5. Conclusion

The quality standards for the alcohol-processed *Rhizoma Ligustici wallichii* included the descriptive criteria according to the Vietnamese Pharmacopoeia V, qualitative analysis by thin layer chromatography using a solvent system of dichloromethane and diethyl ether (2:1, v/v), quantitative analysis with ferulic acid content not less than 0.05%, moisture content not exceeding 12%, total ash content not exceeding 5%, and the extractive compounds not less than 8%. Additionally, further quantitative analysis of prominent markers was recommended to clarify the differences between the raw material and the alcohol-processed *Rhizoma Ligustici wallichii* samples, providing a precise basis for establishing comprehensive standards. The initial results of this research contributed to the evaluation of post-processing herbal quality and laid the groundwork for further studies on the chemical composition and pharmacological effects of processed material.

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HEPATOPROTECTIVE ACTIVITY OF CRUDE EXTRACT AND GANODERIC ACIDS IN *GANODERMA NEO-JAPONICUM* FRUIT BODY AND BIOMASS

Nguyen Quoc Cuong[1,*], Vo Huy Sang[2], Hoang Anh Tuan[2], Pham Van Phuc[3], Bui Thi Minh Dieu[1]

[1]Institute of Food and Biotechnology, Can Tho University, Vietnam;

[2]Bu Gia Map Nation Park, Binh Phuoc, Vietnam;

[3]Stem Cell Institute, University of Nature Sciences, Ho Chi Minh City, Vietnam

*Corresponding author: nguyenquoccuong.ma@gmail.com

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Summary

Hepatoprotective Activity of Crude Extract and Ganoderic Acids in *Ganoderma neo-japonicum* Fruit Body and Biomass

The mushroom *Ganoderma neo-japonicum* (GNJI) is a new mushroom recently found in Bu Gia Map National Park on the decomposing bamboo stump (*Schizostachyum* sp.) which has been cultured and studied in recent years. In this study, the hepatoprotective activity of ganoderic acids (GAs) and crude extract (Ext) from GNJI fruit body (Frb) and biomass (Mas) were determined. The mice were administered GAs and crude extract with one dose of 16 mg/kg or 32 mg/kg body weight (bw) per day for 21 days before hepatic damage induced by paracetamol (acetaminophen) 400 mg/kg bw. The results of relative liver weights, liver histopathological observation (surface parenchyma, histology of lobules), and serum SGOT/SGPT levels showed that GAs from fruit body (FrbGAs) and biomass (MasGAs) had hepatoprotective activity compared to silymarin (16 mg/kg bw). After 21-day administration of FrbGAs or MasGAs at a dose of 16 mg/kg or 32 mg/kg bw, the parenchyma, lobules of the liver, and serum SGOT/SGPT levels restored nearly normal as same as physiological control. Specially, FrbGAs and MasGAs at 32 mg/kg bw showed the best hepatoprotective activity.

Keywords: *Ganoderma neo-japonicum, Hepatoprotective, Ganoderic acids, Biomass.*

1. Introduction

The liver is an important organ responsible for the detoxification and metabolism of animals. The damage in the liver causes disorders to normal detoxification and metabolism of cells, and tissues and even death for the body. Since its clinical introduction in 1955, acetaminophen (*N*-acetyl-*p*-aminophenol; APAP; paracetamol) has become the most widely used analgesic-antipyretic in the United States [1]. Acetaminophen is a component of hundreds of over-the-counter and prescription medications used worldwide. Acetaminophen or paracetamol is usually visible in every medical box of the family in Vietnam. Using paracetamol for a long time is the popular reason for liver damage [2].

G. neo-japonicum Imazeki (GNJI) has been known as a lignin-degrading mushroom by Jo et al. [3]. It was then studied to improve the cellulose degradation activity by this same author's publication [4]. The culture process of GNJI mushroom was supplemented with methionine by Lee et al. [5] to effectively increase the production of ergothioneine and was supplemented with tryptophan by Park et al. [6] to increase the efficiency of phenolic compounds. Ethanol extraction of GNJI had abilities to manage type 2 diabetes mellitus [7]. Antioxidant activity of GNJI was found with ethanol precipitation from the mycelia [8],[9]. Cryptoporic acids H and I were active substances in GNJI fruit body with strong antioxidant capacity [10]

Therefore, this study aimed to observe the hepatoprotective of crude extract and ganoderic acids which are made from fruit body and biomass of *G. neo-japonicum* Imazeki.

2. Materials and methods

Mushroom materials

GNJI fruit bodies in Bu Gia Map National Park were collected (C18), washed, and dried at 45°C with moisture content lower than 20%, and finally ground, vacuum sealed, and stored at room temperature. The process of morphological description and DNA sequencing for identification was carried out at the laboratory of the Institute of Biotechnology Research and Development, Can Tho University.

