EMPIRICAL STUDY OF COMBINATION EFFECT OF CAMELIA SENENSIS AND CURCUMA LONGA L IN REDUCING CISPLATIN-INDUCED OXIDATIVE STRESS IN WHITE RATS

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ABSTRACT

Cisplatin is an anti-cancer which has good results for the treatment of malignancy cases. But the accumulation of excessive reactive oxygen species (ROS) will result in the inefficiency and impaired function of the antioxidant enzymes synthesis. The aim of this study is to determine expression of the decreased levels of the antioxidant enzyme, Superoxide Dismutase (SOD) Catalase (CAT), glutathione peroxidase (GPx) and damage to lipid peroxidase as indicated by increased levels of Malondialdehyde (MDA). When the antioxidant enzyme system unable to cope with excessive oxidative stress, the body requires intake of exogenous antioxidants that are able to capture and neutralize these free radicals so that further reactions that cause oxidative stress can stop and cell damage can be avoided. Ginkgo biloba, as well as combinations Camellia sinensis L in green tea and Curcuma longa in turmeric. All three of them are polyphenols, rich in exogenous antioxidant content, so they are expected to reduce oxidative stress due to cisplatin induction. This was a randomized post-test only control group laboratory experimental design in rat to determine the effectiveness of intravenous Ginkgo biloba, as well as combinations Curcuma longa L and Camellia sinensis administration can reduce oxidative stress based on the activity of the antioxidant enzyme Superoxide Dismutase (SOD) Catalase (CAT), Glutathione Peroxidase (GPx) and Malondialdehyde (MDA) in blood serum levels. 25 male Rattus norvegicus Wistar strain divided randomly into 5 groups, each group consisting of 5 rats, group 1 as a control group without treatment, group 2 was only given cisplatin 20 mg/kgBW/iv, group 3 was given cisplatin 20 mg/kgBW/iv continued with combination therapy Cammelia Senensis Extract at a dose of 50 mg/KgBW/day and Curcuma Longa L Extract at a dose of 75 mg/KgBW/day; Group 4 was given cisplatin 20 mg/kgBW/iv followed by combination therapy Cammelia Sinensis Extract at a dose of 75 mg/KgBW/day and Curcuma Longa Extract at a dose of 100 mg/KgBW/day, group 5 was given cisplatin 20 mg/kgBW/iv followed by therapy with Ginkgo Biloba at a dose of 1.44 mg/KgBW/day. All groups were terminated on day 7. Data were analyzed statistically using SPSS. Data were analyzed using ANOVA and p <0.05 was used. Combination Curcuma longa L in turmeric and Camellia sinensis L in green tea showed its effectiveness in reducing oxidative stress on the 7th day after administration, as evidenced by the highest levels of Superoxide Dismutase (SOD) Catalase (CAT), glutathione peroxidase (GPx) expression and the lowest levels of MDA expression.

Keywords: Cisplatin, Oxidative Stress, Superoxide Dismutase, Malondialdehyde

INTRODUCTION

In the gastrointestinal system, urinary system, as well as head and neck. Although it has good results for treating cases of malignancy, there are limitations to its clinical use. This is because the effect of cisplatin is not only damaging the cancer cells, but also the healthy body cells. The mechanism of this destruction is through the destruction of the cell's Deoxyribonucleic Acid (DNA), causing big side effect. Some of the side effects identified are peripheral neuropathic, nephropathic, bone marrow toxicity, and ototoxicity.1,2,3

Excessive accumulation of cisplatin will suppress the antioxidant enzyme system that should be able to remove and neutralize this increase in superoxide. Under normal circumstances there is a balance between formation of ROS and antioxidant activity in the body, resulting in inefficiency and impaired function of antioxidant enzyme synthesis.4 But if the increased amount of ROS exceeds the capacity of antioxidant enzymes to neutralize them, it can cause oxidative damage to cell components. The main site of ROS reactions is on the plasma membrane, this is because the plasma membrane has a structure consisting of polyunsaturated fatty acids (PUFA) which are very easily oxidized. This event is called lipid peroxidation, characterized by the formation of hydrogen peroxide (H_2O_2), epoxides, malondialdehyde (MDA). Cell damage due to excessive ROS occurs in proteins, lipids and DNA. ROS are metabolized through cellular oxidation-reduction reactions and are formed naturally as by-products in normal aerobic metabolic processes then neutralized by enzymatic scavengers in the form of natural endogenous antioxidants in the body, such as; Superoxide Dismutase (SOD), Glutathione S-transferase (GST), Heme Oxygenase-1 (HO-1), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), Catalase (CAT), and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) (Sheth, 2017).

