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Original Research Article

In-silico and *In-vivo* Long-term Safety Studies of Mangosteen Rind Ethanol Extract (*Garcinia mangostana* Linn.)

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ABSTRACT

Mangosteen rind has been used extensively to treat a variety of diseases. However, research on the use of mangosteen, especially over the long-term, remains limited. The aim of this study was to evaluate the toxicity of ethanol extract of mangosteen rind (EEMR) to ensure its safety. The *in silico* method used the Protox-II web server to test the safety of marker compounds found in EEMR (xanthones, alpha-mangostin and gamma-mangostin). The *in vivo* method involved the oral feeding of EEMR to rats at doses of 100, 200 and 400 mg/kg bw. The researchers observed signs of toxicity and mortality over a period of six months. The study showed a high degree of organ safety, with a safety class of 4 for xanthone and alpha-mangostin and 5 for gamma-mangostin. In addition, consumption of any dose of EEMR to rats showed no toxic effect on body weight, haematology or relative organ weight, nor was it the cause of mortality in the animal model. However, its use at 200 and 400 mg/kg bw influenced triglyceride, GOT and creatinine levels and caused liver cell abnormalities in rats. EEMR 400 mg/kg bw also showed adverse effects on the kidneys. Based on these findings, it can be concluded that the gamma-mangostin compound in mangosteen rind is safer than xanthones and alpha-mangostin, while EMMR at a dose of 100 mg/kg bw is relatively safe for long-term use. However, doses of 200 mg/kg bw and 400 mg/kg bw require further evaluation.

Keywords: Ethanol extract of mangosteen rind, *in silico*, *in vivo*, safety

Introduction

Toxicity tests have been used to determine the toxic effects of a substance on a biological system. The data obtained from toxicity tests can then be used to determine the level of risk that may arise if humans are exposed to the test substance. Toxicity tests in animal models are expected to describe the presence of clinical biochemistry, physiological and pathological responses by several test parameters, generally in the form of macropathological observations of each organ and tissue, haematology, clinical biochemistry, histopathology, and other toxic symptoms that can be described by the behaviour of the test animal or even its mortality.[1] However, it takes a very large amount of time and money, as well as many test animals, to obtain all the toxicological results for a single chemical. Therefore, predicting the toxic effect of a substance computationally (*in silico*) has become an option to reduce the shortcomings of the *in vivo* method.[2] By combining these two methods, *in vivo* and *in silico*, it was expected that it would be possible to describe the toxicology of a drug candidate, especially those derived from plant extracts that contain many chemical compounds.

Traditional medicines have been used to treat various diseases.[3] These substances can be isolated or derived from the plant's bioactive compounds. Each part of the mangosteen rind (pericarp, epicarp and endocarp) is rich in chemical compounds.

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Some of the compounds identified are xanthones, alpha-mangostin and gamma-mangostin, which have been studied for their effects as traditional medicines.[4,5,6]

Mangosteen (*Garcinia mangostana* Linn.) has been used in medicine as antidiabetes mellitus,[7] antihepatotoxic,[7] antidyslipidemic,[8] antiobesity,[9] anticancer,[10] antitumorigenic,[11] antiproliferative,[11] antihistamine,[11] antidepressant,[12] anti-inflammatory,[13,14] antioxidant,[15,16,17] analgesic,[14] antibacterial,[15] antipyretic,[14] immunomodulator,[18] renoprotective,[19] neuroprotective,[20] enhances wound healing,[21] and may reduce lung damage.[22] Mangosteen rind extract has also been formulated into various medicinal dosage forms, including lotions, creams and films.[23,24,25] Nevertheless, several studies have reported that certain herbal extracts induce adverse effects while being utilised.[26] Moreover, it is critical to guarantee the safety of these substances during use, including by conducting toxicity tests.[27]

Mangosteen rind has been the subject of several previous studies evaluating its safety. Mangosteen rind ethanol extract can be safely fed to rats at 5000 mg/kg bw for 14 days.[28] Meanwhile, hydro-extracts of mangosteen rind have been shown to alter renal histopathology in female animals when fed at a dose of 100 mg/kg bw for three months.[29] However, consumption of aqueous extract: ethanol (1:1) for three months showed no toxic effect up to a dose of 1200 mg/kg bw, although male rats showed an increase in bilirubin.[30]