GNJI biomass was harvested from the culture process (culture medium containing 90% rice, 5% cornstarch, and 5% rice bran). These biomass samples and fruit bodies were harvested to produce extracts (fruitbody extract - FrbExt, biomass extract - MasExt) and ganoderic acids (fruitbody ganoderic acids - FrbGAs, biomass ganoderic acids - MasGAs) fractions which were used to test for hepatoprotective activity.

Preparing fruit body and biomass crude extracts (Ext)

The mushroom powder (biomass and fruit body) was produced by pureeing in the herbal blender with a 0.45 mm sieve. The extract was prepared by extracting the mushroom sample in hot water at 90°C in a water bath for 24 hours at a ratio of 1:10 (w/v) and concentrating at 50°C until 50% volume remained. The samples were added 30% ethanol at a ratio of 1:1 (v/v), evaporating at 50°C to 50% volume, and cooling at 2-5°C for 8 hours. After removing the precipitate, the extract was evaporated at 50°C until 50% volume remained [11]. The quality of the extracts was initially evaluated based on GAs concentrations using thymol as standard at 245 nm [12].

Preparing fruit body and biomass ganoderic acids (GAs) fraction

Every gram of sample (biomass and fruit body) was suspended in 15 mL of 70% ethanol and extracted two times; 24 hours each. After removing biomass with a cellulose filter, the supernatant was dried at 50°C under vacuum conditions. The residues were suspended by water and extracted with chloroform. The GAs in the chloroform phase were further extracted by 5% (w/v) $NaHCO_3$. The pH of the $NaHCO_3$ phase was then adjusted to be lower than 3.0 by 2 M HCl. Then GAs were extracted by chloroform and dissolved in ethanol after chloroform evaporated. The GAs were measured at 245 nm using thymol as standard [13].

Animals

The male *Swiss albino* mice, 5-6 weeks old, average weight of 22 ± 2 g were provided by the Pasteur Institute in Ho Chi Minh City and allowed to stabilize for at least one week prior to testing.

Screening hepatoxic dose of paracetamol [14],[15]

Mice were divided into 15 groups (5 mice in each group) in 24 hours. All groups were normally eating and drinking. Mice were administered 0.5 mL of paracetamol in distilled water with doses of 0 mg/kg (control), 300 mg/kg, 400 mg/kg, 500 mg/kg, and 600 mg/kg body weight (bw). Observation of mouse behavior was performed for 7-day paracetamol administration. Mouse blood samples were collected to determine serum SGOT, and SGPT at 48 hours, 72 hours, and 7 days of paracetamol administration. Liver organs were dissected at 48 hours to observe the gross assessment and weight. The damages, necrosis,

congestion, and parenchyma surface were noted in the liver gross assessment.

Experimental model to investigate the hepatoprotective effects [14],[15]

Five mice weighing 22 g ± 2 were separated into treatment and made into 2 groups composed of the control group and the experimental group.

Table 1. Experimental layout table to investigate the hepatoprotective effects

Groups	Treatments	Details
Control	BlkCtrl	Physiological control (normal)
	NegCtrl	Pathological control
	PosCtrl	Reference - silymarin at dose16 mg/kg bw
Experimental	FrbExt_16	Fruit body crude extracts at dose of 16 mg/kg bw
	FrbExt_32	Fruit body crude extracts at dose of 32 mg/kg bw
	MasExt_16	Biomass crude extracts at dose of 16 mg/kg bw
	MasExt_32	Biomass crude extracts at dose of 32 mg/kg bw
	FrbGAs_16	Fruit body ganoderic acids at dose of 16 mg/kg bw
	FrbGAs_32	Fruit body ganoderic acids at dose of 32 mg/kg bw
	MassGAs_16	Biomass ganoderic acids at dose of 16 mg/kg bw
	MassGAs_32	Biomass ganoderic acids at dose of 32 mg/kg bw

Each experiment had five mice. Individually confining mice received drinking water and food normally for 24 hours before the experiment. The control groups continued to administer distilled water to BlkCtrl, NegCtrl groups, and silymarin 16 mg/kg bw to PosCtrl group for 21 days. The experimental groups administered the test extracts and ganoderic acids at the same time as the control groups. On day 22nd, except the normal control group, other groups were administered a single dose of paracetamol (400 mg/kg bw). Then mouse tail blood was collected at 48 hours after paracetamol administration to determine serum SGOT and SGPT. Liver organs were dissected and stored in 0.9 percent sodium chloride to observe the surface and perform hepatic histology specimens.

