RESEARCH ARTICLE

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The Effect of *Moringa Oleifera* Ethanol Extract on Improving Cisplatin Induced Liver Cells Damages

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Abstract

Objective: Cisplatin, a widely used therapy for solid tumors, is associated with hepatotoxicity characterized by cytoplasmic vacuolization of liver cells, sinusoidal congestion, mononuclear and Kupffer cell infiltration, and focal necrosis. *Moringa oleifera* (*M. oleifera*) leaves, rich in flavonoids with antioxidant properties, may mitigate hepatotoxicity. This study aimed to evaluate the effects of ethanol extract of M. oleifera leaves on inflammation, oxidative stress, and liver cell damage in a rat model of cisplatin-induced hepatotoxicity. **Methods:** Thirty male Sprague-Dawley rats were divided into 11 groups, including controls and treatment cohorts. Cisplatin (5 mg/kgBW) was administered as a single dose, followed by a 28-day observation. *M. oleifera* was administered daily at doses of 300, 600, and 1200 mg/kgBW using three regimens: pre-treatment (7 days prior to cisplatin), concurrent treatment (simultaneously with cisplatin), and post-treatment (7 days after cisplatin). On day 28, blood samples were analyzed for alanine aminotransferase (ALT), malondialdehyde (MDA), and nuclear factor kappa beta (NF-κβ), while liver tissues were assessed for cysteine-aspartic proteases (caspase)-3 levels and histopathological changes. **Results:** Pre-treatment with M. oleifera demonstrated the most effective reduction in liver damage, with the 1,200 mg/kgBW dose yielding optimal protective effects across all parameters. Significant differences ($p < 0.05$) were observed in all measured variables across the treatment groups and dosing regimens. **Conclusion:** M. oleifera exhibits a dose-dependent ability to mitigate inflammation, oxidative stress, and liver cell damage caused by cisplatin. The pre-treatment regimen with M. oleifera was the most effective.

Keywords: Cisplatin- *Moringa oleifera*- hepatotoxicity- liver function- antioxidants

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Introduction

Cisplatin is a chemotherapy drug that has been used since 1978 to treat testicular, ovarian, bladder, cervical, head and neck cancer and small cell lung cancer/SCLC [1]. Liver is an organ that is important in metabolism, including drug and poison metabolism, so it is susceptible to damage due to drug use. The damage that occurs can include structural damage such in sinusoids, blood vessels, biliary tract damage and direct damage to liver cells [2–5]. Cisplatin-induced hepatotoxicity occurs in 30-40% of patients receiving chemotherapy, with manifestations ranging from transient elevation of liver enzymes (ALT/AST) to severe hepatocellular necrosis [3].

The mechanism of cisplatin induced hepatotoxicity is caused by the destructive effects of free radicals, lipid peroxidation and decreased glutathione [6]. Nasr. [7] stated that intraperitoneal administration of a single dose of cisplatin 7.5 mg/kgBW caused liver damage in the form of pericentral disorganization, necrosis and apoptotic changes. It is stated that repeated use of high and low doses of cisplatin induces deoxyribonucleic acid (DNA) strand damage in the basal epithelium, resulting in reactive oxygen species (ROS) production and liver cells damage [8]. Oxidative stress plays an important role in hepatotoxicity and reflects mitochondrial damage. This oxidative cascade depletes endogenous antioxidants like glutathione while simultaneously activating pro-inflammatory pathways, leading to cellular apoptosis and necrosis. As demonstrated by Saberianpour et al. [8], cisplatin-generated ROS specifically target hepatocyte membranes through lipid peroxidation, causing structural damage to organelles and DNA. These findings align with prior observations of elevated malondialdehyde (MDA)

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levels and suppressed superoxide dismutase (SOD) activity in cisplatin-treated models [8].

Moringa oleifera leaves are rich in bioactive components, including vitamins (present in fresh leaves), polyphenols (predominantly in dried leaves), alkaloids, tannins, and saponins. Among the polyphenol compounds in dried *M. oleifera* leaves are flavonoids such as myricetin, quercetin, and kaempferol, as well as phenolic acids. Notably, quercetin is a potent antioxidant known for its diverse medicinal properties [9].

Karthivashan et al. [10] through their research on the therapeutic effects of *M. oleifera* leaves showed that this protective effect occurs through increasing the endogenous antioxidant system and modulatory effects on specific proinflammatory cytokines in the kidneys of rats. Another study by Kumar et al. [11] using methanol extract of *M. oleifera* leaves showed that *M. oleifera* can reduce the proliferation of Dalton lymphoma cells by reducing potential changes in the mitochondrial membrane and overall changing cell morphology. Quercetin is one of the compounds contained in *M. oleifera* which is a potential antioxidant and has a direct effect on eliminating free radicals [10–12]. Despite evidence of Moringa's antioxidant properties, no study has yet evaluated the hepatoprotective efficacy of its ethanol extract in a dose dependent manner against cisplatin-induced injury. This study aimed to analyze the effect of ethanol extract *M. oleifera* leaves on inflammation, oxidative stress, and liver cell damage in rat model of cisplatin induced hepatotoxicity.

