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Research Article

Subchronic Toxicity of *Garcinia Xanthochymus* Fruit Bark Extract to Female Rats: Effect on Liver and Kidney

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A B S T R A C T

Objective: *Garcinia Xanthochymus* Fruit Bark has been pharmacologically proven as beneficial as an antidiabetic, antioxidant, detoxifier, and antibacterial agent.

Methods: Subchronic toxicity evaluations were performed for 28 days for the control and experimental groups, and for 42 days for the satellite groups. This study employed 30 female white rats, divided into six treatment groups: the control group (Na cmc 0.5%), the test group receiving ethanol extract of kandis tamarind fruit bark at doses of 250, 500, and 1000 mg/KgBW, the control satellite group (Na cmc 0.5%), and the high-dose satellite group (1000 mg/KgBW). Daily observations included toxic symptoms, body weight, mortality, relative organ weight, macropathology, and histopathology of hepatic tissues.

Results: The subchronic toxicity test results demonstrated that the ethanol extract of *Garcinia Xanthochymus* Fruit Bark, administered at a satellite dose of 1000 mg/KgBW, induced toxic symptoms such as weakness and mortality in test subjects by the fourth week, along with significant macropathological changes in the liver marked by white spots. Histopathological analyses of liver and kidney tissues in the test group administered a dosage of 250 mg/KgBW showed no signs of tissue damage. The test group administered doses of 500 and 1000 mg/KgBW, together with the satellite group at 1000 mg/KgBW, had hepatic tissue damage marked by sinusoidal dilation, cellular necrosis, and central venous alterations.

Conclusion: The findings showed showed that *Garcinia Xanthochymus* Fruit Bark can hurt tissues at doses of 500 and 1000 mg/KgBW. The liver tissue damage was shown by changes in central veins, sinusoidal swelling, and cell death.

Keywords: Subchronic toxicity,*Garcinia Xanthochymus,* Liver histopathology,Kidney histopathology

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INTRODUCTION

Indonesian culture includes traditional medicine. It has been used for ages to prevent, treat, and improve health. Traditional medicine is still utilized in Indonesia and other nations based on inherited and empirical facts [1]. *Garcinia xanthochymus*, known as Kandis acid, is a widespread species in Southeast Asia and belongs to the Clusiaceae family [2]. *Garcinia xanthochymus* is a popular seasoning in western Sumatra [3]. Ethyl acetate fraction with phenolic chemicals, flavonoids, alkaloids, and saponins in *Garcinia xanthochymus* fruit is an antioxidant. *Garcinia xanthochymus* includes xanthan, isocoumarins, and

benzophenone, which contain antibacterial, antioxidant, detoxifying, and antidiabetic properties. Antioxidants limit oxidation by interacting with reactive free radicals [4].

Flavonoid, glycoside, steroid, and triterpenoid components in *Garcinia xanthochymus* have antioxidant and anti-inflammatory properties [5, 6]. Garcinia parvifolia (Miq) fruit Bark ethanol extract contains polyphenolic, flavonoid, and triterpenoid chemicals, according to prior research [7]. A study on the effects of ethyl acetate fraction *of Garcinia xanthochymus* fruit Bark on female white mice's liver and renal function found that the dose directly affected SGPT activity and serum creatine levels ($p < 0.05$) [8].

Toxicity tests measure a substance's toxicity to biological systems and collect typical dose-response data[10]. After 28 days, the sub-chronic toxicity test examines critical organs such the liver and kidneys and assesses a compound's overall effect on test animals [9]. One parameter is the test animals' crucial organ histopathology. To scientifically explain disease following repeated doses of the test agent over a predetermined time [10].

To assess a compound's impact on biological and nonbiological activities, toxicity testing is crucial. A plant having pharmacological potential must be tested before being developed into a product to meet regional or national distribution and licensing criteria [11].

MATERIALS AND METHODS

Laboratory glassware (beaker glass (pyrex), measuring flask (pyrex), measuring cup (Pyrex), watch glass), mortar and pestle, spatula, light microscope, analytical balance, oral sonde, set of surgical tools, animal balance, syringe, parchment paper, aluminum foil, dropper pipette, microtome, and tissue. The materials used in this study include plant materials and chemicals. The plant material used is *Garcinia xanthorhycimus* fruit Bark. The chemicals used are distilled water, Na-CMC 0.5%, 70% ethanol, 10% formaldehyde, and 0.9% sodium chloride, chloroform, and sodium chloride.