A six-month study in Wistar rats treated with ethanol extract of mangosteen rind (EEMR) at doses of 10, 100, 500, and 1000 mg/kg/day showed no apparent pharmacotoxic signs or abnormal haematological values. However, there was an increase in ALT, BUN, and creatinine levels in the test animals when EEMR was fed at a dose of 500 mg/kg/day or 1000 mg/kg/day. Even at a dose of 1000 mg/kg/day, there was a significant increase in AST and hepatocellular degeneration. For this reason, this study stated that EEMR should not be used for long periods of time because it could damage the liver and kidneys.[31] The results of these different studies were predicted to be influenced by the

type of extract and the amount of chemicals in the extract used. Meanwhile, research into the safety of individual compounds in mangosteen rind extract, particularly xanthones, alpha-mangostin and gamma-mangostin, are still limited. Therefore, the aim of the research was to provide a reference for the determination of safe and acceptable EEMR doses, particularly for the safety of long-term human exposure.

Materials and Methods

In Silico

The xanthones, alpha-mangostin, and gamma-mangostin structures from https://pubchem.ncbi.nlm.nih.gov were evaluated using the Protox-II web server that can be accessed at http://tox.charite.de. Toxicity prediction included LD_{50}, mean similarity and prediction accuracy, as well as prediction of organ toxicity (hepatotoxicity) and toxicity endpoints (carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity). The results of the compound toxicity model were categorized as "active" or "inactive".

Experimental Animals

The Institutional Review Board (or Ethics Committee) of the Health Polytechnic Ministry Of Health Bandung approved the protocol for the animal study (protocol code 02/KEPK/EC/II/2022 and 22 February 2022). Prior to the start of the experiment, male and female Wistar rats, aged 8-12 weeks and weighing 250-300 g, were fasted and given ad libitum access to water. The animals were observed for fourteen days to ensure their adaptation to the new environment. In addition, the experimental animals were divided into 4 groups according to dose (1 normal control group and 3 EEMR groups). Each group was randomly assigned and consisted of 10 male and 10 female rats. After fasting for approximately 14 hours, the animals were weighed and dosed with the test substance. The test substance was fed orally in a single dose of 1 ml per 100 g of rat body weight.[1] Rats were dosed daily, or at least five days per week, for six months.

Collection of Plant Material

Borobudur Herbal Medicine Industry, Indonesia, provided this extract (date of manufacture: 7 November 2019). This extract complied with various parameters in the standardisation of the extract based on the regulations of the National Agency of Drug and Food Control of the Republic of Indonesia.[32] Borobudur Herbal Medicine Industry, as the manufacturer, conducted this test on 8 January 2021.

Preparation of Plant Extract

Maceration is used to extract mangosteen peel powder with 70% ethanol. After that, use the freeze-drying technique to concentrate the extract until 500 mg of dry EEMR is obtained. EEMR was suspended in Na.CMC (Natrium Carboxymyle Cellulose) 0.3% with 3 tested doses: EEMR at a dose of 100 mg/kgbb, 200 mg/kgbb and 400 mg/kgbb.

Observation of 6 Months Toxicity Study (In Vivo)

Rat weight gain was monitored twice per week. Rats were weighed daily using an SF-400® digital scale (Surya Fajar, Indonesia) to determine test substance dose. Meanwhile, food consumption was weighed every two days.[1]

Observations of toxic and clinical symptoms in the form of changes in skin, hair, eyes, mucous membranes, secretions, excretions, changes in gait, abnormal behaviour (e.g. walking backwards), convulsions, etc.[1] were conducted daily for 6 months, including observations of mortality in the test animals.

Rats have historically been favoured as animal models for biomedical research on account of their genetic, physiological, and anatomical resemblance to humans. Rodent advantages consist of their small size, effortless upkeep requirements, abbreviated life cycle, and ample genetic material.[33] Each rat was never allowed to come into contact with water before death (to avoid hemolysis) and its blood was collected using a sterile syringe. A syringe was used to slowly withdraw blood from the animals' jugular veins, up to 3 to 5 mL per animal. For haematological examination, a total of 0.5 mL of blood was placed in a microcentrifuge tube (Biologix®, Biologix Group Limited, Indonesia) filled with anticoagulant (EDTA) up to 10 L, and a blood smear was made with 0.5 mL of blood to determine differential leukocytes. It was then placed at room temperature (30°C) for 10 minutes before being transferred to an ice box (Marina Cooler®, Lion Star, Indonesia) and centrifuged at 3000 rpm to obtain the final product, which was dissolved in water and stored in ice cube trays. Serum was centrifuged and stored at low temperature (-20°C)[1] for clinical biochemistry tests.