Mechanisms to fight oxidative stress, either by producing antioxidants from the endogenous antioxidant system or externally through exogenous or secondary antioxidants. So, when the endogenous antioxidant system is not sufficient to overcome excessive oxidative stress, the body will require external intake containing antioxidant compounds to capture and neutralize these free radicals so that further reactions causing oxidative stress can be stopped and cell damage can be avoided. Antioxidants in this group are also called chain-breaking antioxidants, it can protect cells from oxidative stress by detoxifying carcinogens or by reducing oxidative stress (Molina-Jijón, et al., 2011).

Exogenous antioxidants used to prevent and reduce the damage caused by cisplatin to date is Ginkgo biloba extract containing flavonoids. In several studies, these chemicals compounds are known to play a role in preventing sensory neural hearing loss in patients receiving cisplatin chemotherapy (Sampaio et al., 2016). Ginkgo biloba is not a native plant from Indonesia. Meanwhile, Indonesia itself is one of the countries with the largest biological wealth has more than 30,000 species of high-level native plants. Until currently, there are 7000 species of plants in Indonesia known their efficacy (Saifudin, 2011), including; Curcuma longa L in turmeric and Camellia sinensis L in green tea. Both are polyphenolic compounds widely grown in Indonesia and have been widely used as well as researched for their benefits as ant carcinogenic, antioxidant, anti-inflammatory, antiproliferative and antiapoptotic. If both are combined using the right dose, it is expected to be a very potential antioxidant in protecting and reducing the occurrence of apoptosis after cisplatin induction. The synergy of the two lies in the catechin Camellia sinensis L which is a potential source of antioxidants because it has a strong phenolic group capable of regenerating and stabilizing radicals Curcuma longa L by donating one hydrogen atom to the radical curcumin (Alrawaiq, Nadia & Abdullah, 2014). The novelty of this study is the combination of the two antioxidants, Curcuma longa L and Camellia sinensis L extracts which are expected to provide a synergistic effect of antioxidants as adjuvant therapy that is better than the previous therapy, that is Ginkgo biloba in cytotoxic patients because cisplatin is proven by molecular biology by increasing antioxidant enzymes, and decreased oxidative stress.

MATERIALS & METHODS

This research has obtained an ethical clearance letter for experimental animal research published by the Health Research Ethics Commission (KEPK) Regional General Hospital dr. Moewardi Surakarta. This type of research is randomized post-test only control group laboratory experimental design on healthy and adult male Rattus Novergicus Wistar strain rats weighing 150-250 grams, conducted at the Inter-Central Food and Nutrition Laboratory of Gadjah Mada University Yogyakarta in April 2021. The study was conducted to determine the effective dose of several antioxidants; Curcuma longa L in turmeric, Camellia sinensis L in green tea and Ginkgo Biloba after cisplatin induction on the expression of antioxidant enzyme activity Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and lipid peroxide (MDA) levels.

The research sample size was obtained based on the provisions of the WHO, namely at least 5 rats for each group. The rats were adapted for 7 days and placed in specially designed cages with plywood walls and wire netting, paddy husk pads and equipped with drinking water as well as food containers. After the adaptation period is over all those mice were randomly distributed to 5 groups: group 1 as negative control group 5 rats without any treatment; group 2 as the positive control group was only given cisplatin 20 mg/kgBW/iv only; group 3 was given cisplatin 20 mg/kgBW/iv followed by combination therapy Cammelia Senensis Extract at a dose of 50 mg/KgBW/day and Curcuma Longa L Extract at a dose of 75 mg/KgBW/day; and group 4 were given cisplatin 20 mg/kgBW/iv followed by combination therapy Cammelia Sinensis Extract at a dose of 75 mg/KgBW/day and Curcuma Longa Extract at a dose of 100 mg/KgBW/day, group 5 was given cisplatin 20 mg/kgBW/iv followed by therapy with Ginkgo Biloba at a dose of 1.44 mg/KgBW/day. All five groups were terminated on the 7th day. During the treatment, all white rats were fed a standard ration of 10% of body weight and drank ad libitum. Body weight and activity of white rats were observed and recorded every day until the end of the experiment that the mice can move actively so no samples were removed. All treated animals were euthanized with carbondioxide for blood samples on the scheduled termination day then blood sampling was done through the orbital sinus. The blood is allowed to stand until serum is formed, then it is collected for measurement of levels Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and lipid peroxide (MDA) levels, in serum.