Preparation of Ethanol Extract *of Garcinia Xanthochymus* Fruit Bark

The method used is maceration with a ratio of (1: 10), which is as much as 500 g of simplicia powder with 5 liters of solvent. First, put the simplistic powder into a container, then soak it with 75 parts of 70% ethanol solvent, as much as 3.75 liters. The container is covered with aluminum foil and left for 5 days while stirring occasionally, then filtered with filter paper to produce filtrate and residue. The residue is then soaked again with 25 parts of the remaining 70% ethanol, as much as 1.25 liters, then the container is covered with aluminum foil and left for 2 days while stirring every 2 hours. After 2 days, the sample was filtered to produce filtrate and residue. Filtrate 1 and filtrate 2 were mixed, then the ethanol liquid extract obtained was evaporated using a vacuum rotary evaporator at a temperature of 40-50ºC until a thick extract was obtained [12, 13].

Preparation of Test Material Suspension

Before testing on experimental animals, the extract obtained from the extraction of kandis tamarind fruit Bark is made into a suspension to be given orally to rats. Suspensions are preparations containing solid medicinal substances in a fine and insoluble form dispersed in a carrier liquid [14].

Preparation of 0.5% Na-Cmc Suspension

A total of 0.5 g of Na-CMC was sprinkled into a mortar containing 10 ml of hot distilled water. Let stand for 15 minutes and then crushed until a transparent mass is obtained, then crushed until homogeneous, diluted with distilled water, homogenized and put into a 100 mL volumetric flask, sufficient volume with distilled water to 100 mL [15].

Preparation of Suspension of Ethanol Extract Of *Garcinia Xanthochymus*fruit Bark

In the test, 3 dose variations will be used, namely doses of 250 mg/kg bw, 500 mg/kg bw, and 1000 mg/kg bw. A number of 250 mg, 500 mg, and 1000 mg of ethanol extract of*Garcinia xanthochymus*fruit Bark was put into a mortar and added 0.5% b/v Na-CMC suspension little by little while crushed until homogeneous up to 10 mL [16].

Grouping of Test Animals and Administration of Test Preparations

The test animals used were 30 healthy female white rats divided into six treatment groups, each comprising five rats. The division of the test animal groups is as follows:

1. Group I: Control, given a 0.5% Na-CMC suspension
2. Group II: Treatment, given EEGXFB at a dose of 250 mg/ KgBW
3. Group III: Treatment, given EEGXFB at a dose of 500 mg/ KgBW
4. Group IV: Treatment, given EEGXFB at a dose of 1000 mg/ KgBW
5. Group V: Satellite, given 0.5% Na-CMC suspension
6. Group VI: Satellite, administered EEGXFB at 1000 mg/ KgBW

The test preparation was administered orally using an oral sonde every day for 28 days, and observation continued for the satellite group until 42 days [17].

Observation of Subchronic Toxicity

Toxic Symptoms

Oral test preparation occurred daily for 28 days. Two hours were observed after the test preparation had been given. Observations of toxic and clinical signs including tremor, salivation, diarrhea, weakness, animal motions like walking backward and using the stomach. The observation methods include:

a. Salivation
   The salivary expenditure of rats administered an ethanol extract of Garcinia xanthochymus fruit Bark is compared

to the control using filter paper.

b. Diarrhea
   Fecal output of rats administered Garcinia xanthochymus fruit Bark ethanol extract vs. control utilizing filter paper.
c. Tremor
   Ethanol extract of Garcinia xanthochymus fruit Bark causes tremors in animals.
d. Limp
   Animals administered Garcinia xanthochymus fruit Bark ethanol extract are monitored for general activity.
e. Animal Movement
f. In animals administered ethanol extract of Garcinia xanthochymus fruit Bark, stomach-walking and reverse walking are observed [18].

Body Weight

Mice weights were taken weekly for 28 days to establish test preparation volume. Weight changes were monitored weekly. Survival animals were weighed and slaughtered after the study [19].

Animal Mortality

Rat mortality was observed from day 1 to day 28, and rats that died during the time of administration of the test preparation were immediately autopsied [20].

