JURNAL KEDOKTERAN SYIAH KUALA
www.journal.usk.ac.id/jks
ORIGINAL RESEARCH
P-ISSN: 1412 – 1026
E-ISSN: 2550 – 0112

The Effect of Temulawak Extract (*Curcuma xanthorrhiza roxb*.) on Serum Albumin in Wistar Rats Induced by Paracetamol

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ARTICLE INFO

Keywords:
Albumin Serum, *Curcuma xanthorrhiza roxb,*
Paracetamol, temulawak

Article History:
Received: 14/01/2024
Accepted: 12/12/2024
Published Online: 30/12/2024

ABSTRACT

Introduction: Paracetamol is a drug that is often used as an analgesic and antipyretic. However, the incidence of poisoning due to Paracetamol is still relatively high. Liver damage due to a lethal dose of Paracetamol causes an increase in liver serum enzymes and a decrease in albumin levels. Various plants have been extensively researched to act as hepatoprotectors. Temulawak *(Curcuma xanthorrhiza)* contains secondary metabolites, including xanthorrhizol and curcumin, which function as hepatoprotection by capturing free radicals, lipid peroxidase, and reducing hepatocyte apoptosis.
Objective: This study aims to determine the effect of ginger extract on serum albumin levels induced by Paracetamol in Wistar rats.
Methods: This research is an experimental study (pretest-posttest with control group). A total of 25 rats were divided into five groups: Group I (K1) was only given distilled water; Group II (K2) was given Paracetamol 2000 mg/kg BW; Group III (P1) given 800 mg/kgBW ginger extract on days 8-14 and Paracetamol 2000 mg/kgBW on day 19-21 for three days; Group IV (P2) given ginger extract 1600 mg/kgBW on day 8-14 and Paracetamol 2000 mg/kgBW on day 19-21 for three days; and group V (P3) given ginger extract 3200 mg/kgBW on day 8-14 and Paracetamol 2000 mg/kgBW on days 19-21 for three days. Serum albumin levels were measured before and after treatment.
Results: Temulawak extract at 1600 and 3200 mg/kgBW had a better hepatoprotective effect than 800 mg/kgBW. Statistically, the difference is significant with a p=0.000 (p<0.05).
Conclusion: Ginger extract has a significant effect on changes in serum albumin levels in Wistar rats induced by toxic doses of Paracetamol.

INTRODUCTION

Paracetamol is a drug that is often used as an analgesic and antipyretic with a therapeutic dose of 4 grams/day. After entering the body, Paracetamol will be metabolized into toxic metabolites such as *N-acetyl-benzoquinone imine* (NAPQI) by cytochrome P450, a liver enzyme (Saide et al., 2019). The incidence of poisoning due to Paracetamol is still relatively high (Febriani et al., 2020). It becomes an analgesic for mild to moderate pain (nociceptive pain). Paracetamol can reduce body temperature through a central effect mechanism by inhibiting prostaglandin production mediated by cyclooxygenase 3 (COX-3) in the central nervous system. Side effects in irritation, erosion, and stomach bleeding do not occur when administering Paracetamol, nor do respiratory and acid-base balance disorders (Przybyła et al., 2021)(Sharma & Mehta, 2014). Fatal toxic effects due to long-term use of Paracetamol at toxic doses in adults is 7.5 grams – 10 grams and 150 mg/kgBW – 200 mg/kgBW in children aged 1-6 years are the cause of hepatotoxicity and

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https://doi.org/10.24815/jks.v24i3.36849

nephrotoxicity (Hidayati & Kustriyani, 2020). Paracetamol overdose will cause hepatotoxicity by saturating the sulfation pathway and producing excess NAPQI so that GSH (Glutathione) storage in the liver is reduced and NAPQI cannot be excreted (Xie et al., 2015)(O'Brien et al., 2015). Glutathione deficiency results inhibits the conjugation of remaining metabolites so that ROS can damage DNA, proteins, and lipids in hepatocyte cells. Massive ROS and GSH depletion increase result in hepatocyte cell damage and acute hepatic necrosis (Chamchoy et al., 2022). The function of necrotic hepatocyte cells will decrease, characterized by an increase in liver enzymes in the blood and a decrease in liver products, one of which is albumin(Levitt & Levitt, 2016). Liver damage caused by lethal doses of Paracetamol causes an increase in liver serum enzymes and a decrease in albumin levels(Sedky et al., 2019). Albumin is the most abundant protein in serum. Albumin synthesis occurs in hepatocytes, accounting for 10% of total hepatic protein synthesis. The average expected level of albumin in healthy conditions is around 4.3 grams/mL, or 60% of total plasma protein. Elevating the antioxidant levels can repair hepatocyte cells damaged due to oxidative stress. The curcumin content can repair hepatocyte cells and increase serum albumin levels (Kim et al., 2014).

