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Research Article

STUDY OF HEPATOPROTECTIVE ACTIVITY OF STINGLESS BEE PROPOLIS AGAINTS TOXICITY OF DRUGS

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ABSTRACT

Objective: The aim of this study is to determine the effect of stingless bee propolis supplementation as a hepatoprotector on the prevention of Drug-induced liver injury (DILI) and the effect of healing and restoring nutrition for DILI patients due to drug induction.

Methods: The literature review starts from problem identification, library data collection, reading, taking notes, analyzing, and processing the data obtained and then compiling it into a systematic review.

Results: The results of a literature study conducted show that propolis has a good hepatoprotective ability against drugs that cause DILI cases such as the anti-tuberculosis, antibiotic, and antipyretic groups as indicated by the serum glutamic pyruvic transferase, serum glutamic oxaloacetic transferase, total bilirubin, glutathione, and superoxide dismutase values that are close to normal values. In addition, propolis supplementation can accelerate the healing and restoration of the nutritional status of DILI patients. All active compounds contained in propolis such as phytochemicals and lipopolysaccharides work to protect the liver from the toxic effects of DILI through antioxidant mechanisms.

Conclusion: Overall, the data from this literature study show that the hepatoprotective activity of propolis has the potential to complement drug therapy to reduce hepatotoxic effects and can conclusively beneficial to accelerate the restoration of nutritional status for DILI patients.

Keywords: Antioxidant, Drug-induced liver injury, Hepatotoxic Hepatoprotective, Propolis.

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INTRODUCTION

Drug-induced liver injury (DILI) is a diagnosis in patients who have liver damage caused by consuming drugs that are liver damage or hepatotoxic [1]. DILI can be caused by drugs that are hepatotoxic including anti-tubercular drugs (Rifampicin, Isoniazid, and Pyrazinamide) [2-4], nonsteroidal anti-inflammatory drugs (Ibufenac, Acetaminophen) [5], anti-retroviral drugs (Ritonavir and Indinavir) [6], anti-hyperlipidemic drugs (Statins, Atorvastatin) [7,8], anesthetic agents (CCl4, Chloroform) [9], and antibiotics (tetracycline and monocycline) [10].

The DILI study found that antibiotic, antibacterial, and anti-tuberculosis drugs were drugs with high hepatotoxic effects compared to other drugs. Based on the diagnostic criteria for hepatotoxicity and the population studied, the reported incidence of anti-TB hepatotoxicity increased from 2% to 28% [11]. The study by the US DILIN cohort reported 46% cases of anti-TB hepatoxicity [10]. In India, cases of anti-TB hepatotoxicity were reported in 58% and cases of death due to hepatotoxicity of anti-TB drugs reached 9.5% [12]. For this reason, the WHO strongly encourages research with the main aim of finding new drugs to tackle drugs that are more effective and with the lower side effects than existing drugs [13]. At present, supplements derived from natural products, fruits, vegetables, herbs, and spices have many biological properties and have the potential to fight several diseases [14]. One that is starting to be developed a lot is propolis which has many pharmacological effects.

Propolis is a resin collected by bees from various plants, which mixes with saliva and various enzymes to produce a new, different resin. Propolis has antibacterial, anti-inflammatory, antiviral and other biological activities such as anti-inflammatory, local anesthetics, hepatoprotections, anti-tumor, and immunostimulants [15]. Animal studies have shown that propolis has an antimicrobial effect [16,17], antiviral [18,19], antifungals [20,21], anti-parasitic [22,23], anti-inflammatory [22], and anti-tumor [24]. Propolis contains antioxidants and has anti-inflammatory, immunoregulatory, bacteriostatic and bactericidal effects [22,25]. Bhadauria *et al*. [26] suggested that propolis has the potential as a hepatoprotector in chronic liver injury by maintaining its antioxidant activity.

METHODS

The literature review starts from problem identification, library data collection, reading, taking notes, analyzing, and processing the data obtained and then compiling it into a systematic review. The method is generally divided into two stages; The first stage is the stage related to the data collected, consisting of: Problem identification, determining library sources based on eligibility criteria and inclusion or exclusion criteria, data collection, and sorting based on the suitability of the literature with the topics discussed. The second stage is processing the data that have passed the sorting, consisting of: Data analysis and interpretation and confirmation. Data that have been confirmed will be included in the review, while unconfirmed data will be re-sorted.