Reagents and test kits

Processing	Reagents and test kits	Origination
Determine the ganoderic acids in samples	Thymol	Sigma Aldrich - Singapore
Determine the SGOT and SGPT in serum	SGOT/SGPT Biochemistry Reagent	Anamol Laboratories - India
Stain the hepatic tissues	Hematoxylin & Eosin (H&E)	Morphisto laborchemikalien (DE)
	Xylene	Central Drug House Ltd. - India
	Formaldehyde - F8775-500ML	Sigma Aldrich - Singapore

Analyzing SGOT, SGPT procedure

The blood samples (1.5 mL) were centrifuged and separated for serum and then the serum was quantified SGOT or SGPT level by automatic machine Olympus AU 2700.

Procedure for performing hepatic histology specimens [16]

Sample fixation and formalin removal: livers were dissected, washed in physiological saline, and fixed at 2% formalin (w/w) for an hour. All organs were sliced to 5 mm thin and continued to fix in 4% formalin (w/w) for 24 hours, then soaked under gently running water for 48 hours to remove formalin.

Dehydration and ethanol reduction process: samples were moved sequentially through the vials containing alcohol with concentrations of 50, 70, 80, 90, and 99.5% (w/w). Tissue samples were transferred to the mixture of xylene and 99.5% ethanol w/w (1:1) and then moved to pure xylene. Samples were stored in each vial for an hour.

Paraffin impregnating: hepatic tissues were transferred to the mixture of xylene and paraffin (1:1) and then incubated at 60°C for 24 hours. Continued to keep samples in the mixture of paraffin and beeswax (1:1) twice at 60°C in 24 hours.

Paraffin molding: the molds were 3 cm x 1.5 cm x 1.5 cm in size and made by carton paper. The samples were moved quickly to molds with the hot mixture of paraffin and beeswax (60°C, 1:1) then kept the molds at room temperature for 24 hours up to solidified. Samples in solidified paraffin would be stored at 4-8°C. Samples were sliced gently with 3-5 µm thickness by microtome. Tissues were next imbued with Hematoxylin and Eosin Harris.

Hematoxylin and Eosin Harris imbuing process [16]

Transferring sliced samples three times in a xylene tank for 5 minutes each time to remove paraffin, then soaked twice in an alcohol tank for 5 minutes with sequence concentrations of 100, 95, 80, and 70% (w/w). Gently wash the samples with distilled flowing water for 5 minutes. The samples were dyed in a hematoxylin Harris tank (appendix A2 of Vietnam National Standards 8730-14:1015) for 3-5 minutes and washed with distilled flowing water for 5 minutes. Continued dyeing samples in Eosin 1% (w/w) tank (appendix A3 of Vietnam National Standards 8730-14:1015) then washed with distilled running water for 5 minutes. Embedding samples to alcohol 15 times for 2 minutes each time through concentrations of 95 and 100% (w/w) in sequence. Samples were embedded 15 times for 2 minutes each in two xylene tanks sequence then soaked in a third xylene tank for 10 minutes. Samples were fixed with Baume Canada on glass lame.

Statistical analysis

Actual data about concentrations of SGOT, SGPT, body weight, and liver weight per 10g body weight were analyzed with ANOVA statistics by the Statgraphic Centurion XV program.

3. Results and discussion

Quantitative analysis of ganoderic acids (GAs) in test extracts

The triterpene structure in ganoderic acids absorbed the light at wavelength 245 nm. The fruit body, biomass crude extract, and two GAs fractions were determined as the amount of ganoderic acids by this UV measurement method. The GAs contents are shown in Table 2.

Table 2. The content of ganoderic acids in crude extracts

Crude extracts	Ganoderic acids (mg/g)	Fractions	Ganoderic acids (mg/g)
Fruit body	1.193	Fruit body GAs	4.760
Biomass	0.621	Biomass GAs	5.397

Hepatoxic dose of paracetamol

The results of mouse activity observation are shown in Table 3.

Table 3. The activity observation results of mice in 7 days after paracetamol administering

Doses of paracetamol (mg/kg bw)	Mouse activity
0	Normal
300	Normal
400	4/20 mice stopped eating and sedentary after administering and returned normal after 48 hours up to 7 days
500	13/20 mice stopped eating and were sedentary after administering, 6 mice still ate little and were sedentary for 48 hours, 2 mice died in 60 hours and otherwise, all others were normal up to 7 days
600	20/20 mice stopped eating and were sedentary after administering, then ate little and were sedentary in 48 hours, 4 mice died in 60 hours, 7 died more for 72 hours and otherwise, all others were normal up to 7 days

After 48-h paracetamol administration, five mice were weighed and collected blood serum to determine serum SGOT and SGPT contents. The livers were dissected for weighing and gross examination (Table 4). The mice lost weight at doses of 500 mg/kg and 600 mg/kg after 48 hours of paracetamol administration (Table 4). Otherwise, liver organs were enlarged and liver relative weight was increased with statistical significance ($p<0.05$) at all doses (Table 4). Hepatic tissues were seriously damaged, showing complete necrosis and congestion in hepatic lobules,the rough and heterogeneous parenchyma after 48 hours of paracetamol administration at doses of 500 mg/kg and 600 mg/kg (Table 4). These damages may be the reasons for making the death of mice after 60 hours of paracetamol administration.