Temulawak is a medicinal plant native to Indonesia called *Curcuma javanica*. Temulawak is cultivated in Indonesia and Southeast Asian countries such as Malaysia, Thailand, Vietnam, and the Philippines (Rahmat et al., 2021). Curcumin is the main compound and plays a role in hepatoprotective activity. Curcumin can inhibit Gluthation s-transferase (GST) and cytochrome P450 activity. The mechanism of the preventive effect of curcumin is related to inhibiting lipid peroxidation and oxidative stress. Curcumin restores the ratio of Bcl-2 and Bax, thereby reducing paracetamol-induced hepatocyte apoptosis (G. Li et al., 2013). Xanthorrhizol is an active compound from ginger that is responsible for hepatoprotective activity. The mechanism of the hepatoprotective effect of xanthorrhizol is related to its ability to regulate the DNA binding of transcription factors, nuclear factor kappa B (NF-αB), and activator protein-1 (AP-1)(Kim et al., 2014). The pharmacological properties of xanthorrhizol include anti-inflammatory, nephroprotective, and hepatoprotective properties. The antioxidant activity of xanthorrhizol was also found in research conducted by Jantan et al., that the most significant decrease in hydrogen peroxide in the brain of mice was induced by lipid peroxidase after intervention with xanthorrhizol (Jantan et al., 2012).

The content of xanthorrhizol and curcumin, which function as a hepatoprotector, can be observed through the mechanism of capturing free radicals and suppressing the synthesis of gene transcription factors in the immune and inflammatory process Tumor Necroting Factor--α (TNF-α), namely Nuclear factor kappa B (NF-kB) (Handayani et al., 2021). In previous research, administering doses of ginger ethanol extract of 400, 800, and 1600mg/kgBW to mice showed positive results in reducing serum Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) levels, which increased due to paracetamol induction, resulting in damage to hepatocyte cells(Hadinata, 2016). Therefore, researchers wanted to know the effect of ginger extract on serum albumin levels in rats induced by Paracetamol.

METHODS & MATERIALS

This research is a quasi-experimental study (pretest-posttest with a control group design) to determine the effect of administering Temulawak extract (*Curcuma xanthorrhiza robx.*) as an antioxidant on serum albumin levels examined at the beginning and end of treatment in Wistar rats induced by Paracetamol. This research was conducted at the Pharmacology Laboratory of FK

UII and the FMIPA UII Laboratory with the ethical number 23/Ka.Kom.Et/70/KE/V/2023 from The Ethics Committee of the Faculty of Medicine, Universitas Islam Indonesia.

Male Wistar rat (*Rattus norvegicus*) aged 8-12 weeks with a body weight of 150-200 grams with the inclusion criteria: clean, smooth, and shiny fur, no mucus in the nose and mouth, clear eyeballs, solid, not liquid, stool consistency, and active and constantly moving, divided into five groups (5 animals per group). Temulawak powder was obtained from the Jamu Sanctification Clinic, Tawangmangu Center for Research and Development of Medicinal Plants and Traditional Medicine (B2P2OOT). Extracts were made using the maceration extraction test method conducted at the Faculty of Mathematics and Natural Sciences, UII. Temulawak powder was weighed, and a maceration process was conducted using a magnetic stirrer. After the ginger powder is macerated, it is separated and purified to remove unwanted compounds so that a purer extract is obtained using a Buchner funnel. This process results in a filtrate, which is dried using a Vacuum Rotary Evaporator at a temperature of 70°C for 5 hours. Wistar rats were placed in a comfortable environment with a room temperature of 27˚C. The cage used is clean and has a husk floor. One cage can contain a maximum of five mice. We acclimatized the sample before the intervention by adjusting the lighting settings to 12 hours of light and 12 hours of darkness. Wistar rats were given BR AD 2 pellets daily, and drinking water was provided ad libitum. A Wistar rat is kept in a 40x30x20 cm^3 cage. Rat acclimatization was carried out for seven days.