Materials and Methods

Material

The ethanol extract *M. oleifera* leaves used in the study were obtained from Borobudur Natural Herbal Industry, located in Semarang, Central Java. Ethanol extract of *M. oleifera* leaves analysis was conducted at Markherb PT. EBM Scientific and Technology in Bandung, West Java. The quercetin content in dry powder of *M. oleifera* was 10.73 ± 0.31 mg quercetin equivalent (QE) per gram of sample. Ethanol was selected as the extraction solvent due to its superior safety profile for therapeutic applications, its higher yield of flavonoids (e.g., quercetin) compared to methanol [12].

Study Design and Animal Model

The animal experiment was conducted in the laboratory of the Center for Food and Nutrition Studies at Universitas Gajah Mada Yogyakarta, and the examination experiment was carried out at the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Sebelas Maret in Surakarta. This research has received approval from the research ethics committee at the Health Ethics and Research Committee of the Dr. Moewardi Hospital in Surakarta with No. 805/IX/HREC/2021.

The research subjects was male Sprague-Dawley (SD) rats aged around 8 weeks and/or weighing between 150 - 200 g. The number of samples used in the research was calculated using the resource equation method [13]. The formula to calculate the sample size was E = Total number of animals – total number of groups. The E value must be in the range 10 – 20 to get the optimal sample size.

The first group of SD rats in the study was the control group (C1) that given standard food for 28 days, the second group (C2) was the group of rats given cisplatin at a dose of 5 mg/kgBW single dose intravenously and observed after 28 days [14, 15]. The third group was the group given cisplatin at a dose of 5 mg/kgBW single dose along with *M. oleifera* ethanol extract given for 28 days. The dose of *M. oleifera* ethanol extract given refers to previous the research conducted by Alverina et al. [17] 600 mg/kgBW [16]. SD rats given *M. oleifera* ethanol extract will be divided into 3 administration methods with 3 doses. The first treatment group (T1) received *M. oleifera* simultaneously with cisplatin (T1.1 *M. oleifera* 300 mg/kgBW, T1.2 *M. oleifera* 600 mg/kgBW, and T1.3 *M. oleifera* 1200 mg/ kgBW). The second treatment group (T2) received *M. oleifera* for 7 days before giving cisplatin, and *M. oleifera* was continued until day 28 (T2.1 *M. oleifera* 300 mg/kgBW, T2.2 *M. oleifera* 600 mg/ kgBW, and T2.3 *M. oleifera* 1200 mg/kgBW). The third treatment group (T3) received *M. oleifera* 7 days after cisplatin injection and were given until day 28 (T3.1 *M. oleifera* 300 mg/kgBW, T3.2 *M. oleifera* 600 mg/ kgBW, and T3. 3 *M. oleifera* 1,200 mg/kgBW).

Laboratory Examination of MDA, NFκβ, ALT, and Caspase-3

At the end of the study the rats were sacrificed for blood drawn and liver organ collection. Blood sample was taken from the orbital plexus by puncture using a capillary tube. The collected blood was centrifuged at 3,000 rounds per minute (rpm) for 15 minutes to collect serum. Kidney and liver samples were taken surgically. Blood was taken to measure the markers malondialdehyde (MDA), nuclear factor kappa beta (NFκβ), and alanine transaminase (ALT) post-test, and the liver was taken to examine cysteine-dependent aspartate specific protease (caspase)-3 and assess liver cells damage. Evaluation of caspase-3 was carried out using Rat Casp3(Caspase 3) enzyme-linked immunosorbent assay (ELISA) kit, Wuhan Fine Biotech Co., Ltd.(catalog number: ER0143); NFκβ examination was carried out using Rat NF-κβ ELISA kit, Wuhan Fine Biotech Co., Ltd. (catalog number: ER1186); MDA examination was carried out using the OxiSelect™ TBARS assay kit (MDA Quantitation), Cell Biolabs, Inc. USA. (catalog number: STA-330); ALT examination was carried out using TOOL (glutamic-pyruvic transaminase/ GPT) FS* (International Federation of Clinical Chemistry/ IFCC modified), with/without pyridoxal-5-phosphate FS (P-5-P), DiaSys Diagnostic Systems GmbH, Germany (catalog number: 1 207199 10920);

Liver histopathological examination by evaluation of the liver damage, and the determination was using score of these following parameters including: hepatic cord organization disturbance, congestion, inflammation, necrosis, dilated sinusoid, vacuolar and hydropic degeneratin and another injury [17]. The assessment was semiquantitative, score 0: normal, score 1 (mild): 1-25%, score 2 (moderate): 26-50%, score 3 (severe): 51-75%

and score 4 (very severe): 76-100%.

Stastical Analysis

Data on the characteristics of research subjects was presented in the form of a table containing the mean and standard deviation if data were normally distribute. The data normality test in this study used Shapiro Wilks because the number of samples in each group was 6 samples each. Differences between the five groups were assessed using the one-way ANOVA test if the data was normally distributed and homogeneous, the Brown-Forsythe test for data that was normally distributed but not homogeneous and the Kruskal Wallis test if the data did not meet the normality assumption. The differences between the three concentrations of *M. oleifera* ethanol extract on each parameter was evaluated using the Tamhane T2 post hoc test if the data was normally distributed but not homogeneous, the Scheffe post hoc test if the data was normally and homogeneously distributed, and the Dunn post hoc test if the data did not meet the assumption of normality.