RESULTS AND DISCUSSION

Hepatoprotective activity of propolis

Hepatoprotectors are compounds or substances that can protect cells as well as repair damaged liver tissue due to toxic substances [27]. Free radical damage in the body can be overcome with antioxidants. Antioxidant is defined as a substance that can delay, prevent, or eliminate

oxidative damage to target molecules, for example, proteins, lipids, and DNA [28]. Giving hepatoprotectors can be done for prevention or healing (curative) [29]. Table 1 summarizes the literature data searched concerning hepatoprotective activity of propolis.

Studies of propolis as a hepatoprotector *in vivo* with the induction of hepatotoxic DILI drugs have been carried out by several researchers using analytical methods such as the Kruskal–Wallis test, the Mann–Whitney Advanced Test, the ANOVA test, the Tukey HSD Advanced Test, and the *post hoc* test. In general, hepatoprotective compounds have high antioxidant activity [30]. High antioxidant activity will prevent fat peroxidation and tissue damage in the liver caused by the presence of incoming free radical compounds such as hydroxyl radicals, superoxide anions, and nitric oxide [28].

Research conducted by Mahani *et al.* [31] analyzed several propolis obtained from regions throughout Indonesia. The propolis chosen as the best candidate as a complementary hepatoprotector for ATD is the propolis *Geniotrigona incisa* from South Sulawesi province. This propolis has very high antioxidant activity to reduce hepatotoxic effects (IC_{50} 100.05 ppm), low toxicity (854.75 ppm), and strong M.Tbc inhibition (49.84%).

The hepatoprotective effect of propolis with high antioxidant activity was also found to protect the liver from the toxic effects of taking the drug atorvastatin according to Abdelsameea *et al.* [32]. The study using Egypt Native propolis was carried out on 56 albino mice divided into seven groups; Group I (25 mg/kg propolis in 1 ml distilled water), Group II (25 mg/kg atorvastatin in 1 ml distilled water), Group III (50 mg/kg propolis in 1 ml suspension + 20 mg/kg atorvastatin in 1 ml distilled water), Group IV (100 mg/kg propolis + 20 mg/kg atorvastatin in 1 ml distilled water), Group V (80 mg/kg atorvastatin in 1 ml distilled water), Group VI (50 mg/kg propolis in 1 ml suspension + 80 mg/kg atorvastatin in 1 ml distilled water), and Group VII (100 mg/kg propolis in 1 ml suspension + 80 mg/kg atorvastatin in 1 ml distilled water).

After 1 month, hepatocyte degradation was obtained at a dose of 20 mg/kg and 80 mg/kg of Atorvastatin. Atorvastatin can cause acute hepatotoxicity, especially in women aged over 60 years [33]. The group treated with the addition of 50 mg/kg and 100 mg/kg of propolis showed a significant decrease in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels and catalase enzyme activity (CAT), and superoxide dismutase (SOD) as biomarkers of hepatocyte degradation. The addition of propolis treatment can protect the liver from oxidative stress that occurs due to Atorvastatin administration. Banskota *et al.* [34] stated that the hepatoprotective effect of propolis can help reduce *D-galactosamines* which trigger tumor necrosis in rat hepatocytes. Omar *et al.* [35] added that Egypt Native propolis has been shown to have a hepatoprotective effect against the anticancer agent Doxorubicin which causes side effects such as cardiotoxicity, hepatotoxicity, and nephrotoxicity in mice. Polyphenol compounds that act as antioxidants can protect liver tissue damage from oxidative stress caused by consuming drugs.