The data normality test is used to determine the statistical test to be used in this study. The results of the data normality test can be determined if the data is normally distributed then the parametric test used is the ANOVA test. If the results of the Anova test showed that there is a significant difference between the three treatments, both on SOD, CAT, GPx and MDA, so the Post Hoc test will be done with the aim of seeing the differences between treatments.

K (-)=Normal

K (+)=Cisplatin 20 mg/KgBW

P1=Cisplatin 20 mg/KgBW given Camelia Sinensis L extract 50 mg/KgBW and Curcuma Longa L extract 75 mg/KgBW

P2=Cisplatin 20 mg/KgBW given therapy Camelia Sinensis L Extract 75 mg/KgBW : Curcuma Longa L Extract 100 mg/KgBW

P3=Cisplatin 20 mg/KgBW given therapy Ginkgo 1.44 mg/KgBW

(K=control, P=treatment)

Expression of MDA Levels Against Administration of Cammelia Sinensis L Extract combined with Curcuma Longa L and Ginkgo on the 7th day after administration of cisplatin

Expression of MDA levels using the ANOVA test because the data in the study were normally distributed.

Table 1 SIMULTANEOUS DIFFERENCE TEST (ANOVA) OF MDA VARIABLES BASED ON TREATMENT PREPARATION		
Group	MDA (nmol/ml)	
	N	*Mean+SD*
K (-)	5	1.493 +0.189
K (+)	5	9.278 +0.298
P1	5	4.714 +0.270
P2	5	2.239 +0.161
P3	5	3.110 +0.430
p-value		<0.001*

One Way Anova test; * significant at α =5%

Based on table 1, the lowest MDA levels in the group of rats given antioxidant therapy were in the P2 treatment group (Cisplatin 20 mg/KgBW → Camelia Sinensis L Extract 75 mg/KgBW : Curcuma Longa L Extract 100 mg/KgBW), with the average of 2.239 +0.161 nmol/ml, and the highest MDA levels were in the P1 group (Camelia Sinensis L Extract: 50 mg/KgBW : Curcuma Longa L Extract: 75 mg/KgBW) that is 4.714 +0.270 nmol/ml.

Based on the statistical test, there was a significant difference in MDA levels between various preparations of antioxidants with p value=<0,001 (p<0,05), it can be seen in Figure 4.1 as follows.

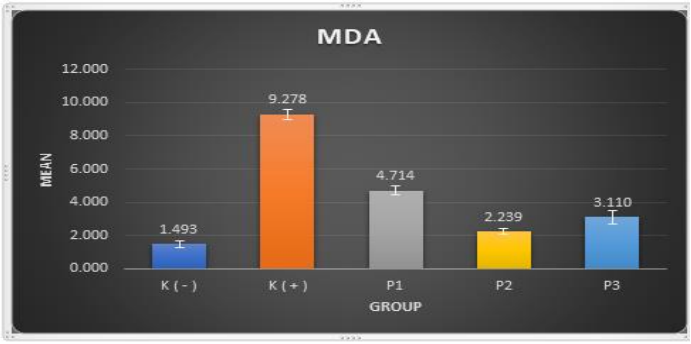

FIGURE 1
BAR CHART OF MDA LEVELS BASED ON TREATMENT PREPARATIONS

Based on the ANOVA test, it was shown that there was a significant difference in MDA levels between the various antioxidant treatment preparations with a p value of <0.05, then the posh hoc LSD test was carried out with the following results obtained.

Table 2 **PARTIAL DIFFERENCE TEST (POST HOC) MDA VARIABLES BASED ON TREATMENT PREPARTIONS**		
Group	**MDA**	
	Diff Mean	**p-value**
K (-) *vs*. K (+)	-7.786	<0.001*
K (-) *vs* P1	-3.221	<0.001*
K (-) *vs* P2	-0.746	0.001*
K (-) *vs* P3	-1.617	<0.001*
K (+) *vs* P1	4,564	<0.001*
K (+) *vs* P2	7.040	<0.001*

K (+) *vs* P3	6.169	<0.001*
P1 *vs* P2	2.475	<0.001*
P1 *vs* P3	1,604	<0.001*
P2 *vs* P3	-0.871	<0.001*

Table 2 explains that the antioxidant treatment group that was most effective in reducing MDA was the P2 group, which had a different mean value with the largest range with the K+ group compared to the treatment with other antioxidants (P1 and P3), which was equal to 7,040, or it can be said that the MDA levels in the K+group were higher by 7.040 nmol/ml compared togroup P2. Thus giving Cammelia Sinensis L extract 75 mg/KgBW and Curcuma Longa L extract 100 mg/KgBW were the most effective in reducing MDA.