All rats were euthanized in their cages (closed containers), which were then filled with 100% carbon dioxide (CO_2). Filling was carried out at a rate of 30-70% of the chamber volume per minute to achieve a balanced gas mixture to induce rapid unconsciousness with minimal animal discomfort.[34] The rats' organs were also removed, weighed and counted against their body weight. Organs which were collected from male rats including liver, heart, lungs, kidneys, adrenals, spleen and testes. In female rats, on the other hand, the observed organs were the liver, heart, lungs, kidneys, adrenal glands, spleen and ovaries. The isolated liver, heart, lung, kidney and spleen were then immediately placed in 10% formaldehyde buffer solution and histopathological preparations were made for examination under a microscope[1] (XSZ-107BN®, ReHaze, China). Unused animal carcasses were stored in a special freezer (AQUA AQF-200W®, Aqua, Japan) in the animal laboratory of the ITB School of Pharmacy.

Statistical Analysis

Data were collected and analyzed using SPSS 20 statistical program with one-way ANOVA, Tukey HSD analysis and $p<0.05$.

Result and Discussion

Through several studies that have been observed, mangosteen rind is one of the plants that is strongly predicted to have a role in the treatment of various diseases.[32] In addition to the efficacy and quality of its use, the different doses used in the treatment of this disease are important variables in determining the safety factor for its use.[1] In this case, toxicity assessment using computational tools, known as "*in silico toxicology*" (computational toxicology), becomes crucial as it can predict the toxicity of a chemical even before it is synthesised.[33] A prediction of acute toxicity was carried out on marker compounds found in mangosteen rind extract. It is expected to provide an overview of the adverse effects of either a single exposure or multiple exposures over a short period of time.[35]

The structure of each xanthone, alpha-mangostin and gamma-mangostin compound used for *in silico* toxicity prediction was obtained from https://pubchem.ncbi.nlm.nih.gov. (Figure 1). Each structure of the three compounds was analysed for toxicity using the Protox-II Web Server, a virtual laboratory for small molecule toxicity prediction. The Protox-II Web Server can be accessed via https://tox-new.charite.de/protox_II.[36] The The results of the analysis, including the average prediction similarity and prediction accuracy, are shown in Table 1. The average similarity and prediction accuracy for each compound was over 50% on average, so it can be concluded that xanthones, alpha-mangostin and gamma-mangostin are similar to compounds in the database. The predicted LD_{50} value and toxicity class for each compound are shown in Table 2. The xanthone compound is toxic at 1680 mg/kg (class 4), alpha-mangostin is toxic at 1500 mg/kg (class 4) and gamma-mangostin is toxic at 3200 mg/kg (class 5).[37,38] Based on these results, gamma-mangostin is found to be less toxic than xanthones and alpha-mangostin.

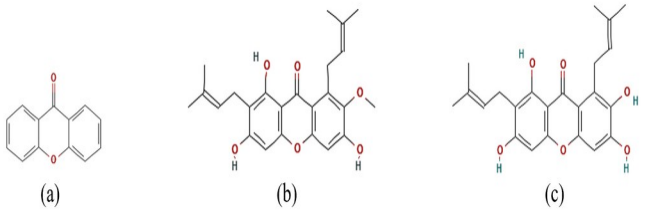

Figure 1: The two-dimensional structure of the xanthone compounds (a), alpha-mangostin (b), and gamma-mangostin (c)

Furthermore, predicted organ toxicity and toxic endpoints (Table 3) support this claim. Based on the predictions of organ toxicity and toxic

endpoints, xanthone compounds are predicted to be carcinogenic, mutagenic and cytotoxic at certain levels. The alpha-mangostin compound is active as an immunotoxicant, whereas the gamma-mangostin compound shows no toxic effects.

