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Antioxidant Associated Antihypertensive Performance of Purified Gambir (*Uncaria gambir* Roxb.) on Prednisone Salt-Induced Hypertensive Rats

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Abstract

AIM: Purified gambir (*Uncaria gambir* Roxb.) is potential for treating hypertension based on the antioxidant activity of its catechin compound. In this study, we investigated the antihypertensive performances of purified gambir on hypertensive rats and the correlation with its antioxidant activity.

METHODS: Rats (2–3-months-old and the body weight [BW] of 200–250 g) were divided into five groups. Groups 1, 2, and 3 were treated orally with water suspension of purified gambir at doses of 2.5; 5; and 10 mg/kg BW once a day for 14 days, group 4 and 5 were captopril treated groups (positive control) and Carboxymethyl Cellulose Sodium suspension treated (negative control) respectively. The systolic blood pressure, diastolic blood pressure, mean arterial pressure, heart rate, blood flow, and blood volume were recorded once a day before and on 1, 3, 7, and 14 days after commencement. The blood was taken before and at the end of recording the Plasma Nitric Oxide (NO) concentrations. Two-way ANOVA was used to analyze the changes of those parameters, except for plasma NO concentrations analyzed by one-way ANOVA, followed by Duncan multirange test (95% confidence interval).

RESULTS: The study showed significant antihypertensive effects ($p < 0.05$), influenced by doses and gambir administration duration. The plasma NO concentrations of animals were increased after administration of purified gambir ($p < 0.05$), although there was no significant difference in plasma NO concentrations in gambir doses.

CONCLUSION: These indicated that purified gambir proceeded antioxidant-associated antihypertensive effects at doses of 2.5–10 mg/kg BW when administered for 14 days on the salt-prednisone induced hypertensive rats. Based on the cardiovascular parameters, the smallest dose (2.5 mg/kg BW) exhibited the best reduction in blood pressure compared to other groups. Regarding NO plasma concentrations, it showed the medium-dose group, 5 mg/kg BW, caused the highest increase of NO plasma concentration.

Introduction

Hypertension is a degenerative disease whose prevalence is increasing every year. This non-communicable disease is a significant risk factor for cardiovascular disorders [1]. Apart from its high prevalence, hypertension has become the highest cause of death globally in 2019 [2]. Reactive Oxygen Species are well-known for preserving cellular homeostasis. However, its overproduction has been reported to cause oxidative stress, which is thought to damage cell structure. It is believed to be responsible for developing several cardiovascular diseases such as hypertension [3].

Medicinal plants have long been known and used by the people of Indonesia to overcome health problems [4]. One of the plants developed to become herbal medicinal plants is the gambir (*Uncaria gambir* Roxb.) plant which is spread in Aceh, North Sumatra, Riau, West Sumatra, Bangka, Belitung, and West Kalimantan [5]. West Sumatra is one of the most important suppliers for the world's gambir needs [6].

Gambir is potential as antibacterial [7], antioxidants [8], antihypertension [9], antihyperglycemic [10], hepatoprotection [11], [12], and atherosclerosis [13]. Gambir contains catechins, tannic catechu acid, quercetin, red catechin, flavonoid and fluorescent gambir [14]. Catechins showed antioxidant and antihypertensive effects in hypertensive rats. Increased plasma Nitric Oxide (NO) concentrations were reported along with a decrease in blood pressure in hypertensive rats and did not cause hypotension in normal rats [15].

In this article, doses variation and the duration of administration of purified gambir (*U. gambir* Roxb.) to the cardiovascular parameters and plasma NO concentrations on hypertension rats will be explored.

Materials and Methods

Water suspension of purified gambir preparation

Purified gambir was prepared as Carboxymethyl Cellulose Sodium (Na-CMC) water suspension of 0.025, 0.05, and 0.1 % w/v.