Table 1: Hepatoprotection activity of propolis

Propolis	Assay Method	Inductions	Analysis method	Biomarker compounds	Reference
Propolis extract *Geniotrigona incisa*	Clinical assay (TB patient)	ATD (Rifampicin, Isoniazid, Pirazinamid, Etambutol)	Kruskal Wallis Test and Mann Whitney Advanced Test	SGPT, SGOT, BT, GSH, SOD	(Mahani *et al.*, 2018)
Propolis ethanol extract *Trigona sp.*	*In vivo* assay (Wistar strain male white rat)	Chlorampenicol	ANOVA and Tukey HSD Advanced Test	SGOT, ALP	(Mahdi *et al.*, 2018)
Malaysian propolis extract *Heterotrigona itama*	*In vivo* assay (Sprague-Dawley strain adult male rat)	Streptozotosin (STZ)	ANOVA and Tukey HSD Advanced Test	AST, ALT, ALP, GGT, BT	(Nna *et al.*, 2018)
Propolis hydroalkoholic extract *Scaptotrigona aff.*	*In vivo* assay (Swiss strain male and female rats)	No induction	ANOVA and Tukey HSD Advanced Test	AST, ALT, TC, TG	(Araujo *et al.*, 2011)
Propolis ethanol extract and nanopropolis *Trigona spp.*	*In vivo (Sprague-Dawley strain female rat)*	7,12-Dimethylbenzaanthracene (DMBA)	Kruskal Wallis Test	Macroscopic observation and histopathological assessment	(Hasan *et al.,* 2014)
Ekstrak etil asetat propolis *Apis mellifera.*	Uji *in vivo* (pada tikus galur *Wistar)*	Epirubicin (antibiotik)	Uji ANOVA	(AST, ALT, SOD, CAT, GSH)	(Chaa *et al.*, 2019)
Egypt Native Propolis	*In vivo* assay (albino male and female rats)	Atorvastatin	ANOVA and *Post-hoc* Test	AST, ALT, SOD, CAT	(Abdelsameean *et al.*, 2013)
Egypt native Propolis ethanol extract	*In vivo* assay (Sprague-Dawley strain adult male rat)	Doxorubicin	ANOVA Test	AST, ALT, Albumin, TP and Histopathological observation	(Ayoub *et al.*, 2016)
European propolis ethanol extract	*In vivo* assay (Wistar-albino strain male and female rat)	Acetaminophen	ANOVA Test	(AST, ALT, ALP)	(Ambardekar *et al.*, 2012)
Bangladeshi Propolis ethanol extract	*In vivo* assay (Wistar-albino strain adult male rat)	Tetracycline	ANOVA and Tukey HSD Advanced Test	SGPT, SGOT, ALB, BT, TC, TG	(Tanvir *et al.*, 2019)
Egypt Native Propolis aqueous extract	*In vivo* assay (Swiss Albino strain male rat)	Octylphenol	ANOVA Test	AST, ALT, BT, SOD, GAT	(Saleh, 2012)

EFFECT OF PROPOLIS ON DRUG TOXICITY IN LIVER

Based on the literature studies, the drugs with the most potential in DILI and still widely used are the Anti-Tuberculosis (ATD) types Rifampicyn and Isoniazid which have hepatotoxicity side effects. Some researchers have found new types of OAT that have a lower hepatotoxicity effect such as quinolone drugs (moxifloxacin and levofloxacin). However, these drugs cause resistance and are quite expensive. Isoniazid itself can produce acetyl hydrazine and hydrazine by N-asetyl transferase and amide hydrolysis enzymes which are toxic metabolites and a strong liver inducer [36]. Consumption of isoniazid together with rifampicin will cause rifampicyn toxicity to the liver, and significantly reduce the survival rate of liver cells [31]. The hepatotoxicity of rifampicyn increases sharply when combined with isoniazid, which is characterized by an increase in acetylhydrazine and hydrazine. Both are toxic metabolites. The mechanism of rifampicyn hepatotoxicity is mediated by oxidative damage [37] while rifampicyn hepatotoxicity through increased ALT concentration and other disorders causes the accumulation of bilirubin [3].

Anti-tuberculosis drugs are known to be hepatotoxic which is characterized by an increase in the concentration of serum glutamic pyruvic transferase (SGPT) biomarkers, serum glutamic oxaloacetic transferase (SGOT), total bilirubin (BT), ALP and decreased concentrations of SOD, Glutathione (GSH), glutathione peroxidase (GPx), and CAT [38]. Clinically the symptoms of ATD toxicity are decreased appetite, decreased body weight (BW), insomnia, skin rash, nausea/vomiting, diarrhea, epigastric pain, fatigue, dizziness/headache, fever, peripheral neuropathy, and dysmenorrhea [39,40]. Free radical activity in TB patients who consumed ATD was very high and had low antioxidant status. This occurs because the oxidative stress mechanism to fight M.tbc infection causes depletion of antioxidants. Propolis has been widely researched and is able to provide hepatoprotective effects both *in vitro*, *in vivo,* and clinically. Research conducted by Bhadauria *et al.* [41], Hashmi *et al.* [42], Cevik *et al.* [43], and Omar *et al.* [35] showed that propolis is an antioxidant and can protect the liver from the toxic effects of drugs and ATD. The effectiveness of propolis in protecting the liver from the hepatotoxicity effects of AOT can be seen from its biomarkers, namely, SGPT, SGOT, BT, GSH, and SOD.