Treatment of experimental animals in this research was carried out for 14 days with a frequency of administration once a day. Control group 1 (K1) received no treatment and was only given distilled water for 14 days. Control group 2 (K2) was given distilled water on days 8-14 and Paracetamol 2000 mg/kgBW on days 19-21 for three days. Treatment group 1 (P1) was given ginger extract at 800 mg/kgBW on days 8 -14 and Paracetamol 2000 mg/kgBW on days 19-21 for three days. Treatment group 2 (P2) was given ginger extract at 1600 mg/kgBW on days 8-14 and Paracetamol 2000 mg/kgBW on days 19-21 for three days. Treatment group 3 (P3) was given ginger extract at 3200 mg/kgBW on days 8-14 and Paracetamol 2000 mg/kgBW on days 19-21 for three days. The administration of paracetamol and ginger extract was calculated according to the rat's body weight, then mixed with a small quantity of water and given orally using a probe.

Pretest blood samples were taken before treatment on day 8; blood was taken via the retroorbital of the Wistar rats using a hematocrit pipette and then placed into an Eppendorf tube. Posttest blood samples were taken on the 22nd day, also using a hematocrit pipette. Serum albumin levels were examined at the LPPT Gajah Mada University. The data obtained was then analyzed using One-way ANOVA statistical software to determine whether there were significant differences between groups.

RESULT

Serum albumin levels were measured before treatment (pretest) and after treatment (posttest). The average results of serum albumin level measurements are presented in Table 1.

In Table 1, all groups have average serum albumin levels in the same range as before treatment. This can be seen from the average serum albumin levels in (K1) 2.71 g/dL, (K2) 2.81 g/dL, (P1) 2.56 g/dL, (P2) 2.77 g/dL and (P3) 2.74 g/dL. Group K1, which was only given standard feed and distilled water without ginger or Paracetamol, did not experience significant differences in albumin levels before and after treatment. In the K2 group, which only received toxic doses of Paracetamol on day 19, albumin levels decreased by 15% from serum albumin levels before treatment with an average of 2.81g/dL to 2.40 g/dL. In the P1 group who were given ginger at a dose of 800 mg/kg BW, their albumin levels increased by 8% from the albumin levels before

treatment with an average of 2.56 g/dL to 2.76 g/dL. In the P2 group who were given ginger at a dose of 1600 mg/kg BW, serum albumin levels increased by 35% from albumin levels before treatment with an average of 2.77 g/dL to 3.75 g/dL. In group P3, those given ginger at a dose of 3200 mg/kgBW experienced an increase in serum albumin levels of 35% from the serum albumin levels before treatment with an average of 2.74 g/dL to 3.70 g/dL. This shows a change in serum albumin levels in the groups given treatment, namely groups K2, P1, P2, and P3.

Table 1. Analysis of pretest and post-test of Serum Albumin level

Group	Serum Albumin level		*P Value**
	***Pretest* (g/dL)**	***Posttest* (g/dL)**	
K1 (Aquadest)	2.71 ± 0.08	2.72 ± 0.29	0.098
K2 (PCT 2gr/kgBW)	2.81 ± 0.13	2.40 ± 0.36	0.040
P1 (TMW 800mg/kgBW + PCT 2gr/kgBW)	2.56 ± 0.28	2.76 ± 0.27	0.208
P2 (TMW 1600mg/kgBW + PCT 2gr/kgBW)	2.77 ± 0.09	3.75 ± 0.13	0.000
P3 (TMW 3200mg/kgBW + PCT 2gr/kgBW)	2.74 ± 0.17	3.70 ± 0.36	0.006

TMW = Temulawak, PCT = Paracetamol

Table 2. Analysis of differences in Serum Albumin Levels between groups (post-test)

Group	Mean +SD	P Value
K1 (Aquadest)	2.72 ± 0.29	<0.001
K2 (PCT 2gr/kgBW)	2.40 ± 0.36	
P1 (TMW 800mg/kgBW + PCT 2gr/kgBW)	2.76 ± 0.27	
P2 (TMW 1600mg/kgBW + PCT 2gr/kgBW)	3.75 ± 0.13	
P3 (TMW 3200mg/kgBW + PCT 2gr/kgBW)	3.70 ± 0.36	

Based on data on albumin levels before and after treatment, *a paired t-test* was conducted to determine whether there was a significant difference in serum albumin levels before and after treatment. The results of data analysis obtained a value of p=0.011 (p<0.05), which showed that there was a significant difference in serum albumin levels before and after treatment (p<0.05). Data from measurements of serum albumin levels were then analyzed using the *One Way ANOVA* statistical test to determine whether there were significant differences in serum albumin levels between post-test treatment groups after being given ginger for 14 days and Paracetamol for three days in Wistar Rats. They obtained a p-value = 0.00, which indicated there were significant differences in serum albumin levels between treatment groups K1, K2, P1, P2, and P3. The results of the ANOVA test, which compared differences between treatment groups at the posttest, showed significant differences. Meanwhile, the results of the ANOVA test between treatment groups in the pretest showed no significant differences (p= 0.211). Then, *a post hoc* test was carried out in the group after treatment (post-test) to compare significant differences between the treatment groups, as seen in Table 3.