Aminal preparation for hypertensive condition

A total of 15 white male rats (weighing 200–250 g, aged 2–3-months-old) were acclimatized to normal laboratory conditions for 7 days before experimental procedures. Food and drink were provided during this period.

After acclimatization, rats were induced using Prednison 5 mg/kg body weight (BW) and NaCl 8% to get the hypertensive rats. An inducer was given orally simultaneously for 14 days. Animals were indicated hypertensive condition if the animal's Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Mean Arterial Pressure (MAP) were increased up to 140 mmHg, 90 mmHg, and 110 mmHg, respectively.

Evaluation of the purified gambir effect on cardiovascular parameters

The hypertensive rats were divided into five groups; every group consisted of three rats. Rats were treated, group 1–3: water suspension of purified gambir at the dose of 2.5 mg/kg BW, 5 mg/kg BW and 10 mg/kg BW, group 4: Captopril 2.25 mg/kg BW (positive control), and group 5: 0.5% Na-CMC suspension (negative control). All treatments in this study were given orally once a day for 14 days.

The cardiovascular parameters such as SBP, DBP, MAP, Blood Flow (BF), Blood Volume (BV), and Heart Rate (HR) of the test animals were measured using the Non-Invasive Blood System from CODA® High Throughput System with 8 Activated Channels (USA). The CODA® mouse rat tail-cuff system was designed to measure the blood pressure in mice and rats accurately. This system utilizes Volume Pressure Recording sensor technology to measure the blood pressure of those animals. Therefore, the condition of the test animal must constantly be monitored and guarded so as not to stress, faint or even die because it will affect the BF to the tail. Cardiovascular parameters were measured on days 1, 3, 7, and 14, respectively. The data were presented the percentage change, which was calculated as the difference of all parameters before and after treatment, using the following formula:

$$\text{Percentage change of blood parameters (\%)} = \frac{\text{Blood parameters at early - Blood parameters at the time}}{\text{Blood parameters at early}} \times 100$$

Evaluation of plasma NO concentrations

A sampling of rat serum was carried out before and on day 14 to examine plasma NO concentrations using the Rat NO Enzyme-Linked Immunosorbent Kit (Bioassay Technology Laboratory – China) and read with the xMArk Microplate Reader® (Bio-Rad Laboratories – Japan). The volume of serum used for testing is 40 microliter. The data presented in the form of percentage changes in plasma NO concentrations before and after administration of purified gambir preparations, the percentage changes which were calculated through the following equation:

$$\text{NO concentrations change percentage (\%)} = \frac{\text{NO conc. at early - NO conc. at the end}}{\text{NO at early}} \times 100$$

Data analysis

All data of all parameters were analyzed using two-way ANOVA except for plasma NO concentration, by one-way ANOVA followed by Duncan's multiple T-test and significance was taken at $p < 0.05$.

Ethical approval

For this experiment, the ethics code approval was obtained from the ethics committee of the Faculty of Medicine, Andalas University, Padang - Indonesia (The registration numbers of 364/UN.16.2/KEP-FK/2021).

Results

The induction caused an increase in cardiovascular parameters to reach the pathological condition of hypertension. The results showed that prednisone salt administration caused a significant increase ($p < 0.05$) in SBP, DBP, and MAP. In contrast, HR, BF, and BV were unchanged ($p > 0.1$) (Table 1).

Table 1: The average cardiovascular parameters of normal and hypertension rats

	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	HR (BPM)	BF (µl/menit)	BV (µl)
Normal	111 ± 2	77 ± 2	88 ± 2	352 ± 10	22 ± 1	80 ± 4
Hypertension	152 ± 1	118 ± 2	129 ± 2	342 ± 11	17 ± 1	65 ± 6

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean arterial pressure, HR: Heart rate, BF: Blood flow, BV: Blood volume.