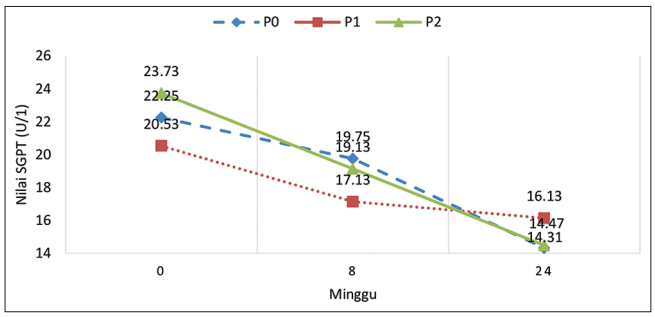

Fig. 1: Average change of the serum glutamic pyruvic transferase concentration Group P0 (ATD + Propolis placebo), P1 (ATD + Propolis 6%) and P2 (ATD + Propolis 30%) (Mahani *et al.*, 2018)

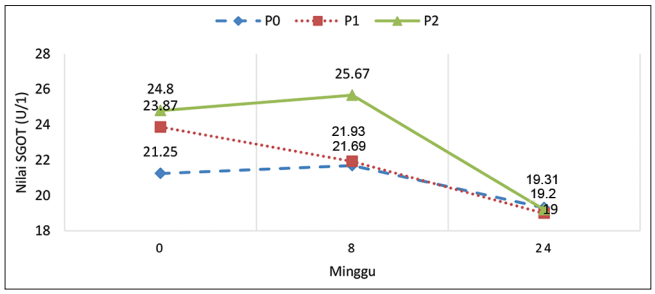

Fig. 2: Average change of the serum glutamic oxaloacetic transferase concentration Group P0 (ATD + Propolis placebo), P1 (ATD + Propolis 6%), and P2 (ATD + Propolis 30%) (Mahani *et al.*, 2018)

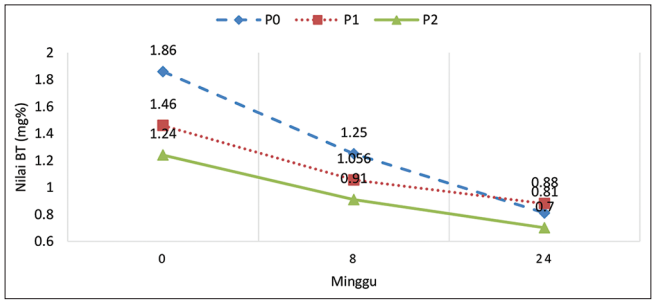

Fig. 3: Average change of total bilirubin concentration in Group P0 (ATD + Propolis placebo), P1 (ATD + Propolis 6%), and P2 (ATD + Propolis 30%)

EFFECT OF PROPOLIS ON SGPT LEVEL

Effect of propolis on SGPT level has described by Mahani *et al.* [31] clinically in three groups, Group P0 (ATD + propolis placebo), P1 (ATD + propolis 6%), and P2 (ATD + propolis 30%). During the intervention, Group P2 experienced the greatest decrease in SGPT and conversely, Group P0 experienced the smallest decrease in SGPT. This shows that the greater the concentration of propolis supplementation, the greater the ability to reduce liver toxicity.

These results are in line with the study conducted by Tanvir *et al.* [44]. SGPT concentrations in the treatment with Tetracycline (TTC) and Bangladeshi propolis were found to be significantly lower when compared to the TTC treatment in rats. TTC is toxic to the liver and can cause necrosis of hepatocytes and an inflammatory reaction [45,46]. Provision of propolis can maintain the integrity of the membrane and inhibits the leakage of enzymes in the blood. This protective mechanism is related to the presence of phenolic compounds (gallic acid, benzoic acid, salicylic acid, pyrogallol, and routine), tannins, ascorbic acid,