Table 4. Liver gross assessment of mice livers at 48 hours after paracetamol administering

Doses (mg/kg bw)	Body weight (g) (mean ± SE)	Liver weight per 10 bw(g) (mean ± SE)	Liver gross assessment
0	22.069^{a} ± 1.030	0.369^{d} ± 0.010	Livers were normal, hepatic parenchyma was homogeneous
300	21.640^{ab} ± 0.820	0.398^{c} ± 0.011	Livers were not damaged, hepatic parenchyma was homogeneous

Doses (mg/kg bw)	Body weight (g) (mean ± SE)	Liver weight per 10 bw(g) (mean ± SE)	Liver gross assessment
400	22.676^{a} ± 0.653	0.550^{b} ± 0.003	4/5 liver organs were necrosis and congestion in some areas; little enlarged livers; the parenchyma was rough, not smooth, and not homogeneous
500	20.361^{bc} ± 1.519	0.553^{b} ± 0.006	5/5 liver organs exhibited complete necrosis and congestion; little enlarged livers; the parenchyma was rough, not smooth, and not homogeneous
600	19.954^{c} ± 1.252	0.569^{a} ± 0.012	5/5 liver organs showed complete necrosis and congestion; enlarged livers; the parenchyma was rough, not smooth, and not homogeneous

** Letter a, b, c, d after values of bw, liver weight per 10 g bw in each column are groups of homogeneous ($p<0.05$).

The mouse tail blood was collected at 72 hours and 7 days to analyze serum SGOT and SGPT contents. Table 5 showed that paracetamol could increase SGOT and SGPT in serum at a dose over 300 mg/kg bw with statistical significance ($p<0.05$). At a dose of 300 mg/kg of paracetamol, SGPT values could be returned to normal while all other groups (at doses of 400 mg/kg, 500 mg/kg, 600 mg/kg) kept markedly high values for 7 days ($p<0.05$).

Table 5. The analysis results of SGOT and SGPT in serum after 7 days of paracetamol administering (mean ±SE)

Doses of paracetamol (mg/kg bw)	48 hours (IU/L)		72 hours (IU/L)		7 days (IU/L)	
	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT
0	81.34^{d} ± 5.39	35.00^{d} ± 3.12	81.20^{d} ± 7.03	35.34^{d} ± 3.16	83.10^{c} ± 6.17	37.00^{c} ± 2.62
300	138.80^{c} ± 3.84	34.52^{d} ± 2.53	136.24^{c} ± 6.60	62.74^{c} ± 4.08	105.34^{b} ± 5.44	42.64^{c} ± 3.53
400	154.96^{b} ± 5.47	102.40^{a} ± 6.10	155.42^{b} ± 8.35	71.40^{b} ± 6.35	127.64^{a} ± 7.18	62.46^{a} ± 5.76
500	172.86^{a} ± 7.08	95.94^{b} ± 3.30	138.76^{c} ± 7.30	75.80^{b} ± 5.68	136.10^{a} ± 8.97	50.58^{b} ± 3.51
600	173.16^{a} ± 10.89	58.66^{c} ± 5.23	183.10^{a} ± 11.47	94.36^{a} ± 6.39	Death	Death

** Letter a, b, c, d after values of SGOT, SGPT in every column are groups of homogeneous ($p<0.05$).

Taking together, the paracetamol dose of 400 mg/kg was chosen since the higher doses of 500 mg/kg and 600 mg/kg caused lethality in mice. This result was suitable with Nguyen Manh Cuong et al. [14] and Tauqeer Hussain Mallhi et al. [15] announcements.

The paracetamol toxicity was explained by the following mechanism. Under therapeutic doses, paracetamol is detoxified in the liver mainly by glucuronidation and sulfation. Part of paracetamol is metabolized by cytochrome CYP2E1 and is converted to a toxic metabolite NAPQI. NAPQI is detoxified by conjugation with glutathione (GSH); thus, GSH content is the critical factor of paracetamol toxicity. Under the condition of paracetamol overdose, glucuronidation and sulfation become saturated, GSH is depleted, and NAPQI is bound to cellular proteins, resulting in oxidative stress and liver necrosis [17].