SBP was significantly decreased by the doses ($p < 0.05$) and duration ($p < 0.05$) of purified gambir administration but not by the interaction of these two variables ($p>0.05$). The three variations of purified gambir dose caused a decrease in SBP, which was not significantly different from the positive control group. The reduction in SBP was higher when gambir use was extended ($p < 0.05$). Table 2 showed the average decline in SBP (%) in the gambir group treated at doses of 2.5, 5, 10 mg/kg BW, positive control and negative control group were 17 ± 2, 14 ± 2,11 ± 2, 17 ± 2, and 0 ± 2. Based on days, the average decrease in SBP (%) on days 1, 3, 7, and 14 were 7 ± 2, 10 ± 2, 13 ± 2, and 19 ± 2, respectively.

Table 2: The effect of purified gambir on SBP

Groups	Decrease of SBP (%) on days ± SE				Average ± SE
	1	3	7	14	
Gambir					
2.5 mg/kg BW	7 ± 4	10 ± 4	26 ± 4	24 ± 4	17 ± 2
5 mg/kg BW	5 ± 4	8 ± 4	20 ± 4	23 ± 4	14 ± 2
10 mg/kg BW	7 ± 4	9 ± 4	6 ± 4	23 ± 4	11 ± 2
Captopril (positive control)	16 ± 4	21 ± 4	10 ± 4	21 ± 4	17 ± 2
Na-CMC (negative control)	−3 ± 5	−1 ± 4	1 ± 4	5 ± 4	0 ± 2
Average ± SE	7 ± 2	10 ± 2	13 ± 2	19 ± 2	

SBP: Systolic blood pressure, BW: Body weight, Na-CMC: Carboxymethyl Cellulose Sodium.

Similarly, DBP also significantly decreased the treatment with gambir ($p < 0.05$). The decrease in DBP was higher when gambir use was prolonged ($p < 0.05$). Gambir at the dose of 2.5 mg/kg BW possesses higher efficacy to reduce DBP than gambir at other doses about 36 ± 6% on day 14. The average decrease in DBP (%) in the gambir group 2.5, 5, 10 mg/kg BW, positive control, and negative control group were 21 ± 2, 13 ± 2, 14 ± 2, 20 ± 2, and −1 ± 2. Meanwhile, the average decrease in DBP (%) on days 1, 3, 7, and 14, respectively, were 7 ± 2, 11 ± 2, 14 ± 2, and 22 ± 2. The data can be shown in Table 3.

Table 3: The effect of purified gambir on DBP

Groups	Decrease of DBP (%) on days ± SE				Average ± SE
	1	3	7	14	
Gambir					
2.5 mg/kg BW	5 ± 5	11 ± 5	31 ± 5	36 ± 6	21 ± 2
5 mg/kg BW	1 ± 5	7 ± 5	23 ± 5	22 ± 5	13 ± 2
10 mg/kg BW	9 ± 5	20 ± 6	4 ± 5	23 ± 5	14 ± 2
Captopril (positive control)	21 ± 5	27 ± 5	11 ± 6	22 ± 5	20 ± 2
Na-CMC (negative control)	−1 ± 6	−10 ± 5	0 ± 5	5 ± 5	-1 ± 2
Average ± SE	7 ± 2	11 ± 2	14 ± 2	22 ± 2	

DBP: Diastolic blood pressure, BW: Body weight, Na-CMC: Carboxymethyl Cellulose Sodium.

MAP was significantly affected by the administration of purified gambir ($p < 0.05$). The percentage rate of reduction in MAP seemed to increase gradually when gambir use was prolonged ($p < 0.05$). The gambir dose of 2.5 mg/kg BW showed the best lowering effect in treated gambir groups. The average decrease in MAP (%) in the gambir group 2.5, 5, 10 mg/kg BW, positive control, and negative control group were 18 ± 3, 15 ± 3, 12 ± 3, 21 ± 3, and -2 ± 3. Based on days, the average decrease in MAP (%) on days 1, 3, 7, and 14, respectively, were 6 ± 2, 10 ± 2, 15 ± 2, and 20 ± 2. The data can be shown in Table 4.