Results

Biochemistry Results in Blood and Liver Tissue Homogenate Sample

The study were divided into 5 groups SD rats consisting of a control group (C1), a cisplatin group (C2), a group of cisplatin and *M. oleifera* given simultaneously (T1), a group of cisplatin and *M. oleifera* given 7 days before cisplatin administration (T2) and a group of cisplatin and *M. oleifera* given 7 days after cisplatin administration (T3). The dose of *M. oleifera* given consisted of 3 doses (300 mg/kgBW, 600 mg/kgBW and 1200 mg/kgBW) in each treatment groups (T1, T2, and T3).

Table 1 and Figure 1 showed that *M. oleifera* at a dose of 1200 mg/kgBW was the one that improved inflammation, oxidative stress and liver cell damage in

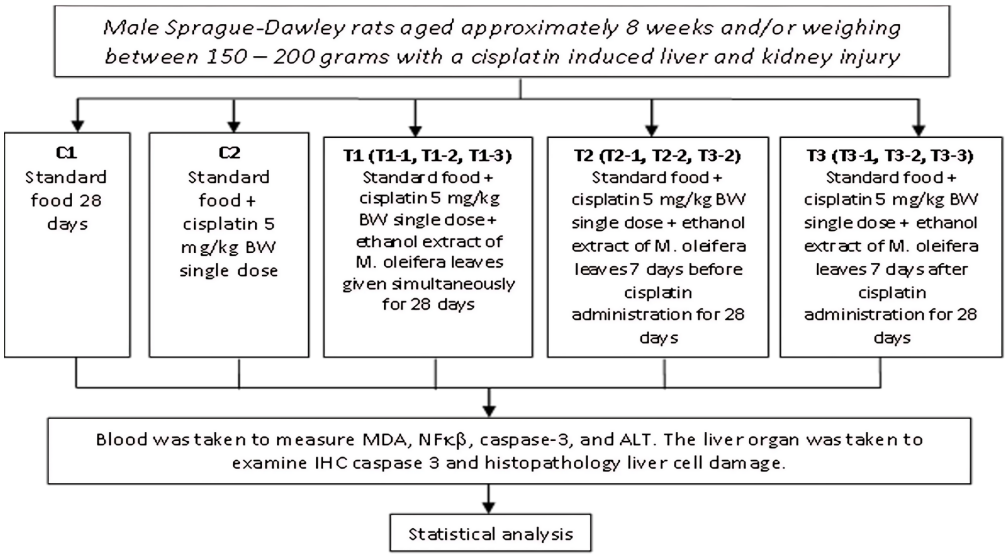

Figure 1. Research Framework

Table 1. Mean and Standard Deviations of NFkb, Caspase-3, ALT and MDA Examination in 11 Groups of SD Rats

Group	NFkb (ng/mL)	Caspase 3 (ng/mL)	ALT (IU/L)	MDA (mmol/mL)
C1	32.85 ±3.97	1.60 ±0.07	17.96 ±0.31	0.68 ±0.18
C2	632.98 ±9.96	11.74 ±0.47	55.43 ±1.72	10.10 ±0.38
T1.1	153.91 ±6.39	6.89 ±0.16	27.27 ±1.20	5.16 ±0.28
T1.2	122.43 ±4.51	4.16 ±0.38	23.06 ±0.51	3.63 ±0.18
T1.3	67.09 ±9.46	2.57 ±0.14	20.07 ±0.50	2.58 ±0.18
T2.1	120.48 ±5.46	6.39 ±0.13	27.11 ±0.57	3.57 ±0.43
T2.2	74.23 ±9.47	3.78 ±0.29	20.47 ±0.57	2.56 ±0.23
T2.3	63.19 ±2.81	1.94 ±0.08	19.26 ±0.59	1.90 ±0.09
T3.1	203.90 ±7.18	7.11 ±0.22	32.13 ±1.04	5.59 ±0.24
T3.2	141.25 ±5.03	4.99 ±0.13	25.81 ±1.28	4.10 ±0.17
T3.3	132.00 ±4.58	3.00 ±0.08	24.84 ±1.08	3.05 ±0.14
p-value	<0.001**	<0.001**	<0.001**	<0.001**

C1= Normal control; C2 = control negative (Cisplatin 5 mg/kgBW); T1.1 = Cisplatin 5 mg/kgBW + M.oleifera 300 mg/kgBW; T1.2 = Cisplatin 5 mg/kgBW + M.oleifera 600 mg/kgBW; T1.3 = Cisplatin 5 mg/kgBW + M.oleifera 1200 mg/kgBW; T2.1 = M.oleifera 300 mg/kgBW → Cisplatin 5 mg/kgBW → M.oleifera 300 mg/kgBW; T2.2= M.oleifera 600 mg/kgBW → Cisplatin 5 mg/kgBW → M.oleifera 600 mg/kgBW; T2.3= M.oleifera 1200 mg/kgBW → Cisplatin 5 mg/kgBW → M.oelifera 1200 mg/kgBW; T3.1= Cisplatin 5 mg/kgBW → M.oleifera 300 mg/kgBW; T3.2 = Cisplatin 5 mg/kgBW → M.oleifera 600 mg/kgBW; T3.3 = Cisplatin 5 mg/kgBW → M.oleifera 1200 mg/kgBW; Brown-Forsythe test (if the data was normally distributed but not homegen); * Significant if p<0.05; ** Significant if p<0.01