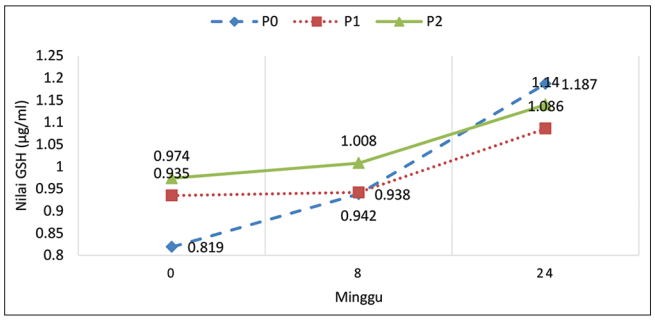

Fig. 4: Average change of the Glutathione concentration in Group P0 (ATD + Propolis placebo), P1 (ATD + Propolis 6%), and P2 (ATD + Propolis 30%)

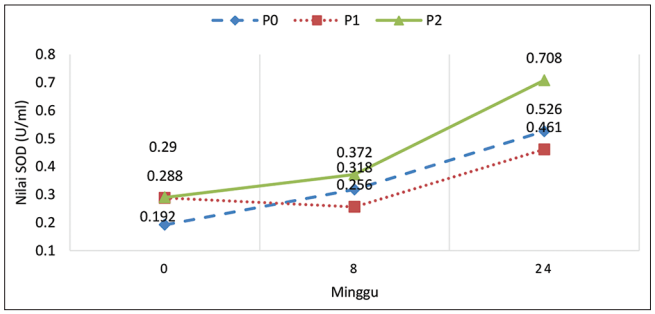

Fig. 5: Average change of the superoxide dismutase concentration in Group P0 (ATD + Propolis placebo), P1 (ATD + Propolis 6%), and P2 (ATD + Propolis 30%)

and Vitamin E in propolis extract which have inhibitory activity on membrane fat peroxidation with strong antioxidant activity [47-49].

These results are also supported by the research of Saleh [50] which states that aqueous extract of propolis can reduce the damage and hepatotoxicity effects on liver cells induced by 4-*tertiary-octylphenol* (4-tert-OP). ALT levels in mice given 4-tert-OP induction showed a significant increase when compared to controls, while mice given propolis extract had lower ALT levels than controls. In this case, propolis is believed to help repair liver damage caused by 4-tert-OP. The flavonoid compounds in propolis have been shown to have a hepatoprotective effect by binding to heavy metal ions thereby suppressing the formation of free radicals.

EFFECT OF PROPOLIS ON SGOT LEVEL

Besides SGPT, Mahani *et al*. [31] also measured the concentration of SGOT in three groups. The SGOT concentrations in the P0 and P2 groups experienced a surge in the eighth week, increasing 0.44 and 0.87 U/l, respectively. This is in contrast to the previous SGPT concentration. In the P1 group, it actually experienced a decrease of 1.93 U/l. SGOT, like SGPT, is indispensable in medicine because it acts as a protein metabolite transporter [30]. The increase in SGOT is the body's homeostasis mechanism to carry out the optimal biotransformation of metabolites for treatment. ATD consumption will produce large amounts of ATD radical metabolites. This metabolite is needed to kill M.tbc. one of the ATDs that are often used and known to be very toxic is isoniazid. The compounds in these drugs are considered foreign by the body, so they are converted by the liver into metabolites, namely, hydrazine, acetyl hydrazine and monoacetyl hydrazine. These metabolites are more soluble and can be accepted by the body, but are radical and toxic [37,51]. This radical nature will kill M.tbc. The transformation of drug compounds into various metabolite compounds is carried out by transporter proteins, namely, SGPT and SGOT.

This is in line with the research of Mahdi *et al*. [30] that showed the provision of Trigona sp. can reduce SGOT and ALP levels significantly in Chloramphenicol-induced rats. Chloramphenicol in large doses will damage liver cells and increase SGOT and SGPT levels. According to Saba *et al*. [52], Chloramphenicol has been shown to have a hepatotoxic effect, causing elevated levels of liver enzymes in the blood. This study was conducted in five groups; negative control, positive control (400 mg/kgBW Chloramphenicol), Group I (400 mg/kgBW Chloramphenicol + 8 mg propolis), Group II (400 mg/kgBW Chloramphenicol + 16 mg propolis), and Group III (400 mg/kgBW Chloramphenicol + 24 mg propolis). In the negative control, the SGOT concentration was in the normal range. This is because the body is able to balance the number of free radicals and antioxidants. The highest SGOT concentration was found in positive control. This is due to increased oxidative stress in the body due to Chloramphenicol.