Relative Organ Weight

Liver and kidney organs to be weighed (absolute) should be dried first with absorbent paper and then weighed immediately [21].

Macroscopic Observation of Liver and Kidney Organs

Dead mice are immediately autopsied and their livers and kidneys examined. Visual observations include liver and kidney color, surface, and consistency [22].

Histopathology of Liver and Kidney Organs

Rats that died were immediately removed from their organs. At the end of the period of administration of the test preparation, all living rats were autopsied. The liver and kidney organs were washed with 0.9% sodium chloride and placed in a 10% formaldehyde diaper solution. Histopathological preparations were made and then viewed under a microscope [23]. A histopathological examination was performed based on the procedures applied in the Histology Laboratory of the University of North Sumatra.

Data Analysis

Data on the number of dead test animals were analyzed statistically using SPSS with the Two-Way Analysis of Variance (ANOVA) method followed by Tukey's posthoc test todetermine significant differences in body weight and relative organ weight.

RESULTS AND DISCUSSION

Subchronic Toxicity Testing Results of Observation of Toxic Symptoms

Talel 1. ObservationofToxicSymptoms

Treatment	Na Cmc 0,5%	EEGXFB 250 Mg/Kgbb	EEGXFB 500 Mg/Kgbb	EEGXFB 1000 Mg/Kgbb	Na CMC 0,5%	EEGXFB 1000 Mg/Kgbb
Salivation	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-
Tremor	-	-	-	-	-	-
Weakness	-	-	-	+	-	+
Backward walking	-	-	-	-	-	-
Abdominal walking	-	-	-	-	-	-

The experimental animals used for this subchronic toxicity test study were 24 female white rats divided into six treatment groups. The number of test animals was increased by 1 in each treatment group to avoid animal death during acclimation. Previously, acclimation was carried out first on test animals in the Pharmacology Laboratory of the University of North Sumatra for 7 days to avoid stress during treatment. During the acclimation process, 3 test animals were found dead and separated from the surviving test animals [24].

If the preparation stage has been completed, then the test is carried out by giving 0.5% Na cmc suspension as negative control and satellite, suspension of ethanol extract of *Garcinia xanthochymus* fruit bark as treatment and satellite at a dose of 250 mg / KgBW, 500 mg / KgBW, 1000 mg / KgBW. The test preparation was administered for 28 days for each group, and observations were made on day 29 for the control and treatment groups. Observations continued on day 42 for the satellite group [24].

The toxic effects observed in table 1 show that rat test animals have normal activity in the control group with 0.5% Na cmc, the treatment group with 250 mg/KgBW and 500 mg/KgBW, and the satellite group. After that, rat test animals in the treatment group at 1000 mg / KgBW and the satellite

group at the highest dose showed abnormal activity like limping, demonstrating the relationship between dose and toxic effects. Due to dose, substances can induce side, unfavorable, and hazardous consequences [25].

BODY WEIGHT OBSERVATION RESULTS

Table 2: Results Average Body Weight of Rats

	Averagebodyweight (g) ± SD					
	1	**2**	**3**	**4**	**5**	**6**
G1	127.75 ±18.08	130.50 ±18.21	140.25 ±15.96	145.50 ±12.81		
G2	127 ±4.96	133 ±5.35	141.75 ±8.65	153.50 ±11.03		
G3	126.75 ±1,5	135.50 ±5.80	136.50 ±7.93	143.75 ±8.01		
G4	151 ±22.90	181 ±24.50	180.50 ±21.87	180.50 ±19.36		
G5	123.25 ±4.64	137.25 ±10.59	146.25 ±12.84	140.75 ±20.45	132.50 ±21.79	136.75 ±20.91
G6	114.75 ±12.57	126.75 ±13.25	135.50 ±12.23	131.50 ±18.80	101.50 ±8.73	102.75 ±8.18

The results of weekly observations of the body weight of rat test animals showed that in each test group, from the first to the fourth week, the body weight of the rats increased. Furthermore, the body weight of rats in the satellite group experienced weight loss in the fifth and sixth weeks [26].