HR of hypertension significantly influenced rats by dose ($p < 0.05$), duration ($p < 0.05$), and the interaction between the two ($p < 0.05$). Interestingly, the HR, if animal treated with gambir at 10 mg/kg BW, increased in HR beginning of treatment but then decreased as the duration of treatment was prolonged. The HR of positive control animals increased during evaluation, whereas most treated animals showed a decrease. The decrease in HR was higher when the use of gambir was more prolonged ($p < 0.05$). The average reduction in HR (%) in the gambir group 2.5, 5, 10 mg/kg BW, positive control, and negative control group were −0 ± 2, −1 ± 2, 4 ± 2, 10 ± 3, and −18 ± 3. Based on days, the average decrease in HR (%) on days 1, 3, 7, and 14, respectively, were −3 ± 3, −3 ± 2, −3 ± 3, and 5 ± 2. The data can be shown in Table 5.

Table 4: The effect of purified gambir on MAP

Groups	Decrease of MAP (%) on days ± SE				Average ± SE
	1	3	7	14	
Gambir					
2.5 mg/kg BW	6 ± 5	11 ± 5	29 ± 5	25 ± 5.251	18 ± 3
5 mg/kg BW	2 ± 5	7 ± 5	24 ± 5	25 ± 5.251	15 ± 3
10 mg/kg BW	9 ± 5	13 ± 5	4 ± 5	23 ± 5.251	12 ± 3
Captopril (positive control)	19 ± 5	25 ± 5	18 ± 5	22 ± 5.251	21 ± 3
Na-CMC (negative control)	−6 ± 5	−6 ± 5	1 ± 5	5 ± 5.251	-2 ± 3
Average ± SE	6 ± 2	10 ± 2	15 ± 2	20 ± 2	

Data with negative marks indicated an increase and vice versa. MAP: Mean arterial pressure, BW: Body weight, Na-CMC: Carboxymethyl Cellulose Sodium.

Table 5: The effect of purified gambir on HR

Groups	The average change of HR (%) on days ± SE				Average ± SE
	1	3	7	14	
Gambir					
2.5 mg/kg BW	−4 ± 5	−3 ± 5	−1 ± 5	7 ± 5	−0 ± 2
5 mg/kg BW	−2 ± 5	3 ± 5	−1 ± 5	−3 ± 5	−1 ± 2
10 mg/kg BW	−2 ± 5	−8 ± 5	11 ± 5	18 ± 5	4 ± 2
Captopril (positive control)	22 ± 6	7 ± 5	2 ± 5	7 ± 5	10 ± 3
NaCMC (negative control)	−32 ± 9	−13 ± 5	−26 ± 9	−1 ± 5	−18 ± 3
Average ± SE	−3 ± 3	−3 ± 2	−3 ± 3	5 ± 2	

Data with negative marks indicated an increase and vice versa. HR: Heart rate, BW: Body weight, Na-CMC: Carboxymethyl Cellulose Sodium.

The doses of purified gambir were significantly influenced to the animal BF ($p < 0.05$), but not by the duration of administration ($p > 0.05$). There is a significant interaction between the two variables ($p < 0.05$). In this study, the BF of rats treated with purified gambir decreased, while the effect on animal BF of negative controls increased slightly. Furthermore, the percentage of reduced BF in rats given 10 mg/kg BW was initially smaller than those in the 2.5 and 5 mg/kg BW groups. Then, the administration of gambir was extended to 7–14 days, the average BF of gambir treated the animal with different doses did not affect ($p > 0.05$). The average decrease in BF (%) with gambir doses of 2.5, 5, 10 mg/kg BW, positive and negative control group were 43 ± 6, 26 ± 6, 26 ± 6, −0 ± 6, and −8 ± 6. The average decrease in BF (%) for each duration of administration of 1,3,7 and 14 days were 13 ± 5, 18 ± 6, 15 ± 6, and 23 ± 6. The data can be shown in Table 6.