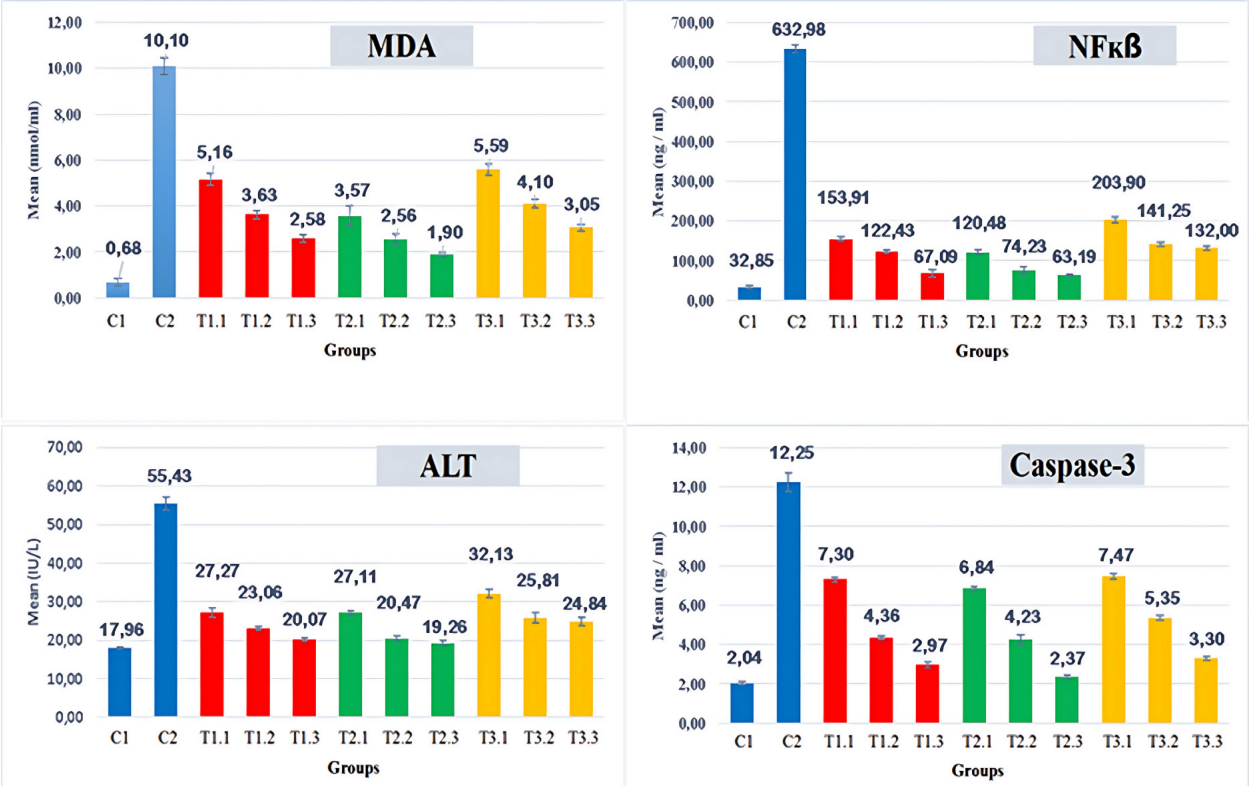

Figure 2. Bar Diagram Comparison of MDA, NFTα and ALT in blood samples and CASP3 in the liver tissue on cisplatin drug induced liver injury model, based on the treatment group preparation. Noted: C1= Normal control; C2 = control negative (Cisplatin 5 mg/kgBW); T1.1 = Cisplatin 5 mg/kgBW + *M.oleifera* 300 mg/kgBW; T1.2 = Cisplatin 5 mg/kgBW + *M.oleifera* 600 mg/kgBW; T1.3 = Cisplatin 5 mg/kgBW + *M.oleifera* 1200 mg/kgBW; T2.1 = *M.oleifera* 300 mg/kgBW → Cisplatin 5 mg/kgBW → *M.oleifera* 300 mg/kgBW; T2.2= *M.oleifera* 600 mg/kgBW → Cisplatin 5 mg/kgBW → *M.oleifera* 600 mg/kgBW; T2.3= *M.oleifera* 1200 mg/kgBW → Cisplatin 5 mg/kgBW → M.oelifera 1200 mg/kgBW; T3.1= Cisplatin 5 mg/kgBW → *M.oleifera* 300 mg/kgBW; T3.2 = Cisplatin 5 mg/kgBW → *M.oleifera* 600 mg/kgBW; T3.3 = Cisplatin 5 mg/kgBW → *M.oleifera* 1200 mg/kgBW

the three treatment groups T1, T2 and T3. Group T2 which a group that received *M. oleifera* before cisplatin administration showed the results closest to group C1, followed by T1 group, and the group that had the least improvement effect was T3 group.