By estimating *in silico* the toxicity of marker compounds found in mangosteen rind, we expect to be able to estimate the toxicity of compounds that play a role in how harmful it is to use mangosteen rind to treat disease. It is also expected that the consumption of the combination of these three compounds simultaneously, will minimize the toxic properties of each compound, as demonstrated by testing the toxicity of mangosteen rind extract containing these three compounds on experimental animals (*in vivo*).

Although *in silico* toxicity studies are very useful and can overcome the shortcomings of the *in vivo* method in terms of time, cost and use of animals, the overall biological state (pharmacokinetics and pharmacodynamics) of the test material may not be available or inaccurate,[39] and it may require specialised software or tools and skills. This is particularly valid for test materials such as plant extracts, which contain many complex compounds that may interact with each other.[40] Therefore, an *in vivo* method is needed and expected to complement the toxicity prediction provided by the *in silico* method.

The results of the acute toxicity test indicated that the ethanolic extract of mangosteen rind was not toxic to rats at a dose of 5000 mg/kg bw for 14 days.[28] However, this finding needs to be confirmed by research, particularly *in vivo* methods to determine safe doses for long-term use.

For approximately 6 months, the use of EEMR at a dose of 500 mg/kg bw in rats could cause a decrease in renal function by several test parameters in the rats used.[31] Therefore, the dose selection in this study used several dose levels, the highest being 400 mg/kg bw in rats. This finding is also supported by several published journals and herbal medicines currently on the market, which use a dose of 400 mg/kg bw as a reference for daily use.[41,42]

The results of this study indicated that giving EEMR to the test animals at each dose level did not cause any significant signs of toxicity based on behavior and vital signs and did not cause mortality in rats. This was also confirmed by the observation of the body weights of the rats over the 6-month period (Figures 2 and 3), which showed that there was no significant difference ($p<0.05$) between the group treated with EEMR and the control group.

Observations on the biochemical examination of rat blood serum were carried out on the last day of the study, including examination of cholesterol, triglyceride, glucose, GPT (glutamic pyruvic transaminase), GOT (glutamic oxaloacetic transaminase), urea and creatinine (Tables 4 and 5). The results of these observations showed a significant difference ($p<0.05$) between the groups of animals given EEMR, especially at the highest dose of extract (400 mg/kg bw), compared with the control group, as indicated by the levels of triglycerides, GOT and creatinine. However, this difference was only seen in female rats and not in male rats. This may be due to the effect of the extract, which has been explained by various research findings that giving EEMR to test animals could reduce triglyceride levels,[43,44] presumably due to the presence of alpha-mangostin in the test sample.[45] Meanwhile, GOT, commonly called AST (aspartate transaminase), is an enzyme that acts as a marker of liver function.[46] The results of the monitoring of GOT levels differed from those of the study, which found no significant difference ($p<0.05$) in the test animals, even at a dose of 400 mg/kg bw.[47] This was presumably due to differences in the contents of the diets consumed. In addition, the increase in urea levels (although not significant) in female rats at the highest test extract dose (400 mg/kg bw) was considered to be an indication of impaired renal function, as shown in the study which justified the 500 mg/kg bw EEMR dose, although this was not supported by an increase in creatinine levels[31] as shown by the results of this study.

Table 1: Prediction of average similarity and accuracy

Compounds	Average similarity (%)	Prediction Accuracy (%)
Xanthone	67.67	68.07
Alpha-mangostin	54.50	67.38
Gamma-mangostin	53.58	67.38

Table 2: Prediction of LD_{50} and class of toxicity

Compounds	LD_{50} (mg/kg)	Class of Toxicty
Xanthone	1680	4
Alpha-mangostin	1500	4
Gamma-mangostin	3200	5