Expression of SOD Levels Against Administration Cammelia Sinensis L Extract combined with Curcuma Longa L and Ginkgo on the 7th day after administration of cisplatin

The data were tested using the ANOVA test because they were normally distributed (appendix). It can be seen in table 3 as follows.

Table 3
SIMULTANEOUS DIFFERENCE TEST (ANOVA) VARIABLE SOD BASED ON TREATMENT PREPARATION

Group	SOD (%)	
	N	*Mean+SD*
K (-)	5	82,295 +3.153
K (+)	5	32,787 +3.666
P1	5	52,787 +3.153
P2	5	73,770 +2.592
P3	5	62,623 +3.914
p-value		<0.001*

One Way Anova test; * significant at α =5%

Based on table 3, the highest levels of SOD in the group of rats given antioxidant therapy were in the P2 treatment group (Cisplatin 20 mg/KgBW → Cammelia Sinensis L Extract: 75 mg/KgBB : Curcuma Longa L Extract 100 mg/KgBW), with the average of 73,770 +2,592%, and the lowest SOD levels were in the P1 group (Cammelia Sinensis L Extract 50 mg/KgBW: Curcuma Longa L Extract 75 mg/KgBW) that is 52,787 +3.153%.

Based on the statistical test, there was a significant difference in SOD levels between various preparations of anti-oxidant with p value=<0,001 (p<0,05). The data can be seen in Figure 2 as follows.

FIGURE 2
BAR CHART OF SOD LEVELS BASED ON TREATMENT

PREPARATIONS

Based on the ANOVA test, it was shown that there was a significant difference in SOD levels between various preparations of antioxidant treatment with a p value of <0.05, then the posh hoc LSD test was carried out with the following results obtained.

Table 4
PARTIAL DIFFERENCE TEST (POST HOC) SOD VARIABLES BASED ON TREATMENT PREPARATIONS

Group	SOD	
	Diff Mean	p-value
K (-) *vs*. K (+)	49,508	<0.001*
K (-) *vs* P1	29,508	<0.001*
K (-) *vs* P2	8,525	0.001*
K (-) *vs* P3	19,672	<0.001*
K (+) *vs* P1	-20,000	<0.001*
K (+) *vs* P2	-40,983	<0.001*
K (+) *vs* P3	-29,836	<0.001*
P1 *vs* P2	-20,984	<0.001*
P1 *vs* P3	-9,836	<0.001*
P2 *vs* P3	11,147	<0.001*

Based on the description above, it can be seen that groups P1, P2, P3, had effective results in increasing SOD. The anti-oxidant treatment group that was most effective in increasing SOD was the P2 group, which had the widest range of different mean values with the K+group compared to the treatment with other antioxidants (P1 and P3), which was -40,983, or it can be said that the SOD levels in the K+group were lower by -40,983% compared to group P2. Thus administrating Cammelia Sinensis L Extract 75 mg/KgBW : Curcuma Longa L Extract 100 mg/KgBW is the most effective in increasing SOD.

Expression of Catalase (CAT) Levels Against Administration of Cammelia Sinensis L Extract combined with Curcuma Longa L extract and Ginkgo on the 7th day after administration of cisplatin

The data were tested using the ANOVA test because they were normally distributed (appendix). Result can be seen in table 5 as follows.

Table 5
SIMULTANEOUS DIFFERENCE TEST (ANOVA) OF CATALASE VARIABLE BASED ON TREATMENT PREPARATION

Group	Catalase (U/ml)	
	N	***Mean+SD***
K (-)	5	5.927 +0.205
K (+)	5	1.800 +0.046
P1	5	4.177 +0.084
P2	5	5.107 +0.173
P3	5	4.583 +0.107
p-value		<0.001

One Way Anova test; * significant at α =5%

Based on table 5, the catalase level in the group of rats given antioxidant therapy was the highest in the P2 treatment group (Cisplatin 20 mg/KgBW → Cammelia Sinensis L Extract 75 mg/KgBB : Curcuma Longa L Extract 100 mg/KgBW), that was the average 5.107 +0.173 U/ml, and the lowest level of Catalase was in the P1 group (Cammelia Sinensis L Extract 50 mg/KgBB : Curcuma Longa L Extract 75 mg/KgBB) that is 4.177 +0.084 U/ml.

