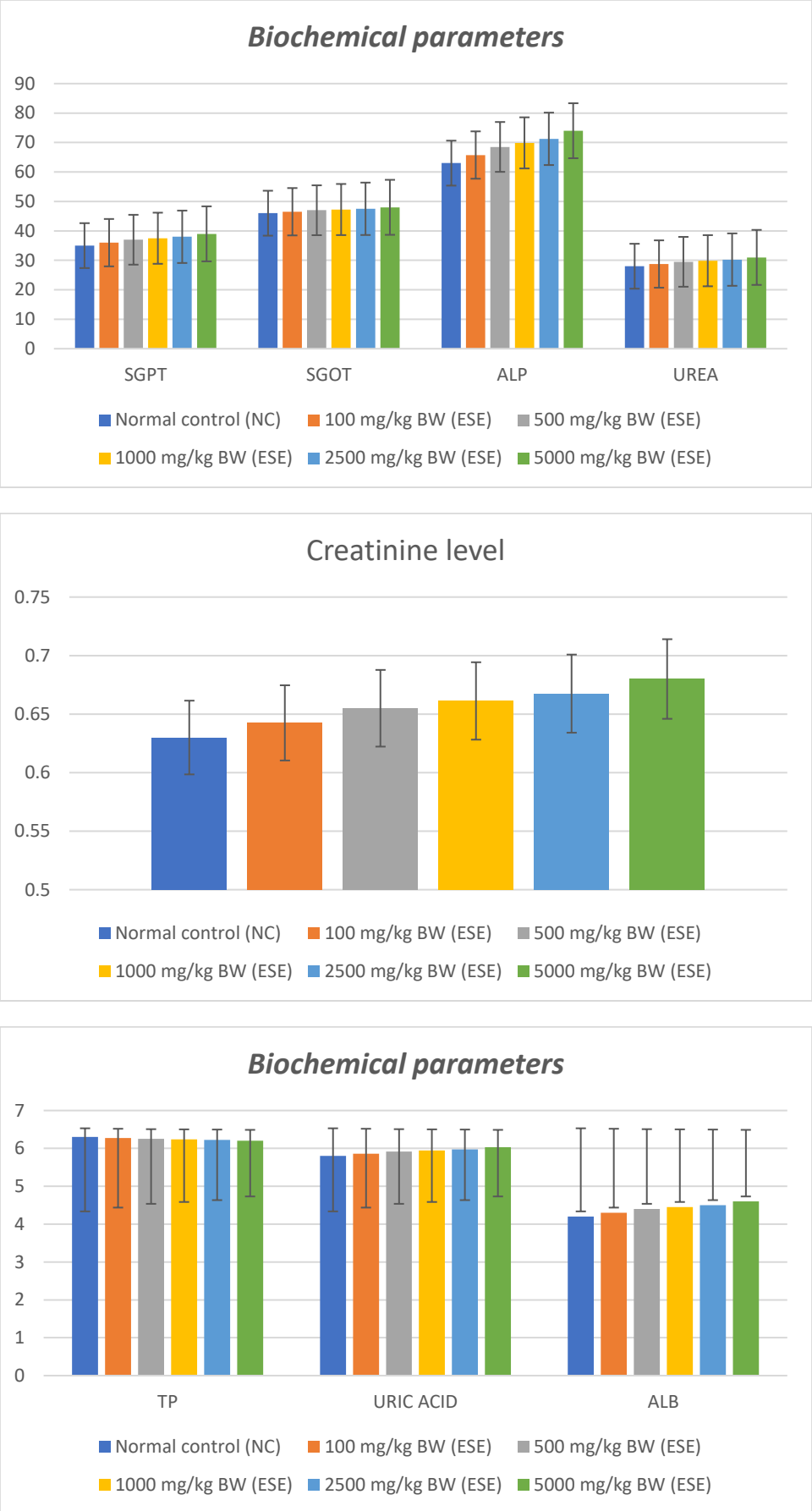


Figure 5: Biochemical analysis of control mice given distilled water and ESE varying doses of extract.