Another study conducted by Ambardekar *et al*. [53] using propolis liposomes in mice induced by acetaminophen or better known as paracetamol. The liver damage in mice in this study was caused by the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) which is produced as a result of biotransformation using the enzyme cytochrome P450 as a catalyst. Under normal conditions, this metabolite is detoxified by GSH. However, high doses of Paracetamol cause an increase in NAPQI so that the detoxification process is ineffective and causes liver cell damage [54]. The results of this study showed that the AST concentration in the group treated with the addition of propolis liposomes was lower than the group treated with Paracetamol induction. Even the AST concentration in the group treated with the addition of propolis liposomes was still lower than the group treated with the addition of ordinary propolis. This showed that propolis in the form of liposomes is more effective as a hepatoprotective agent than ordinary propolis. Propolis in the form of liposomes is more easily absorbed by the tissue because it is nanoparticle size and has a bilayer structure that matches the structure of the cell membrane.

EFFECT OF PROPOLIS ON BT LEVEL

Research conducted by Mahani *et al*. [31] measured BT concentrations in three groups. Normal BT concentrations range from 0.3 to 1.2 mg%. On the other hand, the P1 and P2 groups (the propolis supplementation group) experienced a decrease in BT, namely, 1.05 and 0.91 mg%, respectively. This suggests that administration of propolis can prevent oxidative damage to the liver during the intensive treatment phase. At the advanced treatment stage, BT continued to decline in all groups. At week 24 the BT concentrations of the P0, P1, and P2 groups decreased, namely, by 0.81, 0.88, and 0.70 mg%. The BT concentration in the P2 group was the lowest compared to other groups. This suggests that high doses of propolis supplementation are effective in protecting the liver from oxidative damage due to the hepatotoxic effects of ATD. From SGPT, SGOT, and BT values as a whole, it is clear that the P2 group did not show hepatotoxicity and on the other hand the P0 group showed hepatotoxicity.

These results were also substantiated by the work of of Nna *et al*. [55] with Malaysian propolis in Streptozotocin (STZ)-induced mice. According to Graham *et al*. [56] diabetes disease induced from STZ. STZ is a glucosamine-nitrosourea compound produced from Streptomyces achromogenes which is used clinically as chemotherapy in pancreatic β-cell carcinoma. Diabetes mellitus (DM) can cause oxidative stress in the liver by producing auto-oxidation of glucose and increasing levels of ROS [57,58]. DM is reported to reduce SOD, CAT, GPx, glutathione-S-transferase (GST), and glutathione reductase (GR) [59,60]. The results of this study indicated that BT levels significantly increase in diabetic control compared to normal control. This can be influenced by increased synthesis or the rate of excretion in the liver. In DC, there is an increase in LDH activity and causes the liver to excrete the remaining bilirubin resulting in the accumulation of bilirubin. The addition of propolis treatment can reduce BT levels in STZ-induced mice in addition of metformin. It can be concluded that the combination treatment of propolis and metformin can reduce the hepatotoxicity effect of the liver in DM patients.

This result is also supported by the research of Saleh [50] which states that aqueous extract of propolis can significantly reduce BT levels in liver cells induced by 4-*tertiary-octylphenol* (4-tert-OP) significantly. BT levels in mice given 4-tert-OP induction showed a significant increase when compared to controls, while mice given propolis extract had lower BT levels than controls. High BT levels on induction of 4-tert-OP indicate liver damage. According to El-Kott & Owayss [61] an increase in BT indicates widespread damage to liver cells. The treatment of propolis extract was able to reduce BT levels, so it can be concluded that propolis is hepatoprotective and can reduce the toxic effects of 4-tert-OP on the liver.

EFFECT OF PROPOLIS ON GSH LEVEL

Mahani *et al*. [31] also measured GSH concentrations in three groups. The results showed that at the GSH concentration at the 8th week (the end of the intensive treatment stage), only the P2 group showed a GSH concentration of more than 1, which was 1008 ug/ml. The GSH concentrations of groups P0 and P1 were 0.93 and 0.94 ug/ml, respectively. The highest GSH concentration was found in the P2 group during the intensive treatment stage (week 1–8). On the other hand, the GSH concentration at the same time in the P0 and P1 groups was lower than the P2 group. The difference in GSH concentration indicates that the addition of high doses of propolis (30% concentration) can help reduce radical metabolites so that excessive GSH depletion can be avoided. It can be concluded that high doses of propolis supplementation can create a better GSH status than the other groups, especially in the intensive treatment stage and can protect the liver from the toxic effects of ATD. Similar results were described by Banudevi *et al*. (2005), Chowdhury *et al*. (2006), and Palanisamy *et al*. (2011) High GSH concentrations are an indication of hepatoprotectivity. About 30% propolis supplementation can protect the liver from the hepatotoxic effects of ATD and create good liver antioxidant enzyme status during the intensive treatment phase.

