ORIGINAL ARTICLE

EFFECTIVE ROLE OF THE PROPOLIS AGAINST HEPATOCELLULAR DAMAGE OF TOXIC DRUGS IN ALBINO RATS

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Abstract

Objective: The main purpose of this study is to evaluate the hepatoprotective effects of propolis in hepatocyte injury caused by Anti-tuberculosis treatment (ATT) due to isoniazid and rifampicin.

Methodology: Healthy albino rats with an average weight of 200-250g were included in this study. These rats were divided into main four groups, a group was taken as the control group and then the rest were group B, group C, and group D, designated as experimental groups. The control group had 15 rats with measured weight, they were given distilled water. Group B had 15 rats, they were given with standard dose of rifampicin and isoniazid. Group C had 15 rats, they were also given with standard dose of rifampicin and isoniazid. Group D had 15 rats, they were given a standard dose of rifampicin and isoniazid and also the extract of the propolis we given to this group.

Results: Serum ALT level in experimental group B with group C, and group D was also found to be statistically significant with a p-value < 0.001. ALT serum level was observed high in group B. Multiple comparisons between groups revealed that group B with a significant increase in the serum enzyme AST level in comparison to group A, group C, and group D with p-value <0.001.

Conclusion: This study showed that ethanolic extract of propolis prevents isoniazid and rifampicin-induced hepatotoxicity in albino rats.

Keywords: Propolis, Liver Function Test (LFTs), Anti-tuberculosis treatment (ATTs), Hepato-toxicity, antioxidant, ALT, AST

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Introduction

Propolis is a naturally occurring product derived from plant resins and is collected by the *Apis mellifera* bee. Its composition is quite complex and variable, more than 300 phenolic compounds have been identified so far. Mostly, these compounds are categoryized into three groups, including esters, phenolic acids, and flavonoids. The concentration of phenolic components also varies according to the type of ecoflora. Propolis is widely used for various infections and wound healing due to its numerous chemical substances and enzymatic properties. It also exhibits antimicro-bial, anti-inflammatory, anti-parasitic, anti-oxidant, anti-viral bacteriolysis activity and inhibits bacterial protein synthesis[1,2]. Propolis has many chemical compounds and biological activities, including anti-inflammatory benefits, and is also used to reduce the side effects of anti-tuberculosis treatment (ATT) in tuberculosis. The flavonoids present in the propolis have many uses in pharmacological and biological processes. Tuberculosis is a disease caused by the Mycobacterium tuberculosis, which mainly infects the lungs and also affects bones, lymph nodes, meninges, the gastrointestinal tract, and the genitourinary system [3,4]. The first-line therapy, lasting six to nine months, includes isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin. The second line of drugs includes kanamycin, ofloxacin, cyclomerize, and ciprofloxacin. These drugs also have side effects on different body organs, especially the liver. During the ATT, liver enzyme levels are monitored during therapy, and there may be deranged levels of liver biomarkers and an abnormal rise in ALT and AST [5-7]. Isoniazid, a first-line drug used in ATT, is initially metabolized by liver enzymes, and its byproducts are the main source of damage to hepatic cells. The liver cytochrome system is a defense mechanism against this damage [8,9]. Rifampicin, a drug used in the ATT, is a semisynthetic derivative of rifamycin, which can cause orange coloration of tears and urine, as well as rash, thrombocytopenia, nephritis, and abdominal discomfort. The main hepatotoxic effects are contributed by CYP2E1, which is involved in isoniazid-related hepatotoxicity and also elevates the levels of IL-8 and NO [10,11].

During the treatment of TB, anti-TB drugs induced hepatotoxicity accounts for 2 to 28%.[12] However, the pathogenesis of anti-TB drugs is still unclear. The anti-TB therapies should be clearly monitored for the slow treatment response as well as the side effects caused by these drugs due to various metabolic processes in the liver. In our study, the ethanolic extract of propolis might be responsible for maintaining enzyme levels near normal. In groups C and D, where the ethanolic extract of propolis was administered for 30 days, the levels were significantly lower, almost resembling, those of the control group A. It could be postulated that the hepatoprotective effect of propolis ethanolic extract was due to its ability to inhibit membrane lipid peroxidation and free radical formation, accounted for by its free radical scavenging ability [2,3,7,8,12]. This study showed that the ethanolic extract of propolis prevents isoniazid and rifampicin-induced hepatotoxicity in albino rats. The propolis extract given to the rats showed positive effects in controlling the side effects of the isoniazid and rifampicin, which cause hepatotoxic effects, as seen in clinical biochemical tests. It is worth noting that isoniazid and rifampicin are metabolized in the liver, and due to their complete metabolism in hepatocytes, they cause hepatotoxicity. The hepatoprotective role of propolis is mainly effective due to the role of its antioxidant components, anti-fibrotic, anti-apoptotic and anti-inflammatory behavior. As propolis is rich in some bioactive components that play a role in its therapeutic properties. It scavenges the free radicals,

plays a role in the modulation of inflamematory pathways, and inhibition of lipid peroxidation, and is also involved in the promotion of regeneration of hepatocytes. Further detailed studies are needed to explore propolis's better and other protective roles on hepatocytes due to damage caused by isoniazid and rifampicin-induced liver damage in human beings [7,8,11].