Table 6: The effect of purified gambir on BF

Groups	BF decrease (%) on days ± SE				Average ± SE
	1	3	7	14	
Gambir					
2.5 mg/kg BW	43 ± 12	39 ± 12.14	41 ± 12	48 ± 15	43 ± 6
5 mg/kg BW	25 ± 12	28 ± 12.14	16 ± 12	34 ± 15	26 ± 6
10 mg/kg BW	5 ± 12	5 ± 12	48 ± 15	46 ± 12	26 ± 6
Captopril (positive control)	−11 ± 12	−1 ± 12	−12 ± 15	22 ± 12	−0 ± 6
Na-CMC (negative control)	4 ± 12	18 ± 15	−17 ± 12	−36 ± 12	−8 ± 6
Average ± SE	13 ± 5	18 ± 6	15 ± 6	23 ± 6	

Data with negative marks indicated an increase and vice versa. BF: Bloodflow, BW: Body weight, Na-CMC: Carboxymethyl Cellulose Sodium.

The last cardiovascular parameter observed was the animal BV. It was significantly decreased by the doses ($p < 0.05$) but not by duration ($p > 0.05$). The interaction between these two variables greatly affected the decrease in BV ($p < 0.05$). The BV of the negative control animal has fluctuated. It was increased all of the beginning but then decreased. On the other hand, the BV of gambir-treated animals declined initially. However, the BV of the animals returned to almost normal, except for the animals given gambir. The average decrease in BV (%) of gambir treated doses of 2.5, 5, 10 mg/kg BW, positive and negative control group were 52 ± 7, 40 ± 7, 26 ± 7, 5 ± 7, and −6 ± 8. The average decrease in BV (%) for each duration of administration of 1,3,7, and 14 days were 32 ± 7, 19 ± 7, 18 ± 7, and 26 ± 7. The data can be shown in Table 7.

Table 7: The effect of purified gambir on BV

Groups	BV decrease (%) on days ± SE				Average ± SE
	1	3	7	14	
Gambir					
2.5 mg/kg BW	62 ± 15	54 ± 15	64 ± 15	29 ± 15	52 ± 7
5 mg/kg BW	55 ± 15	48 ± 15	40 ± 15	20 ± 15	40 ± 7
10 mg/kg BW	2 ± 15	7 ± 15	37 ± 15	59 ± 15	26 ± 7
Captopril (positive control)	10 ± 15	1 ± 15	−10 ± 15	21 ± 15	5 ± 7
Na-CMC (negative control)	31 ± 15	−12 ± 15	−42 ± 15	0 ± 18	-6 ± 8
Average ± SE	32 ± 7	19 ± 7	18 ± 7	26 ± 7	

Data with negative marks indicated an increase and vice versa. BV: Blood volume, BW: Body weight, Na-CMC: Carboxymethyl Cellulose Sodium.

The plasma NO concentrations on hypertension rats significantly increased in gambir treated ($p < 0.05$). The medium-dose group, 5 mg/kg BW, caused the highest increase in plasma NO concentrations. The average changes in plasma NO concentrations (%) in the gambir group at doses of 2.5, 5, 10 mg/kg BW, positive and negative control group were 12 ± 8, 18 ± 8, −5 ± 2, 18 ± 10, and −18 ± 10. The data can be shown in Table 8.

Table 8: The effect of purified gambir on plasma NO concentrations

Treatments	Doses (mg/kg) BW	Plasma NO concentrations changes (%) ± SE
Na-CMC	-	-18 ± 10^{a}
Gambir	2.5	12 ± 8^{b}
Gambir	5	18 ± 8^{b}
Gambir	10	$-5 \pm 2^{a, b}$
Captopril	2.25	18 ± 10^{b}

(−) a decrease in plasma NO concentration. (+) an increase in plasma NO concentration. NO: Nitric oxide, Na-CMC: Carboxymethyl Cellulose Sodium.