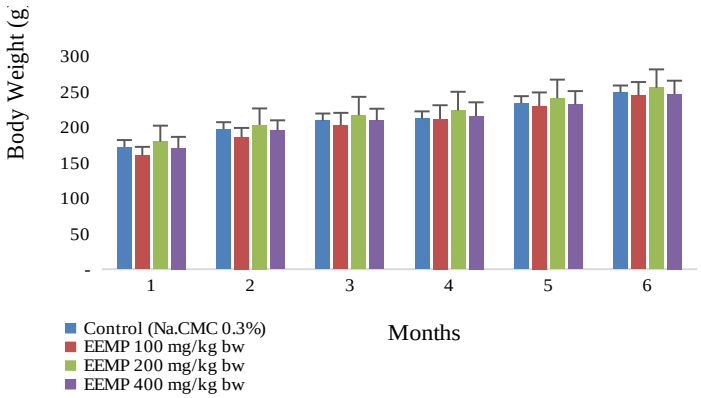

Figure 2: Weight chart of male rats

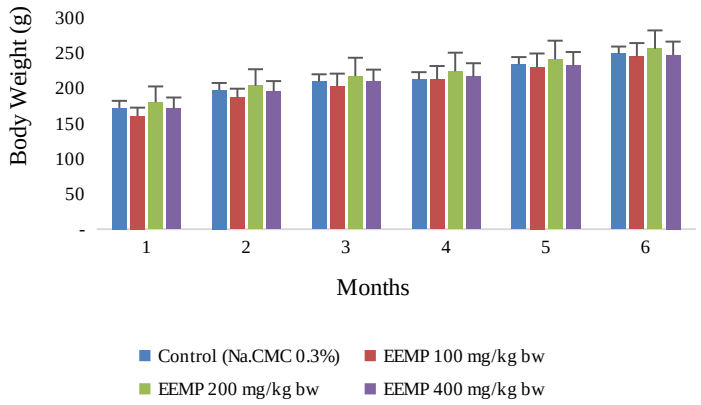

Figure 3: Weight chart of female rats

Table 3: Prediction of organ toxicity and toxicity endpoints

Compounds	Hep	Car	Imm	Mut	Cyt
Xantone	Inactive	Active	Inactive	Active	Active
Alpha-mangostin	Inactive	Inactive	Active	Inactive	Inactive
Gamma-mangostin	Inactive	Inactive	Inactive	Inactive	Inactive

Hep (Hepatotoxicity), Car (Carcinogenicity), Imm (Immunotoxicity), Mut (Mutagenicity), Cyt (Cytotoxicity)

Urea and creatinine are commonly used for screening tests of renal function, which essentially reflect glomerular filtration rate (GFR). However, at all stages of renal failure, serum creatinine is a much more reliable indicator of renal function than urea, because urea is more

likely to be influenced by diet and physiological conditions unrelated to renal function.[48]

Observations were made on the haematological parameters of rat blood serum, including Hb (haemoglobin), WBC (white blood cells), MCH (mean cell haemoglobin), MCHC (mean cell haemoglobin concentration), RBC (red blood cells), Hct (haematocrit), MCV (mean cell volume), RDW (red cell distribution width), PLT (platelets) and MPV (mean platelet volume). This is shown in Tables 6 and 7. Haematological parameters are one of the most important parameters in assessing the toxicity of a test sample. The interaction of the toxin or its metabolites with cellular constituents may result in significant changes in haematological parameters that sooner or later affect the exposed organ or tissue.[49] No clear pharmacotoxic signs or abnormalities in haematological values were found in male or female Wistar rats given EEMR daily for six months at doses of 100, 200 and 400 mg/kg bw.

The rats used in the experiment were necropsied and subjected to a careful macropathological examination of each organ (liver, heart, lungs, kidneys, and spleen). Each dissected organ and tissue were immediately placed in a 10% formaldehyde buffer solution and prepared for histopathology before microscopic examination. Organs to be weighed (absolute weight) were first dried with absorbent paper before being weighed. The relative weight analysed is the absolute organ weight divided by the body weight.[1] The data obtained were statistically analysed using the method of analysis of variation (ANOVA), as shown in Tables 8 and 9. Based on the observations, there was no significant difference ($p<0.05$) between the extract groups at different doses when compared to the control group.

In addition to their relative weights, the histology of these organs was examined, as shown in Figures 4 and 5. Necrosis of hepatocytes was observed in male animals at the 400 mg/kg bw dose. This was also seen in the liver histology of female animals at a dose of 200 mg/kg bw and it was much more pronounced at 400 mg/kg bw. In addition, renal histology showed thickened glomeruli with thickened basement membrane, proliferating mesangial cells and absence of Bowman's space in male rats given the test extract at 400 mg/kg bw. This supports previous findings on blood biochemical parameters, which indicated that at this dose there were abnormal values in several test parameters related to liver and kidney function. Meanwhile, other organs (heart, lungs and spleen) showed no damage at any dose of the extract. This suggests that the use of extract doses needs to be monitored, particularly at a dose of 400 mg/kg bw when used over a long period, e.g. 6 months of treatment. In addition, this study suggests that the safest dose of extract for long-term use is 100 mg/kg bw.