The statistical test of rat body weight and normality showed that the control group had a significance value of p=0.957, the dose group had 329, the dose group had 224, the dose group had 301, and the satellite group had 795. Satellite group dose of 1000 mg/KgBW has p=0.251 significance value. With p = 0.027 (p>0.05), the control, treatment, and satellite groups did not differ. This reveals that giving rats ethanol extract of Garcinia Xanthochymus fruit bark for 28 days for the control and treatment groups and 42 days for the satellite group did not change body weight [27]. Toxic symptoms and body weight indicate poisoning. Test animals are monitored daily for harmful signs and weighed occasionally. Reduced weight growth is a simple but sensitive hazardous effect indicator [27].

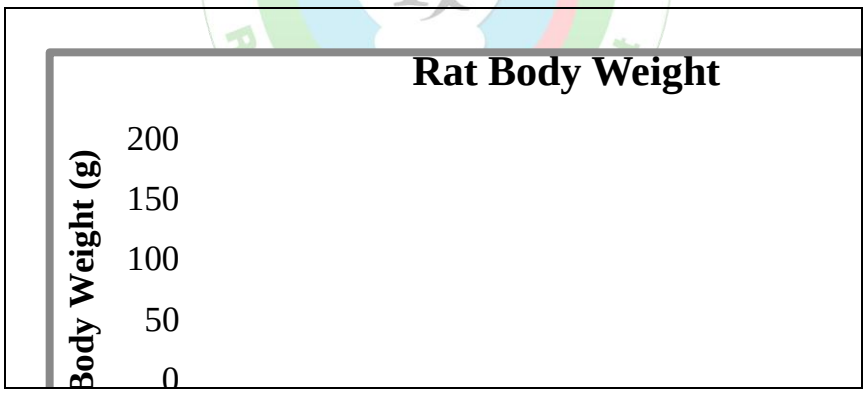


Figure 1. Body Weight Chart of Rats

Observation of Mortality of Test Animals

Table 3: Mortality Observation Results of Test Animals

Treatment	**Number of Rats**	**Number of Dead Rats**
Na cmc 0,5%	4	0
EEGXFB 250 mg/kgBW	4	0
EEGXFB 500 mg/kgBW	4	0
EEGXFB 1000 mg/kgBW	4	2
Satellite, Na cmc 0.5%	4	0
Satellite, EEGXFB 1000 mg/kgBW	4	1

The test preparation at 250 mg/KgBW, 500 mg/KgBW, and satellite Na cmc 0.5% did not kill rats in the control group. In contrast, two rats died in week 4, day 4 at a dose of 1000 mg / KgBW, and one died in week 4, day 5 in the satellite group. Because rats are given a suspension of ethanol extract of kandis tamarind fruit bark daily for 28 days, organ damage can kill them. Secondary metabolite chemicals in Garcinia Xanthochymus fruit bark ethanol extract kill rats. Flavonoids are poisonous and can kill in big concentrations. A drug is dangerous, and the dose and manner of delivery influence poisoning, although the dose is most significant [28].

OBSERVATION RESULTS OF RELATIVE ORGAN WEIGHT

Table 4: Observation Results of Relative Organ Weight

Group	RelativeOrganWeight (g)		
	Liver	Left kidney	Right kidney
Na cmc 0,5%	6.80±0.44	0.64±0.01	0.72±0.02
Dosage 250 mg/kgBW	6.01±0.24	0.6±0..8	0.66±0.05
Dosage 500 mg/kgBW	5.70±0.28	0.60±0.03	0.64±0.06
Dosage 1000 mg/kgBW	6.79±0.27	0.71±0.03	0.73±0.04
Satellite, Na cmc 0,5%	5.73±0.74	0.63±0.04	0.66±0.03
Satellite, dosage 1000 mg/kgBW	5.99±0.45	0.67±0.04	0.70±0.04

The average relative organ weight of rats shows that the left kidney is larger than the right kidney because other organs do not obstruct it. Right kidney is close to liver to reduce size and under liver to avoid sticking [29].

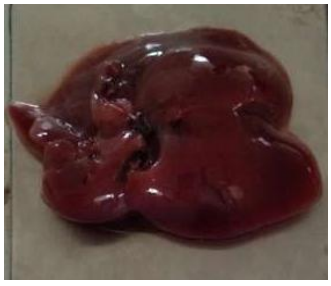
Statistical analysis using Two-way anova revealed no significant difference in liver and kidney weights between rats in the control and treatment groups after ethanol extract of *Garcinia Xanthochymus* fruit bark and satellite groups ($p=0.60$, $p<0.05$). Thus, oral administration of *Garcinia Xanthochymus* fruit bark ethanol extract in various doses does not influence liver and kidney organ weight [30].