Nna *et al*. (2018) demonstrated same result with *Malaysian* propolis in STZ-induced mice. Chronic diabetes can cause major tissue damage to the liver by increasing oxidative stress [65,66], inflammation [57,66,67], and apoptosis [66,67]. In addition, DM can reduce levels of GPx, GST, and GR [59,60]. The results of this study indicated that GSH levels in Groups I, II, and III increased when compared to DC. The highest GSH levels were found in Group III. It can be concluded that the treatment of propolis and metformin is most effective in increasing the antioxidant status of the liver.

Similar results were also shown in the study of Chaa *et al*. [68]. In this study, epirubicin as an antibiotic increased biomarker compounds of oxidative stress in the liver (lipid peroxidation) and decreased endogenous antioxidant agents such as GSH, catalase, and SOD activity. Propolis from Tigzirt managed to restore this oxidative stress in liver tissue. This antioxidant effect is due to the presence of phenolic acids and flavonoids in propolis, as well as its ability to scavenge free radicals. The group of rats that were given propolis supplementation 250 mg/kg for 19 days showed a significant increase (86.51 U/g) compared to the control (76.08 U/g). The group of rats that were only given epirubicin showed a significant decrease in GSH levels (12.78 U/g) compared to the control group. This shows that the ethyl acetate propolis extract is able to restore the antioxidant status of the epirubicin-induced liver.

EFFECT OF PROPOLIS ON SOD LEVEL

Based on research conducted by Mahani *et al*. [31], the P2 group shows the highest SOD concentration in the 8th week (end of the intensive treatment stage) with a concentration of 0.372 U/ml plasma. While the P0 and P1 groups had only 0.318 and 0.256 U/ml plasma concentrations, respectively. At week 24 (end of intervention), P2 group had increased SOD to 0.708 U/ml plasma. Meanwhile, the SOD concentrations of the P0 and P1 groups were only 0.526 and 0.461 U/ml of plasma. This shows that the presence of high doses of propolis can create a better antioxidant enzyme status than the other groups and is a strong indication of hepatoprotectivity. This result is in line with Ramappa and Aithal [51] and Tostmann *et al*. [3] which stated that SOD restoration is an indication of hepatoprotectivity.

This result is also similar with the research done by Nna *et al*. [55] The results of this study indicate that the levels of SOD in Groups I, II, and III have increased when compared to DC. The highest SOD levels were found in Group III. It can be concluded that the treatment of propolis and metformin is most effective in increasing the antioxidant status of the liver. Propolis is able to improve the antioxidant status of the liver directly, indirectly, or both. Directly, propolis can directly trap ROS in the liver. Indirectly, propolis can increase the antioxidant status of the liver by reducing levels of fasting blood glucose so that it will limit the formation of ROS. An increase in antioxidant enzymes in diabetes models with the addition of propolis treatment was also found in studies using propolis from Taiwan [69], Egypt [70], China [71,72], and Brazil [72].

EFFECT OF PROPOLIS ON NUTRITIONAL STATUS

Based on the literature studies, the effects of propolis for TB sufferers include the ability to fight M.tbc infection and synergize with OAT so that it has the potential as an ingredient to accelerate the healing process for pulmonary tuberculosis sufferers. In addition, the ability of propolis as a hepatoprotector has the potential to protect the hepatotoxic effects of OAT which, in turn, is useful for accelerating the recovery process of the nutritional status of patients with pulmonary tuberculosis. Restoration of nutritional status of patients with pulmonary tuberculosis can be measured by knowing changes in BW and body mass index (BMI) [31].

One of the nutritional statuses that are measured to determine the effect of propolis is BW. Based on a study conducted by Mahani *et al*. [31], the OAT induction group without propolis supplementation experienced a decrease in BW until the 4th week. This reduction in BW is strongly thought to be due to the hepatotoxic effect of OAT, namely nausea, dizziness, and decreased appetite, so that the intake of nutrients is lower than normal. When compared with other groups, the OAT induction group with propolis supplementation did not experience a decrease in BW and immediately increased since the beginning of the intervention. This increase in BW is thought to be due to the fact that 30% propolis supplementation was effective in reducing the hepatotoxic effect of standard doses of OAT. The results of this study indicate a strong indication that the intensive use of OAT has an effect on reducing BW through the hepatotoxic mechanism.