Conclusion

The gamma-mangostin compound in mangosteen rind is safer than xanthones and alpha-mangostin. Research into the molecular dynamics of each compound against specific receptors is expected to further support this conclusion. The recommended dose of EEKM for long-term use is 100 mg/kg bw, while use at doses of 200 and 400 mg/kg bw for more than 6 months in rats requires monitoring. These findings need to be supported by clinical studies, so that it can be widely used by humans

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 4: Male rats clinical biochemistry parameters

Parameters	Control (Na.CMC 0.3%)	EEMR 100 mg/kg bw	EEMR 200 mg/kg bw	EEMR 400 mg/kg bw
Chol (mg/dL)	46.33 ± 7.48	45.14 ± 10.32	45.80 ± 4.91	51.57 ± 7.63
Tri (mg/dL)	114.67 ± 3.83	100.67 ± 15.54	98.33 ± 8.39	106.20 ± 8.04
Gluc (mg/dL)	107.33 ± 5.51	112.33 ± 26.27	91.00 ± 29.70	85.67 ± 5.03
SGPT (U/L)	36.76 ± 9.2	37.47 ± 11.01	31.93 ± 4.6	34.95 ± 4.71
SGOT (U/L)	91.99 ± 20.99	81.20 ± 10.13	79.60 ± 12.73	95.88 ± 23.09
Urea (mg/dL)	16.85 ± 4.17	17.33 ± 2.95	17.40 ± 3.82	18.43 ± 3.87
Crea (mg/dL)	0.34 ± 0.06	0.32 ± 0.04	0.39 ± 0.14	0.43 ± 0.13

*($p<0.05$) vs. male rats control group, Chol (Cholesterol), Tri (Triglicerides), Gluc (Glucosa), GPT (*Glutamate Pyruvate Transaminase)*, GOT (*Glutamic Oxaloacetic Transaminase*), Crea (Creatinine).

Table 5: Female rats clinical biochemistry parameters

Parameters	Control (Na.CMC 0.3%)	EEMR 100 mg/kg bw	EEMR 200 mg/kg bw	EEMR 400 mg/kg bw
Chol (mg/dL)	52.50 ± 16.26	50.01 ± 12.72	40.00 ± 5.65	49.00 ± 22.63
Tri (mg/dL)	143.25 ± 0.35	116.75 ± 1.06	125.50 ± 9.89	95.50 ± 12.02*
Gluc (mg/dL)	130.65 ± 16.05	113.57 ± 15.93	102.08 ± 29.95	90.19 ± 10.69
SGPT (U/L)	42.54 ± 3.13	47.30 ± 16.62	44.13 ± 11.21	34.19 ± 0.78
SGOT (U/L)	84.60 ± 7.21	74.55 ± 7.14	46.15 ± 4.59*	48.18 ± 5.41*
Urea (mg/dL)	19.63 ± 0.39	24.65 ± 0.39	25.20 ± 2.69	31.50 ± 7.78
Crea (mg/dL)	0.42 ± 0.08	0.58 ± 0.10	0.57 ± 0.04	0.85 ± 0.07*

*($p<0.05$) vs. male rats control group, Chol (Cholesterol), Tri (Triglicerides), Gluc (Glucosa), GPT (*Glutamate Pyruvate Transaminase)*, GOT (*Glutamic Oxaloacetic Transaminase*), Crea (Creatinine).