Liver Histopathological Result

Liver damage which shown in liver histopathological examination in this study (Table 2, Figures 2-4) were necrosis of hepatocytes, congested blood vessels, inflammatory cell infiltration, dilated sinusoids and vascular and hydrophic degeneration of hepatocytes [17]. The percentage of liver damage experienced by rats that received cisplatin was 66,7% moderate degree necrosis of hepatocytes, 100% severe congested blood vessels, 100% moderate degree inflammatory cell infiltration, 100% severe dilated sinusoid and 83,3% very severe vascular and hydroiyaaphic degeneration of hepatocytes.

Administration of *M. oleifera* improved liver cells damage in all treatment groups and the most optimal dose to repair the damage was 1,200 mg/kgBW in the T2 (T2.3) treatment group. Administration of *M. oleifera* 1,200 mg/kgBW before administration of cisplatin (T2.3 group) improved necrosis of hepatocyte damage to a mild degree by 100%, congested blood vessels to a moderate degree by 83.3% in the three treatment groups (T1, T2, and T3), inflammatory cell infiltration became a mild degree by 100% in the three treatment groups (T1, T2, and T3), dilated sinusoids became a mild degree by 66.7% and vascular and hydrophic degeneration of hepatocytes became a mild degree by 83.3%.

Discussion

The incidence of cancer patients is currently quite high in the world including in Indonesia, so there will also be an increase in the use of cisplatin as a solid tumor therapy. Liver damage due to cisplatin use is still found in daily practice [18]. The liver is the main site of metabolic reactions. After cisplatin enters the body via infusion, it quickly diffuses into various tissues and reaches the highest dose in the liver [19]. Cells exposed to cisplatin show over expression of cytochrome P450 family 2 subfamily E member 1 (CYP2E1), causing increased oxidative stress producing hydrogen peroxide (H2O2), superoxide radical (O2-) and hydroxyl radical (-OH) which in turn causes tissue damage, apoptosis, and acute liver failure [20, 21]. The resulting oxidative stress can also cause a decrease in glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and an increase in MDA causing hepatotoxicity [5]. Habib et al. (2020) in their research stated that administration of a single dose of 10 mg/kgBW cisplatin increased ALT 2-3 times and