These organs were chosen because they synthesize, detoxify, store, and excrete xenobiotics and their metabolites and are vulnerable to hazardous metabolites [31].

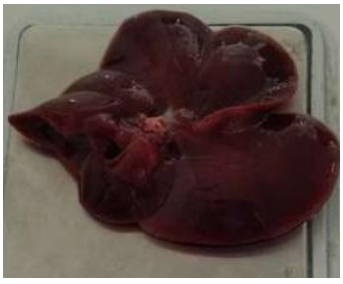
RESULTS OF MACROPATHOLOGY OBSERVATION OF LIVER ORGAN

Table 5: ResultsofMacropathologyObservationofLiver Organ

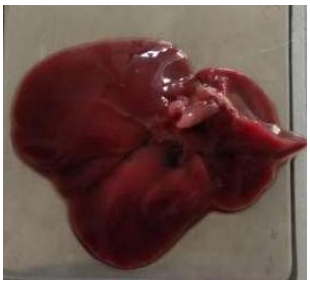
Treatment	Observations		
	Color	Surface	Consistency
Na cmc 0,5%	Brownish red	Slippery	Chewy
EEGXFB 250 mg/ kgBW	Brownish red	Slippery	Chewy
EEGXFB 500mg/kgBW	Brownish red	Slippery	Chewy
EEGXFB 1000 mg/kgBB	Brownish red, white spots present	Slippery	Chewy
Sateliite, Na cmc 0,5%	Brownish red	Slippery	Chewy
Satellite, EEGXFB 1000 mg/kgBW	Brownish red, white spots present	Slippery	Chewy



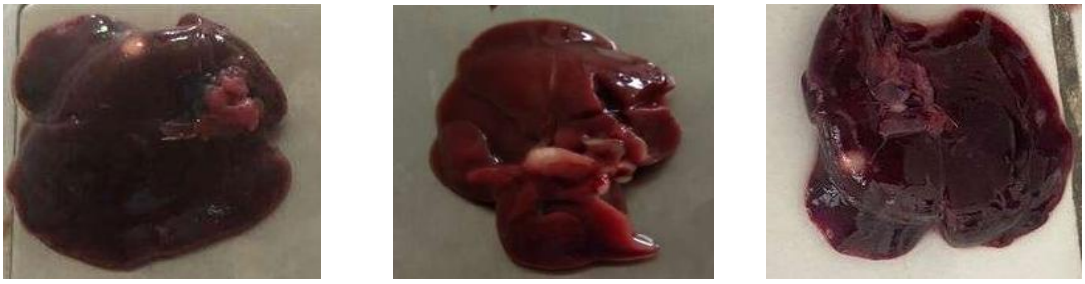
Control0.5% Na cmc



Satellite Na cmc 0.5%



Dose 250mg/kgBW



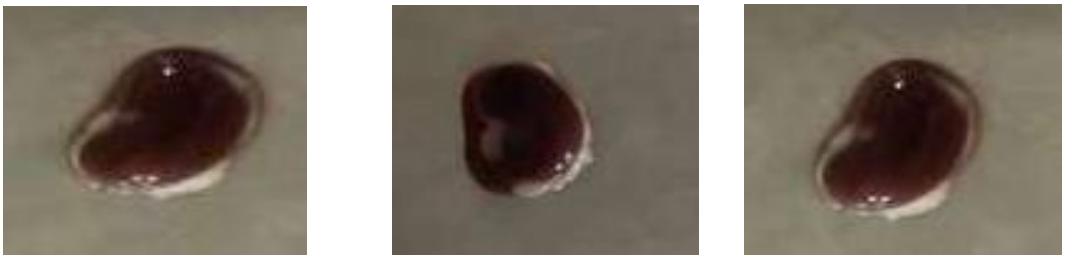
Dose 500 mg/kgBW Dose 1000 mg/kgBW SatelliteDose of 1000 mg/kgBW

Figure 2: Macropathologyofratlivertreatedwith 0.5% Na cmcandethanolextractof*GarciniaXanthochymus*fruitbark.