Table 6: Male rats hematological parameters

Parameters	Groups			
	(Na.CMC 0.3%)	EEMR 100 mg/kg bw	EEMR 200 mg/kg bw	EEMR 400 mg/kg bw
Hb (g/dL)	14.49 ± 0.39	14.68 ± 1.64	14.40 ± 2.30	14.37 ± 0.45
WBC (10^3/mL)	4.53 ± 1.35	7.30 ± 0.85	6.73 ± 2.99	4.59 ± 1.21
MCH (pg)	18.83 ± 5.51	18.87 ± 0.40	19.02 ± 0.68	18.34 ± 0.99
MCHC (g/dL)	33.42 ± 5.52	32.74 ± 5.99	33.06 ± 1.54	35.17 ± 5.59
RBC (10^6/mL)	7.79 ± 0.23	8.41 ± 0.79	8.56 ± 1.56	7.74 ± 0.34
Hct (%)	41.19 ± 2.19	41.14 ± 1.47	39.56 ± 1.04	39.37 ± 1.40
MCV (mm^3)	47.30 ± 0.94	50.36 ± 3.40	51.63 ± 6.07	46.83 ± 1.40
RDW (%)	14.90 ± 1.09	14.23 ± 3.03	15.72 ± 0.81	14.84 ± 0.66
PLT (10^3/mL)	676.11 ± 18.29	674.00 ± 15.72	680.33 ± 33.55	653.78 ± 30.31
MPV (mm^3)	7.00 ± 0.17	7.13 ± 0.15	7.33 ± 0.15	7.21 ± 0.25

Hb (Hemoglobin), WBC (White Blood Cells), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), RBC (Red Blood Cell), HCT (Hematocrit), MCV (Mean Corpuscular Volume), RDW (Red Cell Distribution Width), PLT (Platelet Count), MPV (Mean Platelet Volume).

Table 7: Female rats hematological parameters

Parameters	Groups			
	(Na.CMC 0.3%)	EEMR 100 mg/kg bw	EEMR 200 mg/kg bw	EEMR 400 mg/kg bw
HB (g/dL)	14.35 ± 0.21	14.20 ± 0.99	14.60 ± 0.14	14.45 ± 0.35
WBC (10^3/mL)	4.75 ± 0.07	4.65 ± 0.21	4.78 ± 0.25	6.15 ± 0.92
MCH (pg)	20.20 ± 1.13	19.65 ± 2.76	19.05 ± 2.33	19.30 ± 1.27
MCHC (g/dL)	37.00 ± 0.71	35.00 ± 2.12	36.35 ± 1.20	35.60 ± 1.13
RBC (10^6/mL)	7.37 ± 0.25	7.70 ± 0.71	7.86 ± 0.22	7.81 ± 0.29
HCT (%)	41.90 ± 0.57	41.95 ± 2.33	41.93 ± 0.60	42.25 ± 0.92
MCV (mm^3)	54.30 ± 1.70	54.75 ± 3.89	50.35 ± 1.06	53.55 ± 1.63
RDW (%)	12.55 ± 2.05	11.95 ± 2.05	14.20 ± 0.99	13.20 ± 0.14
PLT (10^3/mL)	662.50 ± 6.63	662.75 ± 1.77	675.50 ± 0.71	671.50 ± 10.61
MPV (mm^3)	7.25 ± 0.50	7.00 ± 0.57	7.55 ± 0.63	7.35 ± 0.07

Hb (Hemoglobin), WBC (White Blood Cells), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), RBC (Red Blood Cell), HCT (Hematocrit), MCV (Mean Corpuscular Volume), RDW (Red Cell Distribution Width), PLT (Platelet Count), MPV (Mean Platelet Volume).

Table 8: Organ weight on macropathological observations of male rats

Organs	Groups			
	Control (Na.CMC 0.3%)	EEMR 100 mg/kg bw	EEMR 200 mg/kg bw	EEMR 400 mg/kg bw
Liver	2.71 ± 0.27	2.62 ± 0.38	3.09 ± 0.27	3.13 ± 0.50
Heart	0.31 ± 0.05	0.29 ± 0.06	0.31 ± 0.04	0.39 ± 0.12
Lungs	0.74 ± 0.12	0.64 ± 0.17	0.68 ± 0.14	0.83 ± 0.20
Kidneys	0.63 ± 0.07	0.59 ± 0.05	0.71 ± 0.09	0.73 ± 0.16
Adrenal Glands	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Spleen	0.38 ± 0.07	0.37 ± 0.09	0.40 ± 0.07	0.40 ± 0.16
Testicles	0.89 ± 0.10	0.92 ± 0.10	0.94 ± 0.21	0.87 ± 0.17