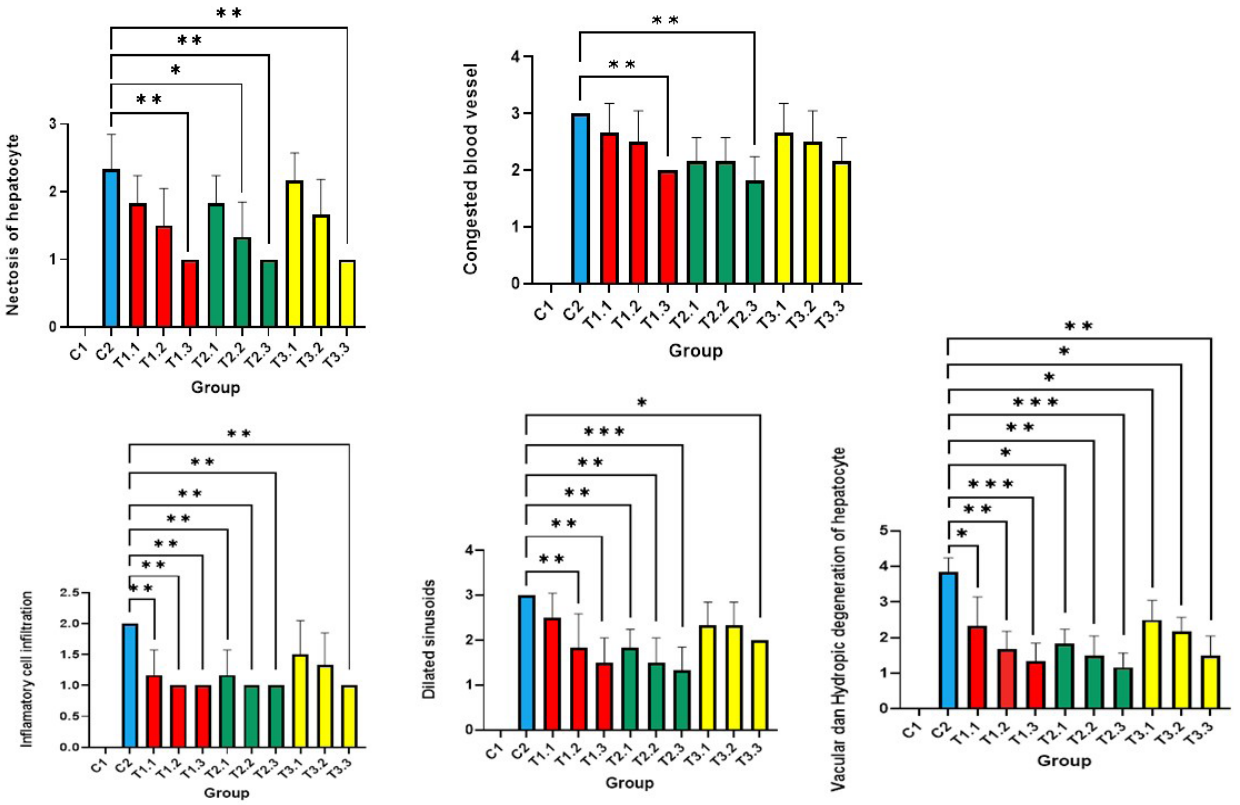

Figure 3. Semiquantitative scoring for necrosis of hepatocyte, congested blood vessel, inflammatory cell infiltration, dilated sinusoid, and vascular and hydropic degeneration of hepatocyte. Data were mean ± SEM. *p<0.05, **p< 0.01 and ***p< 0.001, significant difference with positive control (C2). C1= Normal control; C2 = control negative (Cisplatin 5 mg/kgBW); T1.1 = Cisplatin 5 mg/kgBW + *M.oleifera* 300 mg/kgBW; T1.2 = Cisplatin 5 mg/kgBW + *M.oleifera* 600 mg/kgBW; T1.3 = Cisplatin 5 mg/kgBW + *M.oleifera* 1200 mg/kgBW; T2.1 = *M.oleifera* 300 mg/kgBW → Cisplatin 5 mg/kgBW → *M.oleifera* 300 mg/kgBW; T2.2= *M.oleifera* 600 mg/kgBW → Cisplatin 5 mg/kgBW → *M.oleifera* 600 mg/kgBW; T2.3= *M.oleifera* 1200 mg/kgBW → Cisplatin 5 mg/kgBW → M.oelifera 1200 mg/kgBW; T3.1= Cisplatin 5 mg/kgBW → *M.oleifera* 300 mg/kgBW; T3.2 = Cisplatin 5 mg/kgBW → *M.oleifera* 600 mg/kgBW; T3.3 = Cisplatin 5 mg/kgBW → *M.oleifera* 1200 mg/kgBW

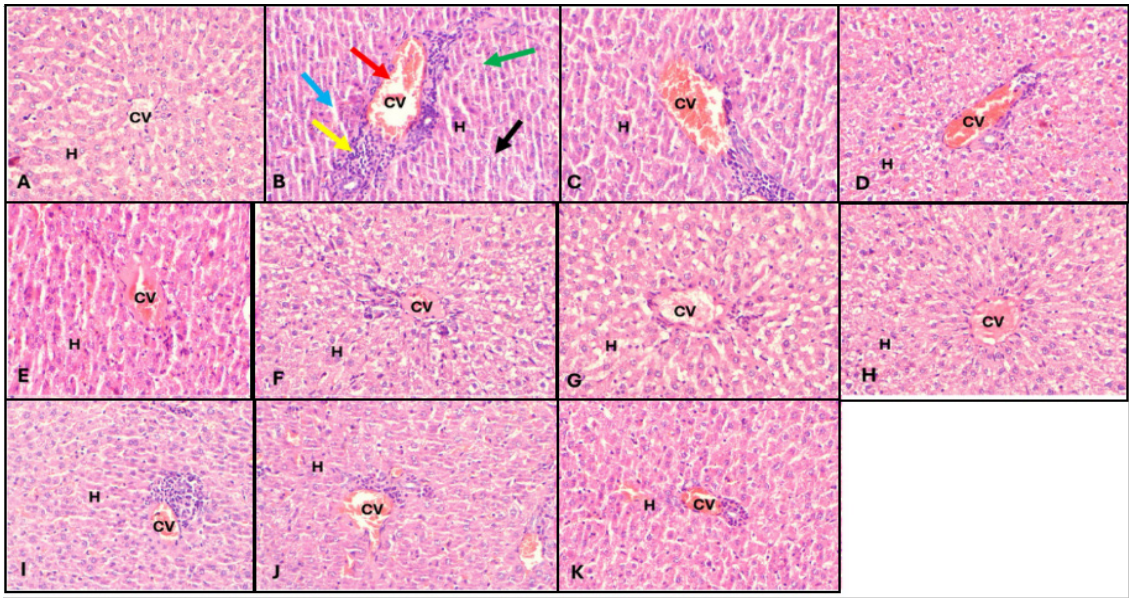

Figure 4. Histopathological Picture of Liver. Histopathological features of the liver of normal control SD group showed normal histology, central vein and well organized radially arranged hepatic cords. (A). Cisplatin induced rats group showed disrupted organization of hepatic cords, congested blood vessels (red arrow), inflammatory cell infiltration (yellow arrow), necrosis (green arrow), dilated sinusoids (blue arrow), and vacuolar and hydropic degeneration in hepatocytes (black arrow) (B). Liver injury and histopathological architecture were amelioted in T1.1 group (C), T1.2 group (D), T1.3 group (E), T2.1 group (F), T2.2 group (G), T2.3 group (H), T3.1 group (I), T3.2 group (J) and T3.3 group (K). H&E-stained sections with 200x magnification Abbreviation: CV: central vein; H: Hepatocyte

MDA 4.9 times when compared to the control group [22]. Another research by Nasr (2013) stated that administration of a single dose of 7.5 mg/kgBW intraperitoneal (i.p.) cisplatin caused liver damage in the form of pericentral disorganization, necrosis and apoptotic changes [7].