Intensive administration of OAT can reduce a person's nutritional status as a result of the hepatotoxic effect. In contrast, supplementation of 20 drops of propolis with a concentration of 30% on OAT can reduce the effects of decreasing nutritional status due to OAT consumption. Even being able to improve the nutritional status of the subject is better than the base line. This shows a strong indication that propolis supplementation with a concentration of 30% in OAT every day can accelerate the recovery of the subject's nutritional status. The recovery of BW and BMI is faster and greater as shown by the high-dose propolis group which is able to reduce nausea since the beginning of the intervention. In addition, the recovery of their weight and body mass is also an indication that their nutrients are no longer used to fight infection as a result of their recovery in the 5th week. The effect is, after the 5th week these nutritional resources are used to restore their nutritional status.

This is confirmed by the research of Chaa *et al*. [68]. A very significant weight loss occurred in mice that received only epirubicin (G1) injections. G4 mice (rats given 250 mg/kg EAP) experienced significant weight gain up to the 19th day of supplementation. Mice in G1 (control) and G4 did not show any behavioral changes indicating that propolis extract could inhibit epirubicin hepatotoxicity. Significant changes in physical activity and behavior occurred in mice on G2 (which only received epirubicin) such as: Drowsiness, hypoactivity, hair straightening, tachycardia, difficulty breathing, and loss of balance.

IDENTIFICATION OF ACTIVE COMPOUNDS OF PROPOLIS AS ANTI HEPATOTOXICITY NUTRACEUTICAL

Based on the literature studies, there are 11 active compounds that are strongly suspected of having an antihepatotoxicity/antioxidant role. These compounds fall into two categories, namely, phytochemicals and lipids. Active compounds classified as phytochemicals are: (1) 2,6-Dimethoxyphenol, (2) Methyl-α-d-glucopyranoside, (3) 1,6-Anhydro-Beta-D-Glucopyranose, (4) (2S, 3R) -3-Allyl-3-methylapfelsaure-4-ethylester, (5) 5-Azulenemethanol, 1,2,3,3a, 4,5,6,7-octahydro-alpha, alpha, 3,8-tetramethyl, and (6) 1,4,5-Trimethylnaphthalene. Like anti-tuberculosis compounds, glycosides (methyl-alpha-D-glucopyranoside and 1,6-Anhydro-Beta-D-Glucopyranose) also act as the main hepatoprotector compound with a concentration of 40.57%. While active compounds classified as lipids are: (1) Tridecanoic acid, (2) Tetra decanoic acid, (3) Pentadecanoic acid, and (4) Hexadecenoic acid. 2,6-dimethoxyphenol [73], 1,6-anhydro-beta-d-glucopyranose compounds [74], (2S, 3R) -3-Allyl-3-methylapfelsaure-4 -ethylester [75,76], 1,4,5-trimethylnaphthalene showed reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical compounds. They can work to reduce radical metabolites by binding to them. Likewise, methyl-α-d-glucopyranoside and 5-azulenemethanol, 1,2,3,3a, 4,5,6,7-octahydro-alpha, alpha, and 3,8-tetramethyl compounds act as hepatoprotectors. They work by reducing radical metabolites. This is based on the results of research by Kokanova-Nedialkova *et al*. [77] and Vinholes *et al*. [78] which showed that the two compounds had a strong ability to reduce radical compounds (DPPH). Glycerol (propanetriol) is a lipid compound that acts as a hepatoprotector, but is not antioxidant. Glycerol has the ability to strengthen membranes and cell walls to deliver antibacterial agents [79]. This role is very important because it can protect the membranes and cell walls of the liver, which interact a lot with radical metabolites used to fight M.tbc.

CONCLUSION

Supplementation of stingless bee propolis has been shown to act as a hepatoprotector and has a positive effect on patients with drug-induced DILI by reducing liver oxidative damage, which is characterized by decreased levels of SGPT, SGOT, and BT as well as increased levels of SOD and GSH. Supplementation of bee propolis without stingers has been proven to reduce liver infection so that the nutrients possessed can be used to restore the nutritional status of DILI patients, which is characterized by increased BMI and BB values at the end of the intervention.

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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this study.

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