Table 9: Organ weight on macropathological observations of female rats

Organs	Groups			
	Control (Na.CMC 0.3%)	EEMR 100 mg/kg bw	EEMR 200 mg/kg bw	EEMR 400 mg/kg bw
Liver	3.86 ± 0.76	4.03 ± 0.13	4.01 ± 0.81	3.51 ± 1.23

Heart	0.39 ± 0.05	0.34 ± 0.10	0.33 ± 0.07	0.32 ± 0.03
Lungs	0.84 ± 0.04	0.54 ± 0.23	0.54 ± 0.27	0.51 ± 0.29
Kidneys	0.70 ± 0.15	0.83 ± 0.13	0.70 ± 0.13	0.77 ± 0.16
Adrenal Glands	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.02
Spleen	0.41 ± 0.01	0.41 ± 0.10	0.48 ± 0.03	0.40 ± 0.09
Testicles	0.38 ± 0.07	0.45 ± 0.06	0.52 ± 0.05	0.64 ± 0.22

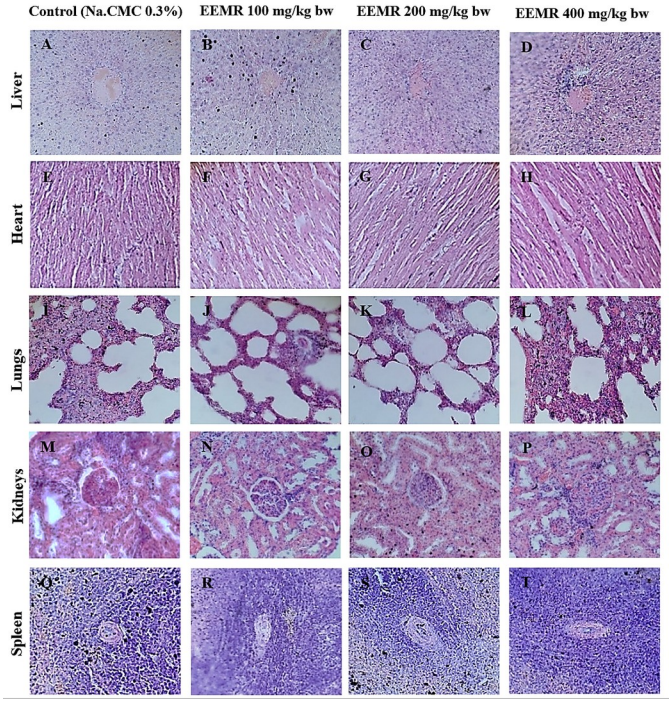

Figure 4: Histopathological examination (HE, 400×) liver (A-D), heart (E-H), lungs (I-L), kidneys (M-P), and spleen (Q-T) in male rats.

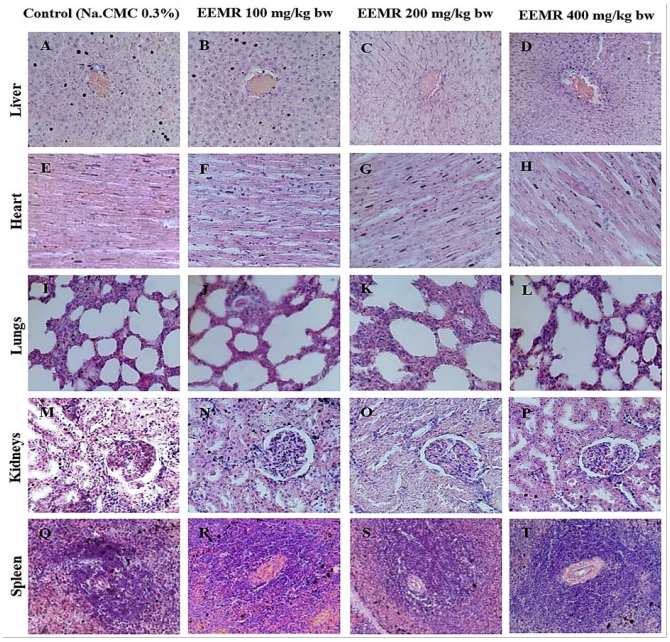

Figure 5: Histopathological examination (HE, 400×) liver (A-D), heart (E-H), lungs (I-L), kidneys (M-P), and spleen (Q-T) in female rats.

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