The hepatoprotective effects of G. neo-japonicum

The mice were administered silymarin, crude extracts, ganoderic acids for 21 days with 0.5 mL per day at doses of 16, 32 mg/kg bw. The body weight of mice in both the control and experimental batches were weighed at 0, 7, 14, and 21 days. The results of body weight observations are shown in Table 6.

Table 6. The mouse body weight of test groups after 21 days using extract (mean ± SE)

Treatments	bw at 0 day (g)	bw at 7 days (g)	bw at 14 days (g)	bw at 21 days (g)
BlkCtrl	21.456^{a} ± 1.166	23.933^{ab} ± 0.921	25.433^{ab} ± 1.180	27.422^{a} ± 1.086
FrbExt_16	21.489^{a} ± 1.012	23.856^{ab} ± 1.395	25.611^{ab} ± 1.449	27.889^{a} ± 1.688
FrbExt_32	21.800^{a} ± 0.555	24.311^{a} ± 0.916	25.678^{ab} ± 1.166	27.856^{a} ± 1.221
FrbGAs_16	21.933^{a} ± 0.851	23.344^{b} ± 0.855	25.444^{ab} ± 1.230	27.344^{a} ± 1.111
FrbGAs_32	21.811^{a} ± 1.193	23.589^{ab} ± 1.056	25.900^{ab} ± 1.227	27.589^{a} ± 0.999
MasExt_16	22.211^{a} ± 1.131	24.111^{ab} ± 1.228	26.278^{a} ± 0.824	28.311^{a} ± 1.431

Treatments	bw at 0 day (g)	bw at 7 days (g)	bw at 14 days (g)	bw at 21 days (g)
MasExt_32	$21.678^{a} \pm 0.950$	$23.556^{ab} \pm 1.049$	$25.867^{ab} \pm 0.986$	$27.389^{a} \pm 0.999$
MasGAs_16	$21.989^{a} \pm 1.236$	$24.044^{ab} \pm 0.989$	$25.867^{ab} \pm 1.062$	$27.722^{a} \pm 1.100$
MasGAs_32	$21.378^{a} \pm 0.893$	$24.144^{ab} \pm 0.938$	$26.378^{a} \pm 1.136$	$27.956^{a} \pm 1.482$
NegCtrl	$21.567^{a} \pm 0.934$	$24.189^{ab} \pm 0.965$	$25.189^{b} \pm 1.107$	$27.489^{a} \pm 0.910$
PosCtrl	$21.822^{a} \pm 0.991$	$23.233^{b} \pm 0.837$	$25.489^{ab} \pm 1.045$	$28.067^{a} \pm 1.414$

** Letter a, b, c, d… after values of bw in every column are groups of homogeneous ($p<0.05$).

The result in Table 6 showed that the body weights of mice administered the test extracts, as well as silymarin, were slightly different at 7 and 14 days but none were statistically different compared to the control after 21 days.

On day 22nd, except for normal control, all mice were administered paracetamol with a dose of 400 mg/kg bw. After 48 h of paracetamol administration, mouse blood serum and liver were collected for analysis. The liver organs were weighed and performed gross and histopathological examinations. The results are shown in Table 7 and Fig. 1.

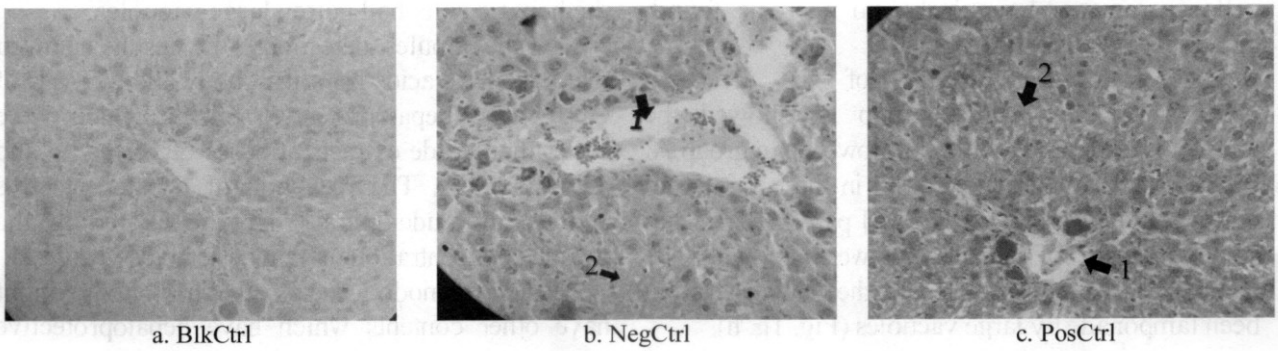
Table 7. The liver weights and gross examination results at 23 days of test groups (mean ± SE)