There was an increase in collagen fibers around blood vessels in the portal vein area and sinusoid walls in mice injected with cisplatin, apart from that there was also a decrease in glycogen content in liver cells due to a decrease in ATP caused by mitochondrial damage. Taghizadeh et al. [2] conducted research on giving cisplatin 10 mg/kgBB single dose (i.p.) and this dose caused degeneration and eosinophilic cytoplasm in the hepatocyte test, sinusoidal dilatation, central venous congestion, and Kupffer cell mononuclear white blood cell proliferation. Ijaz et al. [24] in their research used 10 mg/kgBW cisplatin and it caused hepatocyte necrosis [23]. Bazmandegan et al. [3] research showed that administration of cisplatin 20 mg/kgBW single dose (i.p.) caused liver damage which shown as presence of portal and periportal inflammation, focal lytic necrosis, and confluent necrosis in liver tissue [24]. In this study we saw the effect of administering a single dose of cisplatin 5 mg/kgBW on the liver in group C2, there were moderate liver cell necrosis (66.7%), severe blood vessel congestion (100%), moderate inflammation (100%), severe sinusoid dilation (100%), and very severe hydropic and vascular degeneration (66.7%).

Administration of cisplatin chemotherapy also causes oxidative stress, apoptosis and inflammation. This inflammation induced by cisplatin causes activation of NFκβ and TNF-κβ [3, 25, 26]. The cancer treatment strategy is inducing cancer cell death through accidental cell death (ACD) or programmed cell death (PCD) mechanisms [27]. Pyroptosis is an ACD whose activation is triggered by caspases [28]. In this study it can be seen that a single dose of cisplatin (5 mg/kgBW) will cause an increase of NFκB, Caspase-3, ALT and MDA when compared to the control group. The administration of *M. oleifera* with various doses causes a decrease in the above parameters although not yet close to the concentration as in the control group.

M. oleifera is a plant that grows and that can reach a height of 10 meters, abundantly in Africa and Asia, belonging to the Moringaceae family. Moringa contains flavonoids and phenolic acids, which are useful for body health. The phenol compound in Moringa protects liver cells from inflammation and liver cell damage [10, 29, 30]. All parts of the *M. oleifera* plant have properties, but the most abundant part is the leaves. Fresh leaves contain lots of vitamins A, C and E, which act as antioxidants which protect the body from the harmful effects of free radicals, pollutants and toxins. Dried leaves contain flavonoids (quercetin, myrecitin and kaempferol), where quercetin is a powerful antioxidant by inhibiting MDA and NFκβ so that inflammation does not occur and there is no damage at the liver cell walls and necrosis of liver cells [10, 31].

M. oleifera plays a role in the regeneration process and has hepatoprotective properties, repairs liver fibrosis, reduces liver damage and hepatotoxicity's effects caused by chemicals/pharmacological substances, and diminishes liver myeloperoxidase activity [30]. Moringa keeps the

Table 2. Liver Histopathological Result

Group	Necrosis of Hepatocyte			Congested Blood Vessel			Inflammatory Cells Infiltration			Dilated Sinusoid			Vacular dan Hydropic degeneration of hepatocyte		
	N	1 -25%	26 – 50%	51 – 75%	N	1 -25%	26 – 50%	51 – 75%	N	1 -25%	26 – 50%	51 – 75%	N	1 -25%	26 – 50%	51 – 75%
C1	100%	0%	0%	0%	100%	0%	0%	0%	100%	0%	0%	0%	100%	0%	0%	0%
C2	0%	0%	66.70%	33.30%	0%	0%	0%	100%	0%	0%	100%	0%	0%	0%	0%	16.70%
T1.1	0%	16.70%	83.30%	0%	0%	0%	33.30%	66.70%	0%	83.30%	16.70%	0%	0%	16.70%	33.30%	50%
T1.2	0%	50%	50%	0%	0%	0%	50%	50%	0%	100%	0%	0%	0%	33.30%	66.70%	0%
T1.3	0%	100%	0%	0%	0%	0%	100%	0%	0%	100%	0%	0%	0%	66.70%	33.30%	0%
T2.1	0%	16.70%	83.30%	0%	0%	0%	83.30%	16.70%	0%	83.30%	16.70%	0%	0%	16.70%	83.30%	0%
T2.2	0%	66.70%	33.30%	0%	0%	0%	83.30%	16.70%	0%	100%	0%	0%	0%	16.70%	83.30%	0%
T2.3	0%	100%	0%	0%	16.70%	83.30%	0%	0%	0%	100%	0%	0%	0%	83.30%	16.70%	0%
T3.1	0%	0%	83.30%	0%	0%	0%	33.30%	66.70%	0%	50%	50%	0%	0%	66.70%	33.30%	0%	0%	0%	50%	50%
T3.2	0%	33.30%	66.70%	0%	0%	0%	50%	50%	0%	66.70%	33.30%	0%	0%	0%	66.70%	33.30%	0%	0%	83.30%	16.70%