Table 6: Results of Macropathology Observations of Kidney Organs

Treatment	**Observations**		
	Color	**Surface**	**Consistency**
Na cmc 0,5%	Brownish red	Slippery	Chewy
EEGXFB 250 mg/ kgBW	Brownish red	Slippery	Chewy
EEGXFB 500mg/kgBW	Brownish red	Slippery	Chewy
EEGXFB 1000 mg/kgBW	Brownish red	Slippery	Chewy
Satelit, Na cmc 0,5%	Brownish red	Slippery	Chewy
Satelit, EEGXFB 1000 mg/kgBW	Brownish red	Slippery	Chewy

Control 0.5% Na cmc. Satellite Na CMC 0.5% Dose 250 mg/kgBW



Dose 500 mg/kgBW Dose 1000 mg/kgBW SatelliteDose of 1000 mg/kgBW

Figure 3. Renal macropathologyofratstreatedwith 0.5% Na cmcandethanolextractof*GarciniaXanthochymus*fruitbark.

Based on the results of neuropathological observations of liver organs in Table 4.4 in the normal group with the administration of Na cmc 0.5%, the treatment group with a dose of 250 mg / KgBW and a dose of 500 mg / KgBW and a satellite group with a normal group with the administration of Na cmc 0.5%, it was found that the liver was still in a normal state. Normal liver is brownish red, has a spongy consistency, and smooth surface. The liver was found to be abnormal in the treatment group, with a dose of 1000 mg/KgBW, and in the satellite group, with the highest dose of 1000 mg/KgBW. The abnormal liver is brownish-red with white spots, a spongy consistency, and a smooth surface [32].

The liver is involved in the metabolism of food, as well as most drugs and toxicants. Food substances, narcotics, and toxicants that enter through the digestive tract after being absorbed by the intestinal epithelium will be carried by the veins to the liver. There fore, the liver is an organ that has the potential to suffer poisoning be fore other organs. The presence of spots or white spots on the liver is one of the

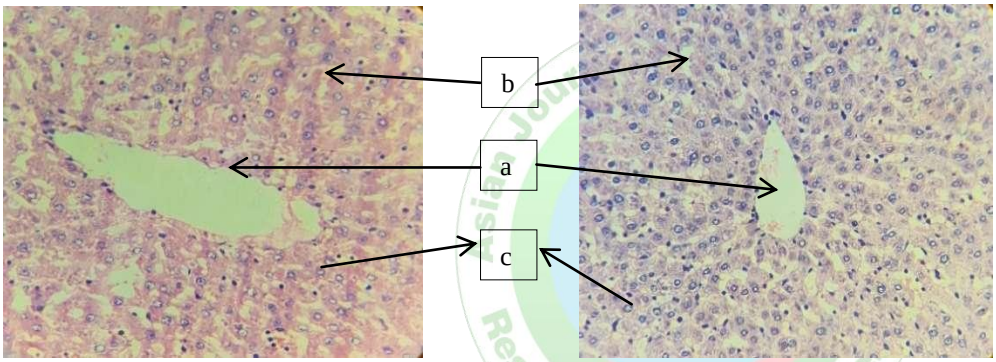
parameters for the occurrence of toxic effects aimedat obtaining in formation about the toxicity of the test substance related to the target organ and the impact on that organ [33].