Treatments	Body weight (g)	Liver weight per 10 bw (g)	Liver surface monitoring		
			Rough surface	Necrosis	Congestion
BlkCtrl	$27.533^{ab} \pm 1.585$	$0.397^{b} \pm 0.006$	+	-	-
FrbExt_16	$27.978^{a} \pm 0.707$	$0.398^{b} \pm 0.008$	-	-	+
FrbExt_32	$27.611^{ab} \pm 1.284$	$0.394^{b} \pm 0.005$	-	-	+
FrbGAs_16	$26.456^{b} \pm 0.990$	$0.394^{b} \pm 0.005$	+	-	-
FrbGAs_32	$27.211^{ab} \pm 1.365$	$0.398^{b} \pm 0.007$	+	-	-
MasExt_16	$26.722^{ab} \pm 1.384$	$0.395^{b} \pm 0.008$	-	-	+
MasExt_32	$26.822^{ab} \pm 1.130$	$0.396^{b} \pm 0.005$	-	-	+
MasGAs_16	$26.867^{ab} \pm 0.847$	$0.396^{b} \pm 0.006$	+	-	-
MasGAs_32	$26.967^{ab} \pm 1.652$	$0.395^{b} \pm 0.006$	+	-	-
NegCtrl	$26.356^{b} \pm 2.210$	$0.566^{a} \pm 0.008$	-	+	+
PosCtrl	$26.411^{b} \pm 2.375$	$0.394^{b} \pm 0.006$	-	-	-

** Letter a, b, c, d… after values of bw, liver bw in every column are groups of homogeneous ($p<0.05$); +: positive, -: negative.

Mouse body weights after 48 hours using paracetamol at all experiments were not different from physiological control with statistical signification. Besides, Mouse relative liver weights (mice liver weight per 10g bw) at all experimental batches were not different in statistics.

The results of the gross examination in Table 7 showed that the tissues remained rough, with no signs of necrosis or congestion in the FrbGAs_16, FrbGAs_32, MasGAs_16, and MasGAs_32 treatments. This means that the ganoderic acids in GNJI induced restoration of the liver after paracetamol intoxication better than other extract treatments. Besides, in comparison to NegCtrl, fruit body crude extracts, and biomass crude extracts could prevent necrosis at the hepatic lobules after paracetamol intoxication. Therefore, although these extracts could not prevent congestion in the portal vein and were not as good as the ganoderic acids fraction, they could have the ability to hepatic protection against paracetamol intoxication.