Table 3. *Post Hoc Bonferroni Test* Results for Serum Albumin Levels

Comparison group	P value
K1-K2	0.797
K1-P1	1,000
K1-P2	<0,001*
K1-P3	<0,001*
K2-P1	0.473
K2-P2	<0,001*
K2-P3	<0,001*
P1-P2	<0,001*
P1-P3	<0,001*
P2-P3	1,000

* P <0.05: There is a significant difference

In the table above, there are significant differences in serum albumin levels between K1 and P2, K1 and P3, K2 and P2, K2 and P3, P1 and P2, P2 and P3 as shown by the value of p=0.000 (p<0.05). There was a significant difference in group K1 compared to groups P2 and P3 with a value of p=0.000 (p<0.05). This shows a significant difference in serum albumin levels between the group that was only given distilled water for 14 days and the group that was given ginger extract at 1600 mg/kgBW and 3200 mg/kgBW for 14 days.

DISCUSSION

According to the results of the phytochemical testing, the extract of Temulawak (Curmumin xanthorrizha) includes a lot of secondary metabolites that function as a hepatoprotector. In in vivo research, the antioxidant activity of temulawak curcumin can increase antioxidant activity characterized by increased levels of the enzymes catalase, glutathione reductase, superoxide dismutase, glutathione peroxidase, and glutathione S-transferase as well as reducing lipid peroxidase levels (Rohman et al., 2020). Typically, albumin levels in male Wistar rats aged 8 - 16 months range from 3.0 – 5.1 gr/dL(Khasanah et al., 2015) . Wistar rats that received a toxic dose of paracetamol induction without prior administration of ginger experienced a 15% decrease in albumin levels. Fatal toxic effects due to long-term use of Paracetamol at toxic doses in adults is 7.5 grams – 10 grams and 150 mg/kgBW – 200 mg/kgBW in children aged 1-6 years are the cause of hepatotoxicity and nephrotoxicity (Hidayati & Kustriyani, 2020). The cause of toxic doses of Paracetamol can increase levels of NAPQI metabolites by liver microsomal cytochrome P450 enzymes, which will induce oxidative stress resulting in GSH depletion, which triggers damage to liver cells (Win et al., 2018).

The decrease in serum albumin levels due to hepatocyte cell damage induced by toxic doses of Paracetamol in this study was also studied in research conducted by Boddupally et al. (2023). This study shows the histopathological picture of liver cells induced by toxic doses of Paracetamol. The histopathological picture of hepatocyte cells induced by toxic doses of Paracetamol shows stenosis, necrosis, and lymphatic infiltrates in the periportal area, giving the impression of acute liver injury. When hepatocyte cells undergo lysis, the liver's function in producing albumin protein is disrupted so that albumin secretion into the blood vessels decreases (Boddupally et al., 2023).

The Wistar rats given ginger extract before being induced by Paracetamol experienced increased albumin levels. As seen in Table 1 above, the treatment group given ginger extract at 1600 and 3200 mg/kgBW achieved a higher average serum albumin level than those given ginger extract at a dose of 800 mg/kgBW. Temulawak (*Curmumin xanthorrizha*) has the active substances

curcumin and xanthorrizhol, which act as an anti-inflammatory and hepatoprotector by suppressing the synthesis of Prostaglandin E2 (PGE2) and Nitric oxide (NO) and inhibiting cyclooxygenase-1 (COX-1) and Inducible nitric oxide synthase (iNOS). So, the Wistar rats that were given ginger extract before being induced by a toxic dose of Paracetamol did not experience a decrease in albumin levels. Temulawak also has antioxidant activity, which can increase glutathione (GSH) levels in the liver(G. Li et al., 2013; Oon et al., 2015). Based on the paired t-test, the group before and after treatment obtained a value of $p=0.011$ ($p<0.05$). This indicates a significant difference in serum albumin levels before and after treatment. The administration of ginger extract as a hepatoprotector inhibits cyclooxygenase-2 (COX-2) and increases the activity of antioxidant enzymes such as catalase and glutathione. The high antioxidants in ginger extract ward off free radicals and prevent the formation of oxidative stress so that damage to hepatocyte cells does not occur. Albumin is synthesized in healthy hepatocytes. Secreted albumin protects hepatocyte cells by maintaining GSH levels(Rohman et al., 2020).