Based on the results of the statistical test, there was a significant difference in catalase levels between various preparations of antioxidant with p value=<0,001 (p<0,05). The data can be seen in Figure 3 as follows.

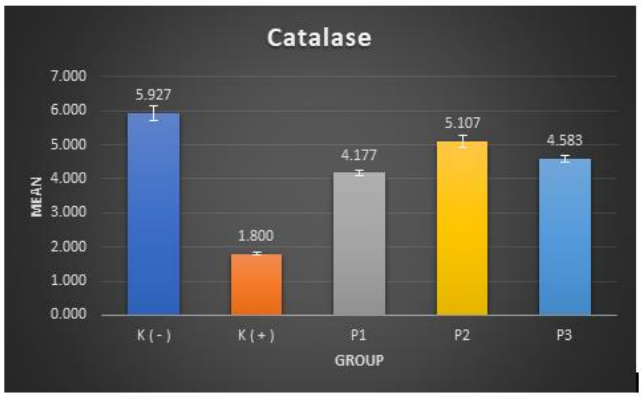

FIGURE 3
CATALASE BAR CHART BASED ON TREATMENT PREPARATION

Based on the ANOVA test, it was known that there was a significant difference in catalase levels between various preparations of antioxidant treatment with a p value of <0.05, then the posh hoc LSD test was carried out and the following results were obtained.

Table 6
PARTIAL DIFFERENCE TEST (POST HOC) OF CATALASE VARIABLES BASED ON TREATMENT PREPARATIONS

Group	Catalase	
	Diff Mean	p-value
K (-) *vs*. K (+)	4.127	<0.001*
K (-) *vs* P1	1.750	<0.001*
K (-) *vs* P2	0.820	<0.001*
K (-) *vs* P3	1.343	<0.001*
K (+) *vs* P1	-2.377	<0.001*
K (+) *vs* P2	-3.307	<0.001*
K (+) *vs* P3	-2.784	<0.001*
P1 *vs* P2	-0.930	<0.001*
P1 *vs* P3	-0.407	<0.001*
P2 *vs* P3	0.523	<0.001*

Post hoc LSD test; * significant at α=5%; The sign (-) on the difference mean shows a lower value than the comparison

From table 6, it can be seen that groups P1, P2, P3, had effective results in increasing Catalase levels but the most effective antioxidant treatment group in increasing Catalase was the P2 group. It had the widest different mean value with the K+group compared to the treatment with other antioxidants (P1 and P3), which was -3.307, or it can be said that the level of Catalase in the K+group was lower by -3.307 U/ml compared to P2 group. Thus the administration of

Cammelia Sinensis L Extract 75 mg/KgBW : Curcuma Longa L Extract 100 mg/KgBW is the most effective in increasing Catalase.

Expression of GPX Levels Against Administration of Cammelia Sinensis L Extract combined with Curcuma Longa L and Ginkgo on the 7th day after administration of cisplatin

The data were tested using the ANOVA test because they were normally distributed (appendix). The result can be seen in table 7 as follows;

Table 7
SIMULTANEOUS DIFFERENCE TEST (ANOVA) GPX VARIABLE BASED ON TREATMENT PREPARATION

Group	GPx(U/mg)	
	N	*Mean+SD*
K (-)	5	73,466 +1.485
K (+)	5	24,077 +1.485
P1	5	54,328 +1,600
P2	5	64,823 +2.114
P3	5	60,347 +1,842
p-value		<0.001

One Way Anova test; * significant at α =5%

The highest levels of GPx in the group of rats given antioxidant therapy were in the P2 treatment group (Cisplatin 20 mg/KgBW → Cammelia Sinensis L Extract 75 mg/KgBW : Curcuma Longa L Extract 100 mg/KgBW), with the average of 64,823 +2.114 U/mg, and the lowest GPx levels were in the P1 group (Cammelia Sinensis L Extract: 50 mg/KgBW : Curcuma Longa L Extract 75 mg/KgBW) which was 54,328 +1,600 U/mg.

The results of the statistical test showed a significant difference in GPx levels between various preparations of anti-oxidant treatment with p value=< 0,001 (p<0,05). The bar chart depicting the GPx level data can be seen in Figure 4 as follows.