Material and Methods

Healthy albino rats with an average weight of 200-250 g were under this study. These rats were divided into four main groups: Group A (control group), and then further into Group B, Group C, and Group D as experimental groups. The crude form of propolis was obtained from hives and prepared as ethanol extracts using 100g with 95% ethanol. The dried form was obtained using a dry rotatory evaporator method. The data was analyzed using SPSS 20.0, and the results are presented as mean ± standard deviation (S.D.). ANOVA was used to compare the differences between groups.

Control Group A: This control group had 15 rats with measured weights. They were given distilled water.

Experimental Group B: This experimental group had 15 rats with measured weights. They were given a standard dose of rifampicin and isoniazid.

Experimental Group C: This group had 15 rats, and their weights were also noted. They were also given standard dose of rifampicin and isoniazid.

Experimental Group D: About 15 rats were included in this group and given the standard dose of rifampicin, isoniazid, as well as the propolis extract which we prepared for this purpose.

Results

Serum Level of Alanine Amino Transferase (ALT): In our study, a comparison of the serum ALT levels in experimental Group B with Group C and Group D revealed significant differences. The Serum levels of the four groups were estimated before and after antibiotics (involved in the treatment of Tuberculosis) dose for the experimental purpose. Group B, which received a standard dose of rifampicin and isoniazid, had significantly higher serum ALT levels as compared to other groups. The table 1 shows the serum level of ALT along with mean and standard deviation values before and after treatment in the rats. The before and after ALT serum levels of the control (group A) and three experimental groups B, C, and D were also compared by applying ANOVA which showed that In contrast, Group D with propolis dose showed a statistically significant decrease in serum ALT levels. The p-value was less than 0.001 shows the significance. The mean square value and comparison of ALT serum level by one-way ANOVA are shown in Table 2.

Groups	Serum level of ALT before mean	Serum ALT before the standard deviation	Serum AST before mean	Serum AST after standard deviation
A	28.2	3.0	30.1	2.7
B	27.1	2.5	64.4	3.6
C	25.6	2.8	51.2	2.5
D	26.4	2.6	44.5	3.6

Table 1. Serum Level of ALT

		The sum of all square	df	Mean square	f	P value
Before	Between-group	60.6	3	20.19	2.51	0.068
	Within group	450.0	56	8.04		
	Total	510.6	59			
After	Between-group	9250.3	3	3083	296.55	<0.001
	Within group	582.3	56	10.40		
	Total	9832.6	59			

Table 2. Comparison of Serum Alt Levels for Four Groups with One Way ANOVA.

Serum Level of Aspartate Amino Transferase (AST): AST serum level was also estimated for the control and three experimental groups before and after the experiment. The group C has higher AST levels in serum as compared to other groups as shown in the

mean and Standard deviation values of all four groups A, B, C, and D in Table 3.

Then, the comparison of the AST serum level of these four groups i.e. control group A and experimental groups B, C, and D was performed by one-way ANOVA. The comparison of AST values before and after the experiment was performed and multiple comparisons between groups revealed that Group B had a significantly increased serum AST level compared to Group A, Group C, and Group D. The p-value of this comparative result is 0.001 which shows that the results are significance of results as shown in Table 4.

Group	AST serum level			
	Before		after	
	Mean	SD	mean	SD
A	126.4	4.6	127.07	4.18
B	126.9	5.5	269.13	6.51
C	128.1	3.7	174.13	5.78
D	127.3	4.0	122.87	4.66

Table 3. Serum AST Levels in Animals at the End of Experiment for Four Groups

		The sum of all squares	df	Mean square	f	P value
before	Between the groups	21.8	3.0	7.30	0.35	0.781++
	Within all groups	1138.3	55	20.32		
	Total	1160.2	58			
After	Between the groups	207986.4	3.0	69328.81	2411.24	< 0.001*
	Within the groups	1610.0	565	28.74		
	Total	209596.5	58			

Table 4. Comparison of AST Levels for Animals Before and After in Four Groups with Help of One-Way ANOVA

Discussion

Based on literature studies, the drugs with the most potential in DILI (Drug-Induced Liver Injury) and still widely used are the Anti-Tuberculosis (ATD) types Rifampicin and Isoniazid, which have hepatotoxicity side effects. Some researchers have found new types of OAT (Organic Anion Transporter) 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 through N-acetyl transferase and amide hydrolysis enzymes, which are toxic metabolites and strong liver inducers [13, 14]. Consuming isoniazid together with rifampicin will cause rifampicin toxicity to the liver and significantly reduce the survival rate of liver cells [13]. The hepatotoxicity of rifampicin increases sharply when combined with isoniazid, characterized by an increase in acetylhydrazine and hydrazine, both toxic metabolites. The mechanism of rifampicin hepatotoxicity is mediated by oxidative damage, while the rifampicin hepatotoxicity, through increased ALT concentration and other disorders, causes the accumulation of bilirubin [3]. Antituberculosis drugs are known to be heaptotoxic, characterized by an increase in the concentration of serum glutamic pyruvic transferase (SGPT/ ALT) biomarkers, serum glu-tamic oxaloacetic transferase (SGOT/ AST), total bilirubin (BT), ALP, and decreased con-centrations of SOD, Glutathione (GSH), glutathione peroxidase (GPx), and CAT [15, 16]. 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 neuro-pathy, and dysmenorrhea [17]. Free radical activity in TB patients who consumed ATD was very high, with low antioxidant status. This occurs because the oxidative stress mechanism to fight Mycobacterium Tuberculosis (*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. In different preclinical experiments, animal models are