C1= Normal control; C2 = control negative (Cisplatin 5 mg/kgBW); T1.1 = Cisplatin 5 mg/kgBW + M.oleifera 300 mg/kgBW; T1.2 = Cisplatin 5 mg/kgBW + M.oleifera 600 mg/kgBW; T1.3 = Cisplatin 5 mg/kgBW + M.oleifera 1200 mg/kgBW; T2.1 = M.oleifera 300 mg/kgBW → Cisplatin 5 mg/kgBW → M.oleifera 300 mg/kgBW; T2.2= M.oleifera 600 mg/kgBW → Cisplatin 5 mg/kgBW → M.oleifera 600 mg/kgBW; T2.3= M.oleifera 1200 mg/kgBW → Cisplatin 5 mg/kgBW → M.oelifera 1200 mg/kgBW; T3.1= Cisplatin 5 mg/kgBW → M.oleifera 300 mg/kgBW; T3.2 = Cisplatin 5 mg/kgBW → M.oleifera 600 mg/kgBW;T3.3 = Cisplatin 5 mg/kgBW → M.oleifera 1200 mg/kgBW

liver cell membrane intact so that there is no leakage of enzymes into the blood [32]. Kaempferol, quercetin, isorhamnetin, and apigenin are the most abundant flavonoids in *M. oleifera* leaves. The total phenolic and total flavonoid contents of *M. oleifera* leaves from Nicaragua in 80% methanol extract were 12.33 g/100g and in 70% ethanol extract were 14.07 g/100g. The concentration of quercetin in *M. oleifera* leaves was 1,362.6 mg/kg and kaempferol was 1,933.7 mg/kg, higher than vegetables such as spinach which is contain quercetin: 17.9 mg/kg, kaempferol: 215.3 mg/kg [33]. The quercetin content in the ethanol extract of *M. oleifera* leaves used in this study was 10.73 ± 0.31 mg/gram of dry leaf powder sample. Ethanol was chosen as the extraction solvent due to its optimal balance of safety and efficacy in isolating bioactive compounds from *M. oleifera* leaves. While methanol may yield higher phenolic content, ethanol is more effective for flavonoid extraction which are key for antioxidant and anti-inflammatory effects [34]. Moreover, ethanol's polarity aligns with the solubility of bioactive compounds, as shown by Obialo et al. [34], who reported higher flavonoid concentrations in ethanol extracts than in non-polar solvents [35].

Liver damage due to cisplatin administration is caused by an imbalance between the oxidant and antioxidants system in the liver tissue. Quercetin in *M. oleifera* decreases the effect of oxidative damage caused by cisplatin. The hepatoprotective effect of quercetin is due to an increase in antioxidant effects on liver tissue [36]. Alanine aminotransferase is a specific enzyme found in liver cells. An increase in ALT indicates liver cell damage. *M. oleifera* leaf extracts reduce ALT levels in the circulation by stabilizing the cell membrane, which prevents enzymes from leaking out of the cells [37]. By increasing PVT1 expression, quercetin inhibits inflammation, oxidative stress, cell apoptosis, and reduces mitochondrial structural and functional dysfunction [37]. Gene PVT1 is a long non-coding RNA locus that is overexpressed in transformed cells and is associated with various types of cancer such as breast cancer, ovarian cancer, acute myeloid leukemia, and Hodgkin's lymphoma [38].

The study showed a new discovery regarding the administration of ethanol extract of *M. oleifera* as an antioxidant, which has never been studied previously, and this study has provided novel scientific data that the ethanol extract of *M. oleifera* contained anti-inflammatory, anti-oxidant and anti-apoptotic properties. Increasing the dose of ethanol extract of *M. oleifera* gave a good effect (dose response) on all parameters. Overall, the benefit of this research is by administering ethanol extract of *M. oleifera* can improve liver injury caused by cisplatin, this was shown by a decrease of MDA and NFκβ levels, and also a reduced of ALT activity which evaluated from blood of rats given ethanol extract of *M. oleifera*. There was also an improvement of rats liver histopathology which received ethanol extract of *M. oleifera*. The *M. oleifera* dose that provided the most optimal effect on liver repair was at a dose of 1,200 mg/kgBW.

Nevertheless, this study has several limitations that need to be considered. First, our experimental study used only male Sprague-Dawley rats, while methodologically advantageous for controlling hormonal variability, may limit the applicability of the findings to female subjects. Second, the study was conducted on healthy animal models rather than tumor-bearing subjects, which may not fully replicate the clinical complexity of cancer patients undergoing cisplatin treatment. Third, the experimental protocol did not incorporate hydration therapy, a standard supportive measure in clinical practice that could potentially influence cisplatin-induced hepatotoxicity. Future research should address these limitations by incorporating both sexes, tumor-bearing models, and clinically relevant hydration protocols to enhance the translational relevance of the findings.

Author Contribution Statement

a. BRA – Conception and planning, acquisition of data, analysis and interpretation data, Drafting manuscript; b. BP, BW, VW, S, RC, TDA – Critical revision of the manuscript; c. BRA, VW – Statistical analysis; d. TDA – Administrative, technical, material support; e. BP, BW, VW – Supervision

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Ethical Declaration

Ethical approval for this study was obtained from the Health Ethics and Research Committee of the Dr. Moewardi Hospital in Surakarta No. 805/IX/HREC/2021

Conflict of Interest

The authors declare that there is no conflict of interest.

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