Histopathologic Observation of Rat Organsoverview of Liver Organ Tissue

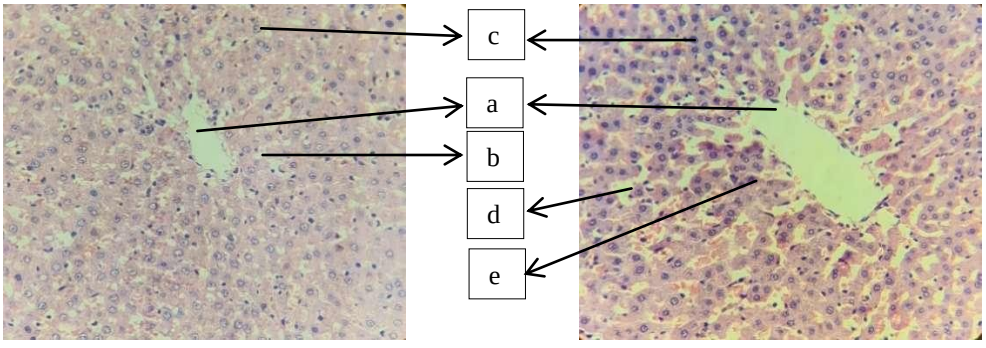
Liver damage can be seen through enzyme levels, organ macropathology, and histopathology results. Based on Figure 4. it can be seen that in the control and satellite groups with the administration of 0.5% Na CMC suspension and the treatment group with a dose of 250 mg/kgBw, there was no damage to the organs seen from the central vein which was still normal, sinusoids arranged radiantly towards the central vein and intact hepatocytes. While in the treatment group with a dose of 500 mg / kgBw, there was damage to the tissue seen from the widening of sinusoids and necrosis cells characterized by shrinking cell nuclei and blackish color (kariopiknosis). Furthermore, the group with a dose of 1000 mg / kgBW found tissue damage characterized by the presence of necrosis cells and central venous congestion, and in the satellite group with a dose of 1000 mg / KgBW found tissue damage characterized by dilated sinusoids, necrosis cells and central venous congestion [34].

Sinusoid dilation indicates liver injury. Necrosis-causing hepatocytes may dilate rat hepar sinusoids. Necrosis causes hepatocytes to become irregular and dilated. High blood toxicant levels can dilate sinusoids and central veins [35].

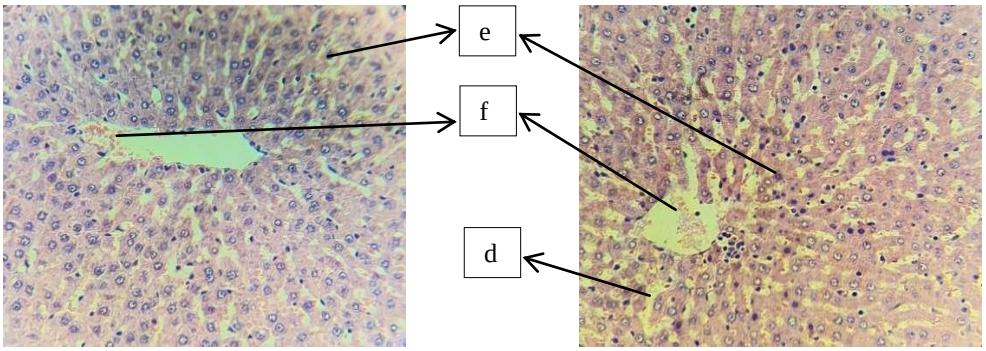
Cell or tissue necrosis occurs in living things. Dead cells have smaller, denser nuclei. Necrosis is hazardous but not always toxic because the liver can regenerate. Endothelial cell lysis causes central vein congestion, making the loop partial and unclear. Because the central vein receives blood from sinusoids, 25% from the hepatic artery, and 75% from the portal vein, which drains blood from the gastrointestinal tract due to intestinal absorption, it might be damaged. A variety of poisonous and benign nutrients and metabolic products are in the central vein [36].