used indi-cating the hepatoprotective role of propolis. For instance, liver damage is caused by a few drugs involved in the treatment of TB, Cancer, cardiovascular issues, and Chemotherapy. Due to the anti-inflammatory and antioxidant properties of propolis, the hepatocytes attenuated due to the side effects of various drugs like carbon tetrachloride, acetaminophen, heavy metals, and ethanol.

In this research work, one control group and three experimental groups underwent examination. Rifampicin and isoniazid drugs, used to treat TB, cause hepatotoxicity, as these drugs are metabolized by the liver causing hepatotoxicity and their side effects are minimized when the experimental organisms (rats) are given ethanolic extracts of propolis. This organic compound limited the side effects of anti-TB drugs, as the results show alterations in ALT and AST levels in groups B and C without propolis treatment. While satisfactory results were obtained in group D, which was treated with propolis, the ALT and AST levels remained within range, whereas hepatotoxic effects were observed in groups without propolis. Several research studies have been conducted to investigate the neutralizing effect of propolis, which also supports this research data. Propolis could be used for the treatment of epatotoxicity as well as it lessens the level of enzymes like AST and ALT, reduces the tissue damage of the liver, and is involved in the inhibition of markers of oxidative stress. Propolis could be declared as a therapeutic agent for liver issues.

Propolis an organic component not only neutralizes the hepatotoxicity of various drugs, but it plays a major protective role against the drug treatments of various other diseases.

Research conducted by Bhadauria et al., Hashmi et al., Cevik et al., and Omar et al. 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. The effect of propolis on SGPT level has been described by Mahani et al. 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 [18, 19]. Besides SGPT, Mahani et al. 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 experienced a decrease of 1.93 U/l. SGOT, like SGPT, is indispensable in medicine because it acts as a protein metabolite transporter [20]. 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 [21]. This study supports our study in terms of neutralizing the hepatotoxicity caused by drugs. The usage of propolis is safe in optimum amounts. However, adverse effects are negligible

related to allergy, dermatitis, and gastrointestinal issues.

A study similar to our work was carried out by a research group in Egypt, which found that propolis plays a protective role against DOX (Doxorubicin), which induces multiple toxic effects, including hepatotoxicity, cardiotoxicity, and chemotherapy side effects. In their findings, propolis was involved in neutralizing the toxic effects of the DOX drug, enhancing its antitumor efficiency, and preventing damage caused by chemotherapy. In the case of cardiotoxicity induced by DOX, which mainly involves an increase in biomarkers of heart damage (CK, CK-MB, LDH, AST, and ALT), the experimental organisms were given propolis before DOX treatment, and it was observed to be involved in neutralizing cardiotoxicity caused by DOX. In the case of hepatotoxicity caused by DOX, damaged heaptocytes due to oxidative stress were overcome when the experimental organisms were pre-treated with propolis, as it is an antioxidant. Additionally, the propolis treated group had comparatively lower levels of ALT and AST than the untreated groups. Thus, propolis, a phenolic compound, is antioxidant, anti-inflammatory, and involved in the deactivation of free radicals involved in hepatotoxicity and other multiple toxic effects [22].

Apart from neutralizing the side effects of anti-tuberculosis drugs, propolis also prevents the leakage of ALT and AST, which get leaked into the blood during the necrosis and swelling of liver tissues. It also promotes the treatment of particular drugs as well as reduces their toxicity. An exhibition of excellent hepatoprotective and antioxidant behavior was observed from this organic compound. However, propolis should not be given to those people who are allergic to bees and their products or have some serious liver issues.

Conclusion

From this study, it is concluded that propolis holds significant importance as a hepatoprotective compound due to its regeneration, antioxidant, and anti-inflammatory responses. Supplementation with stingless bee propolis has been shown to act as a hepatoprotective and has a positive effect on patients with drug-induced DILI (Drug-Induced Liver Injury) by reducing liver oxidative damage, characterized by decreesed levels of SGPT, SGOT, and BT, as well as increased levels of SOD and GSH. Supplementation with stingless bee propolis has been proven to reduce liver inflamemation, allowing the nutrients it possesses to be utilized to restore the nutritional status of DILI patients, characterized by increased BMI and BB values at the end of the intervention. In the future, further study will be performed for a better understanding of the mechanism of its action, and its efficacy in other treatment methods.

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