Discussion

Within ±14 days of NaCl-prednisone induction in this study, a pathologic condition of hypertension was obtained. SBP, DBP, and MAP of the animal were increased up to 151 mmHg, 118 mmHg, and 128 mmHg, respectively. This result is in line with earlier studies using the same inductor [16], [17], [18]. Prednisone is a glucocorticoid hormone with a 0.8 index of mineralocorticoid [19]. NaCl combination of this inducer will enhance the sodium retention effect of prednisone and the water retention. As a result, the animal body fluid, especially the intravascular compartment, will increase. The increase of intravascular compartment will increase the animal's blood pressure [16], [18]. This pathologic condition is similar to hypertension induced by Deoxycorticosterone Acetate-Salt (DOCA-salt) [20], [21].

All three doses of gambir suspension showed an antihypertensive effect on animals. Although there was no significant reduction in blood pressure between the three different doses used in this study, the animals treated with the smallest dose (2.5 mg/kg BW) exhibited the best reduction in blood pressure compared to other groups. This phenomenon was common in the use of antioxidant therapy [16], [22].

Besides lowering blood pressure, the animal HR, BF, and BV were also seen in this study. According to Brzezinski *et al.*, about two-thirds of the systemic resistance to BF is small arteriolar resistance in the systemic circulation. The small internal diameter of the arterioles, but with solid vessel walls, allows massive changes in inner diameter up to four times. A fourfold increase in blood vessel diameter can increase the flow tens or hundreds of times. This result allows arterioles, responding with only slight changes in their diameter to nerve signals or local tissue chemical signals, to shut off BF to the tissue almost entirely or, on the other hand, to cause a significant increase in inflow. BF is proportional to blood pressure but inversely proportional to vascular resistance [23]. That's maybe why the animal BF in this study went down as the blood pressure was decreased and the peripheral BV, even though the blood vessel diameter may increase due to a vasodilation effect produced by NO, which in the animal treated with gambir was increased.

Animal treated with purified gambir showed an increase in NO bioavailability. Catechins are polyphenolic compounds with flavonoid structures that can act directly on the smooth muscle of arterial blood vessels by stimulating the Endothelium Derivate Relaxing Factor, which causes the vascular smooth muscle to relax, leading to vasodilatation [22]. In addition, the flavonoids may also increase the NO Synthase activity and increase NO production. This mediator will reduce oxidative stress conditions in the hypertensive animal and lower blood pressure through vasodilation due to the formation of these blood vessel relaxing factors [24], [25]. That was the reason behind the lowering blood pressure effect obtained in this study. This study's blood pressure-lowering effect aligns with previously reported research on purified gambir's lowering blood pressure in hypertensive and hypercholesterolemia rats [18].

In addition, catechin is reported to act as an inhibitor of angiotensin-converting enzyme activity which plays a key role in regulating arterial blood pressure [26], [27]. The purified gambir contains catechin, which has antioxidant activity [8]. Therefore, when used in sufficient doses, it will benefit the cardiovascular system [16]. However, when antioxidants are used in a large dose, their effectiveness may decrease, but

they may produce side effects. These side effects are perhaps related to the imbalance between antioxidant and pro-oxidant properties [16], [22]. Therefore, it could be understood why purified gambir made a lower effect when the dose was increased.

Conclusion

Based on the cardiovascular parameters, the animals treated with the smallest dose (2.5 mg/kg BW) exhibited the best reduction in blood pressure compared to other groups. Regarding NO plasma concentrations, it showed the medium-dose group, 5 mg/kg BW, caused the highest increase of NO plasma concentration. Based on administration duration, it can be concluded that purified gambir can relatively provide a therapeutic effect on cardiovascular parameters and plasma NO concentration when used for 14 consecutive days. It is necessary to carry out additional studies on the effectiveness of purified gambier as an antihypertensive in hypertensive rats for a longer period and their safety level.

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