In laboratory animals, an acute toxicity study is often conducted by administering a high dose that is, enough to cause the animal to die in accordance with a prior toxicity report. As a result, other from a few early pharmacological investigations, there has never been a report on the toxicity of *Syzygium cumini* seed (ESE) and fruit pulp (EFPE) ethanolic extracts. Neither the current trial nor a prior early pharmacological report (Muruganandan et al., 2001) revealed any mortality. As a result, *Syzygium cumini* seed (ESE) and fruit pulp (EFPE) ethanolic extracts at dosage levels of 100 - 5000 mg/kg body weight are regarded as safe or having very low levels of toxicity.

The OECD standards and the study by Clarke and Clarke (1977) both state that drugs with LD50 values of 1000 and 2000 mg/kg body weight are safe. The plant extract had no influence on FOB, mean body weights, or food consumption, indicating that it has no effect on normal behaviour or appetite suppression (Amidaa et al.,2007). During the 14 days of oral therapy with the seed (ESE) and fruit pulp (EFPE) ethanolic extracts of *Syzygium cumini* at dosages of 100, 250, 1000, 2500, and 5000 mg/kg, no toxicity symptoms or fatalities were noted.

Weight loss that is more than 10% of the starting body weight is considered considerable and is an indicator of a drug's negative effects (Teo et al., 2002; Raza et al., 2002; Ramesh, 2007). The lack of toxic symptoms, as well as no changes in water/food intake or weight growth, suggested that the ethanolic extracts of *Syzygium cumini* seeds (ESE) and fruit pulp (EFPE) at the dosages utilized did not significantly alter the mice. The current study found no discernible change in total body weight, confirming the safety of the greater dosage.

Haematological parameters (such as haemoglobin concentration, platelets, red and white blood cells) did not differ between the control and treated groups during the 14-day study. This suggests that the ethanolic extracts of *Syzygium cumini* seeds (ESE) and fruit pulp (EFPE) were not harmful to the circulating red blood cells or interfered with their ability to produce platelets. Even though the hematopoietic system is one of the most vulnerable to harmful substances (Harper, 1973) and a crucial indicator of both human and animal physiological and pathological conditions (Adeneye et al., 2006; Steven and Mylecrdfaine, 1994), hematopoiesis and leukopoiesis were likewise unaffected.

Regardless of the dosage, the majority of the biochemical parameters in the biochemical investigation (such as SGPT, SGOT, bilirubin, albumin, urea, creatinine, and ALP) were also unaffected by the ethanolic extracts of *Syzygium cumini* that were consumed in the form of seed (ESE) and fruit pulp (EFPE). Higher doses of EJ extract up to 2000 mg/kg did not change the rats' hepatocytes and kidneys, nor their normal metabolism, according to the lack of discernible changes in the levels of ALT and ALP, which are functional indicators of the liver and kidney (Hilaly et al., 2004).Even though there were some notable variations in AST, ALP, and creatinine, they could not be lethal because the levels were within normal ranges and/or lower than those of the control group. In conclusion, it was discovered that oral administration of *Syzygium cumini* seed (ESE) and fruit pulp (EFPE) ethanolic extracts was not harmful to the mice's general behaviour or pathophysiological processes. For the duration of the trial, the extract did not seem to have any harmful effects on mice that were physiologically meaningful. Accordingly, the current investigation concluded that ethanolic extracts of *Syzygium cumini* to seeds (ESE) and fruit pulp (EFPE) were safe for mice up to 5000 mg/kg of body weight. Thus, the current investigation offers enough preclinical proof of EJ stem bark extract's safety.

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ETHICAL CLEARANCE STATEMENT: The Current Research Work Was Ethically Approved by the ethical committee of the Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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