a. BlkCtrl b. NegCtrl c. PosCtrl

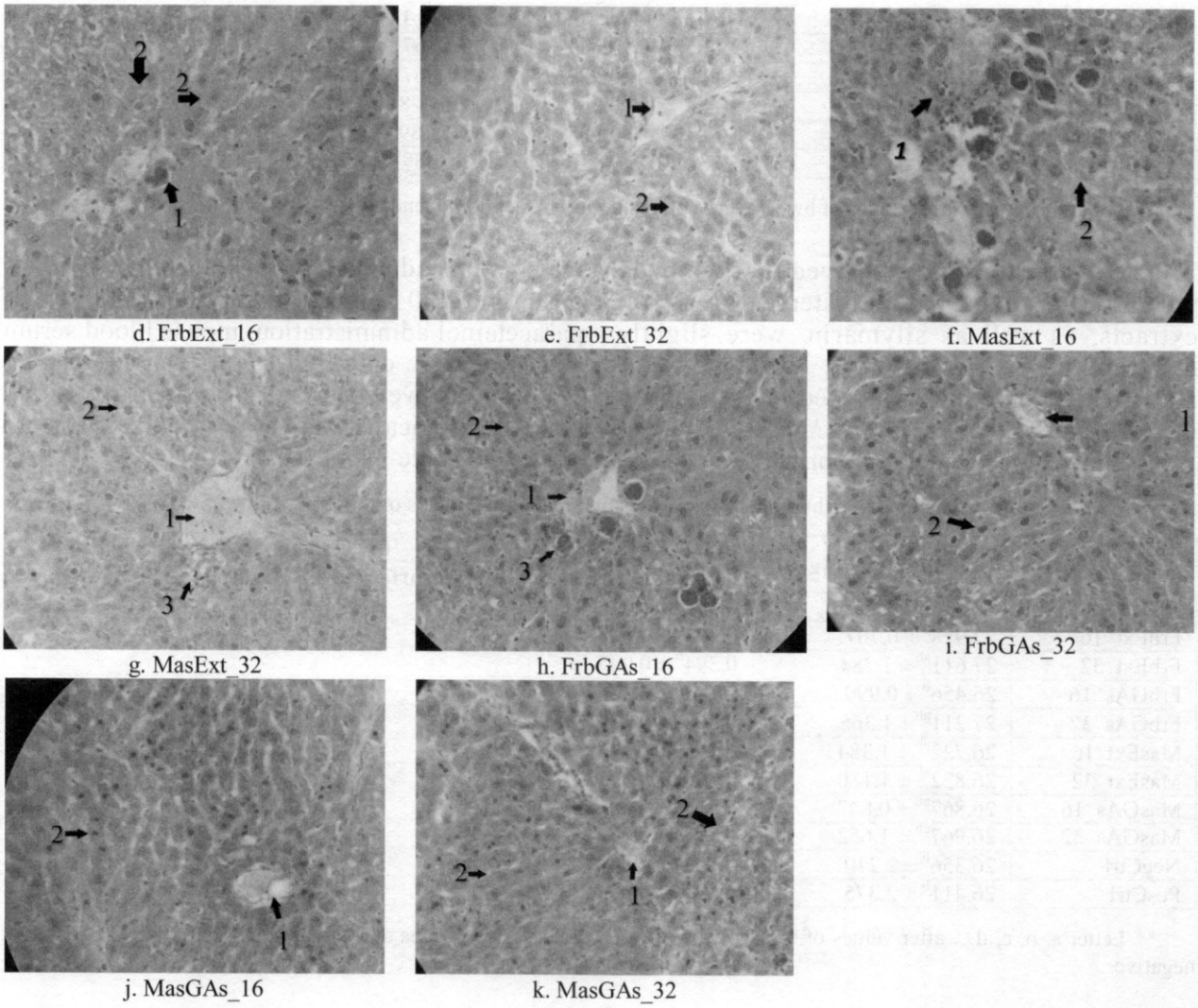


Fig. 1. Histological results of the liver in test groups after paracetamol intoxication
1: central vein, 2: hepatic cells, 3: congestion

The histological results of the liver in FrbExt_32, FrbGAs_32, MasGAs_16, and MasGAs_32 (group A) showed the best restoration at the central vein and tissues (Fig. 1e, i, j, k). The central vein kept normal size as normal control, with decreased diameter compared to negative control. The congestion disappeared around the central veins. The hepatic cells are arranged regularly, no necrosis found, small size, or disappear vacuole.

The histological results of the liver in FrbGAs_16, MasExt_32 (group B) showed the ability to restore the lobules but lower than group A. Decreasing diameter to medium in the central vein but larger than group A. Normal parenchyma with some vestiges of congestion were shown. The hepatic cells were normal with the nucleus having been tamponade by large vacuoles (Fig. 1g, h).

The histological results of the liver in FrbExt_16, MasExt_16 (group C) (Fig. 1d, f) showed that the lobule protection ability was lower than group B as well as the positive control. The central vein shrank to a lower size than the negative control, but larger than group A or B. The parenchyma was restored, no necrosis was shown, vestiges of congestion appeared, and the hepatic cells had gotten large vacuoles.

By the lobules detections, the results showed that ganoderic acids from fruit body and biomass of GNJI have hepatoprotective effects which were better than crude extracts at doses of 16 mg/kg and 32 mg/kg bw. The reduction of hepatoprotective activity in crude extract may be related to the reduced concentration of ganoderic acids (***Table 2***).

Besides ganoderic acids, the fruit body might have other contents which have hepatoprotective

[3],[4],[5],[10]. This made FrbExt_32 have hepatic protection activity as same as others in group A. Although the concentration of ganoderic acids in FrbExt_32 is lower than FrbGAs_16 (infer from Table 2, 32 mg of crude body extract has only 0.038 mg ganoderic acids).

In summary, the FrbExt_32, FrbGAs_32, MasGAs_16, and MasGAs_32 kept the liver rough, with no necrosis and no congestion, with the best restoration at the central vein and tissues. In these treatments the central vein kept normal size as normal control, decreasing the diameter compared to the negative control. The congestion disappeared around the central veins. The hepatic cells are arranged regularly, no necrosis found, small size, or disappear vacuole.