Control0.5% Na cmc Satellite Na cmc 0.5%



Dose 250mg/kgBW Dose 500 mg/kgBW

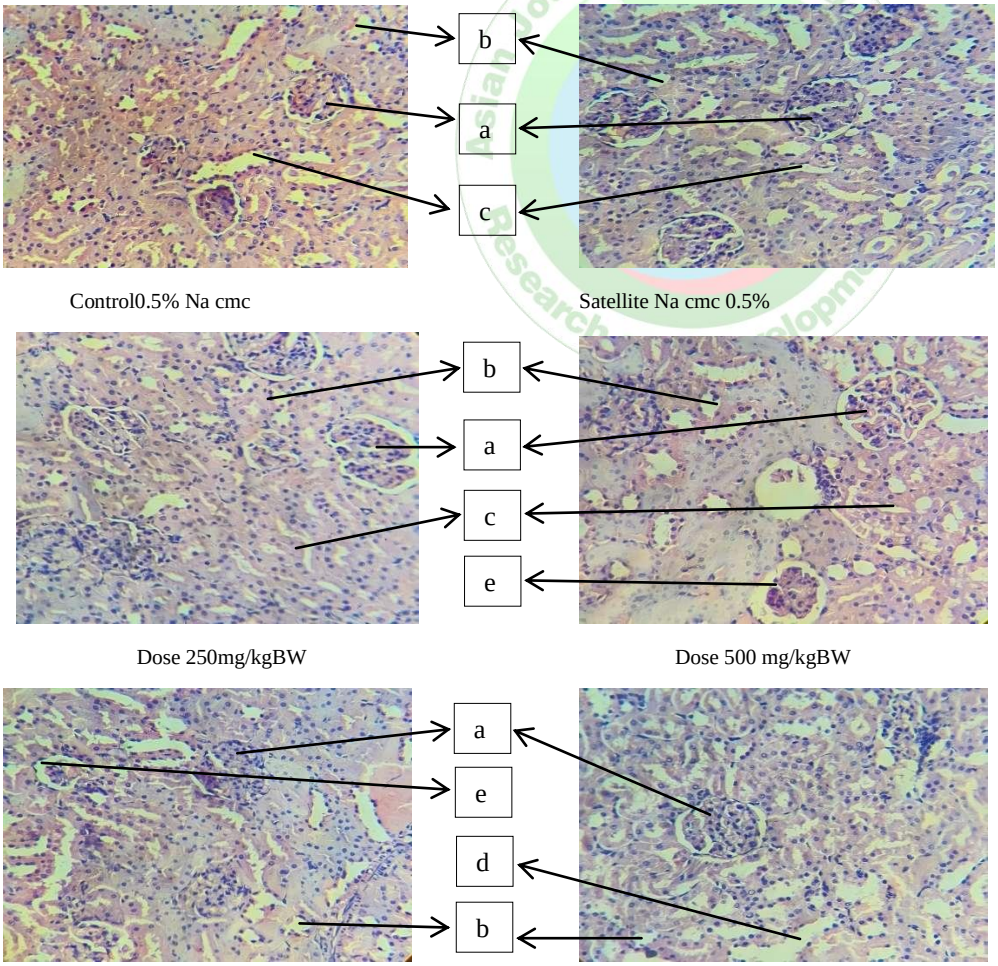


Dose 1000 mg/kgBW SatelliteDose of 1000 mg/kgBW

Figure 4: a. Imageofratliver organ tissueafter EEGXFB administrationwithmagnification (10x40) Centralvein; b. Sinusoids; c. Hepatocytes; d. Dilationofsinusoids; e. Cellnecrosis (karyopicnosis); f. Centralvenouscongestion

The renal organ tissue images in Figure 5 show that the control, satellite, and treatment groups with 0.5% Na CMC suspension and 250 mg/KgBW have normal kidney tissue. The 500 mg/kgBw and 1000 mg/kgBw dose groups widen the bowman space, while the satellite group at 1000 mg/kgBw widens the tubule lumen [37].

Dilation of the bowman's chamber is due to glomerular atrophy, a decrease in tissue size caused by a reduction in the number of cells, or a reduction in cell size that may occur due to slow circulation or oxygen deprivation in the tissue (hypoxia). This damage leads to disruption of the blood filtration process. If theabilityto filter bloodisreduced, blood cells and proteins can leave with the urine or accumulate in the tubules because they can escape the filtration process[38].



Control0.5% Na cmc Satellite Na cmc 0.5%

Dose 250mg/kgBW Dose 500 mg/kgBW

Dose 1000 mg/kgBW SatelliteDose of 1000 mg/kgBW

Figure 5: Renal organ tissueafter EEKBAS administrationwithmagnification (10x40)aGlomerulus; b. Distal tubule; c. Proximaltubule; d. Dilationoftubule lumen; e. Dilationofbowmanspace

CONCLUSION

Ethanol extract of *Garcinia Xanthochymus* fruit bark in the control group, 0.5% Na cmc satellite, 250 mg/kgBw and 500 mg/kgBw had no toxic effects in female white rats after 28 days, but in the 1000 mg/kgBw dose group and satellite, toxic symptoms occurred. Ethanol extract of Garcinia Xanthochymus fruit bark in the control group, satellite Na cmc 0.5%, dose 250 mg/kgBw, did not damage the liver and kidney organs, but 500 mg/kgBw, 1000 mg/kgBw, and satellite 1000 mg/kgBw.

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