One Way ANOVA test showed that the Wistar rats given ginger extract at 800 mg/kg BW dose did not significantly differ from those induced by a toxic dose of Paracetamol. However, the Wistar Rat group given ginger extract at 1600 mg/kgBW and 3200 mg/kgBW significantly differed from those induced by toxic Paracetamol doses. This research shows that ginger extract at 1600 mg/kgBW and 3200 mg/kgBW has a better hepatoprotective effect than ginger extract at 800 mg/kgBW. This is in line with research conducted by Hadinata (2016), which stated that administration of ginger extract at a dose of 1600 mg/kgBW had a significant difference in reducing SGOT and SGPT levels in Wistar rats induced by Paracetamol compared to ginger extract at a dose of 400 mg/kgBW and 800 mg/kgBW (Hadinata, 2016). The higher dose increases the albumin serum level and affects the antioxidant activity as hepatoprotector.

This research is also in line with research by Pramono et al. (2018), which showed that the administration of ginger extract to a group of Wistar rats that were induced by toxic doses of Paracetamol showed improvements in liver histopathology compared to the group that was only induced by Paracetamol. In this research, phytochemical tests were also carried out to determine the presence of compounds contained in ginger, namely curcumin and *xanthorrhizhol(Pramono et al., 2018)*. In the group that was not given temulawak, albumin levels decreased due to the toxic effects of Paracetamol. Albumin can inhibit TNF-α expression directly by downregulating its transcription and indirectly maintaining cellular GSH, which can protect cells from damage caused by reactive metabolites. Downregulation of TNF-α by albumin can inhibit the activation of the pro-inflammatory Nuclear Factor-Kappa B (NF-kB) pathway and leukocyte recruitment and inflammation in the liver (Carvalho & Machado, 2018). Temulawak consists of curcumin antioxidants that can ward off free radicals, prevent oxidative stress, and suppress the formation of NF-kB, which is a transcription factor for several important genes in the immune and inflammatory processes, one of which is to form Tumor Necroting Factor -α (TNF-α) (Handayani et al., 2021). Curcumin restores the ratio of Bcl-2 and Bax, thereby reducing paracetamol-induced hepatocyte apoptosis. The Bcl-2 protein is generally known as an inhibitor of cell apoptosis by preventing mitochondrial membrane depolarization. Bax can inactivate Bcl-2 by forming heterodimers. Curcumin can downregulate Bax mRNA expression and upregulate Bcl-2 mRNA expression. Curcumin also exerts a strong antiapoptotic effect by inhibiting TGF-β as an inducer of caspase-3- apoptosis (G. Li et al., 2013; R. Li et al., 2013).

Research conducted by Masfufatun et al. (2020) reported that giving ginger extract at 1080 mg/200 gr BW to mice effectively reduced SGOT and SGPT levels in mice induced by toxic doses of Paracetamol (Masfufatun et al., 2021). The group given ginger extract had lower MDA levels than those induced by Paracetamol. The administration of ginger extract can suppress the

formation of free radicals in the liver induced by toxic doses of Paracetamol. The curcumin content in ginger can inhibit cytochrome p450 activity and activate the BCL-2 protein. The BCL-2 protein has antiapoptotic activity by preventing depolarization of the mitochondrial membrane so that apoptosis of hepatocyte cells does not occur(G. Li et al., 2013).

CONCLUSION

According to the findings of this investigation, the administration of Temulawak (*C.xhanthoriza*) extract significantly changes serum albumin levels in Wistar rats induced by toxic doses of Paracetamol compared to the control group. Increasing the dose of ginger extract can increase serum albumin levels in Wistar rats induced by Paracetamol. Temulawak extract at 1600 and 3200 mg/kgBW had a better hepatoprotective effect than 800 mg/kgBW. The higher dose of temulawak also effectively increases albumin levels that inhibit TNF-α expression for antioxidant mechanism. This study has limitations in that it did not measure creatinin and histopathology of the liver, which was able to show the more hepatoprotective effect of temulawak.

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