Table 8. The results of serum SGOT and SGPT in test groups

Treatments	SGOT (IU/L)	SGPT (UI/L)
BlkCtrl	$52.22^{a} \pm 4.58$	$25.56^{a} \pm 2.51$
FrbExt_16	$183.33^{g} \pm 14.81$	$102.22^{d} \pm 10.35$
FrbExt_32	$122.22^{e} \pm 12.02$	$80.00^{c} \pm 6.71$
FrbGAs_16	$85.56^{cd} \pm 7.84$	$41.11^{b} \pm 3.86$
FrbGAs_32	$71.11^{b} \pm 6.41$	$43.56^{b} \pm 4.45$
MasExt_16	$195.00^{g} \pm 14.46$	$119.33^{e} \pm 9.21$
MasExt_32	$144.44^{f} \pm 13.33$	$98.33^{d} \pm 9.01$
MasGAs_16	$92.22^{d} \pm 8.04$	$43.33^{b} \pm 3.57$
MasGAs_32	$75.56^{bc} \pm 7.68$	$40.00^{b} \pm 4.09$
NegCtrl	$572.56^{h} \pm 36.66$	$471.22^{f} \pm 36.59$
PosCtrl	$81.11^{bcd} \pm 7.57$	$44.44^{b} \pm 4.61$

** Letter a, b, c, d… after values of SGOT, SGPT in every column are groups of homogeneous ($p<0.05$).

The results of serum SGOT and SGPT were showed in Table 8. MasGAs_32 and FrbGAs_32 were the best statistical significance to hepatic protection activity (both SGOT and SGPT were inhomogeneous group b). The level of SGOT and SGPT were lower than all other experimental batches and as same as the positive control at $p<0.05$. The FrbGAs_16 and MasGAs_16 were successive activity groups (homogeneous groups c, d in SGOT, and group b in SGPT). This means the GAs in both fruit body and biomass have hepatoprotective activity which were related to the administered dose. The statistical analysis showed that GAs affect the SGOT and SGPT levels as same as a positive control at $p<0.05$. Four other treatments FrbExt_16, FrbExt_32, MasExt_16, and MasExt_32 have been significant in reducing SGOT, and SGPT to lower than the negative control but higher than the positive control. This showed that even though the crude extracts had significantly improved liver damage, these extracts had hepatoprotective effects weaker than silymarin. The low content of ganoderic acids in crude extract (Table 2) might relate to the hepatoprotective activity of these four groups.

Mechanistically, paracetamol is assumed to increase the serum levels of SGPT and SGOT, the indices of liver injury, and to decrease GSH content and glutathione reductase activity, which is an essential enzyme for the regeneration of GSH from glutathione disulfide (GSSG). The activities of catalase and superoxide dismutase, the antioxidant enzymes, were expected to be decreased after paracetamol treatment [17].

According to the results of this study, GAs might affect the process of metabolism of paracetamol in cells. This made NAPQI in cells decrease so that low combining to cellular proteins then prevented injury in hepatic parenchyma although mice were administered paracetamol like negative control. By decreasing the NAPQI combining cellular protein according to the above mechanism, GAs kept the cells in normally, not releasing SGOT and SGPT into the serum [17].

In the experiments of Shi et al, the hepatoprotective activity of peptides from *G. lucidum* was evaluated against D-galactosamine-induced hepatic injury in mice. Peptides from *G. lucidum* were administered via gavage daily for 2 weeks at doses of 60, 120, and 180 mg/kg bw, respectively. The best hepatoprotective effects of peptides from *G. lucidum* were observed after treatment with the dose of 180 mg/kg as it was evidenced by biochemical parameters and liver histopathological characteristics which were similar to those of the normal control group [18].

In other way, peptides from *G. lucidum* at a dose of 260 mg/kg bw had hepatoprotective better than control in mice with CCl4-induced liver injuries [19]. Crude polysaccharides from *G. lucidum* at a dose of 200 mg/kg bw had good significance to hepatoprotective effects in male BALB/C mice with CCl4-induced liver injuries [20]. The Kunming mice had liver injuries with α-amanitin induced and then treated with *G. lucidum* aqueous extract. The result showed that at a dose of 500 mg/kg bw, *G. lucidum* aqueous extract had hepatoprotective effects better than silibinin [21]. The crude polysaccharides from *G. applanatum* (GACP) were used to treat liver injuries on carbon tetrachloride-induced. The results showed that the hepatoprotective effect of GACP on liver fibrosis is mainly due to avoiding high elevation of pro-inflammatory cytokine. This result revealed that GACP could be a potential hepatoprotective agent for later clinical therapy [22].

4. Conclusion

Ganoderic acids extracted from fruit body and biomass have significant hepatoprotective activity. The result showed that these ganoderic acids (at doses of 16 and 32 mg/kg bw) can prevent hepatic damage from paracetamol as same as silymarin. Including, the ganoderic acids at a dose of 32 mg/kg bw show the best hepatoprotective activity.

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