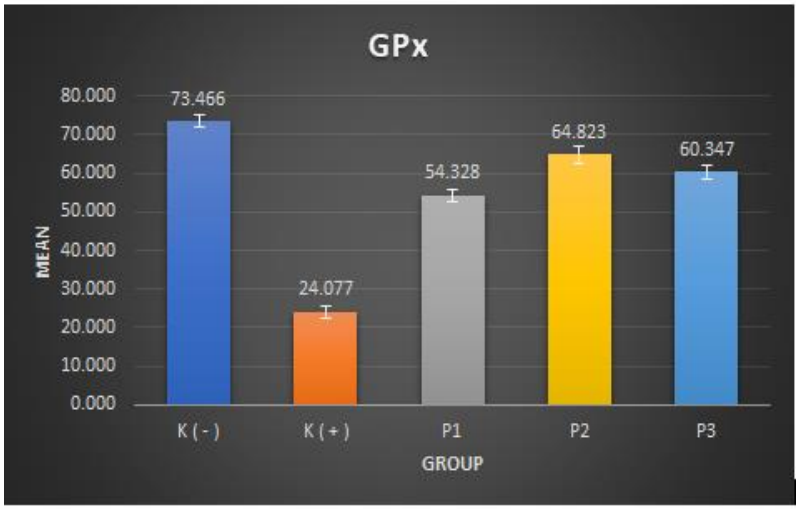

FIGURE 4
BAR CHART OF GPX LEVELS BASED ON TREATMENT PREPARATIONS

Based on the ANOVA test, it was known that there was a significant difference in GPx levels between various preparations of antioxidant treatment with a p value of <0.05, then the posh hoc LSD test was carried out with the following results obtained.

Table 8
PARTIAL DIFFERENCE TEST (POST HOC) GPX VARIABLE BASED ON TREATMENT PREPARATION

Group	GPx	
	Diff Mean	p-value
K (-) *vs*. K (+)	49,389	<0.001*
K (-) *vs* P1	19,138	<0.001*
K (-) *vs* P2	8,643	<0.001*
K (-) *vs* P3	13,119	<0.001*
K (+) *vs* P1	-30.251	<0.001*
K (+) *vs* P2	-40,746	<0.001*
K (+) *vs* P3	-36.270	<0.001*
P1 *vs* P2	-10,495	<0.001*
P1 *vs* P3	-6.019	<0.001*
P2 *vs* P3	4.476	0.001*

LSD post hoc test; * significant at α =5%; The sign (-) on the difference mean shows a lower value than the comparison

Table 8 shows that the values of GPx levels which significantly different from the K+ group (Cisplatin 20 mg/KgBW) are treatment P1, P2, P3, with p value <0.05, also had significantly different results with the K- group (normal) with p<0.05. Groups P1, P2, P3, had effective results in increasing GPx levels on Day 7 after treatment with cisplatin. The antioxidant treatment group that was most effective in increasing GPx was the P2 group, which had the widest range of different mean values with the K+group compared to the treatment with other antioxidants (P1 and P3), which was -40,746, or it can be said that the level of GPx in the K+group is lower by -40,746 U/mg compared to P2 group. Thus administrating Cammelia Sinensis L Extract 75 mg/KgBW : Curcuma Longa L Extract: 100 mg/KgBW is most effective in increasing GPx.

Expression of GSH Levels against Administration of Cammelia Sinensis L Extract combined with Curcuma Longa L and Ginkgo on the 7th day after administration of cisplatin

The data were tested using the ANOVA test because they were normally distributed (appendix). The result of differences between groups can be seen in table 9 as follows.

Table 9
SIMULTANEOUS DIFFERENCE TEST (ANOVA) OF GSH VARIABLES BASED ON TREATMENT PREPARATIONS

Group	GSH (U/mg)	
	N	***Mean+SD***
K (-)	5	7.568 +0.166
K (+)	5	1.552 +0.060
P1	5	6.315 +0.051
P2	5	7.301 +0.048

P3	5	6.872 +0.043
p-value		<0.001*

One Way Anova test; * significant at α =5%

Based on table 9, the highest GSH levels in the group of rats given antioxidant therapy were in the P2 treatment group (Cisplatin 20 mg/KgBW → Cammelia Sinensis L Extract 75 mg/KgBW : Curcuma Longa L Extract 100 mg/KgBW), which was the average of 7.301 +0.048 U/mg, and the lowest GSH levels were in the P1 group (Cammelia Sinensis L Extract 50 mg/KgBW and Curcuma Longa L Extract 75 mg/KgBW) was 6.315 +0.051 U/mg.

The results of the statistical test showed a significant difference in GSH levels between various preparations of anti-oxidant treatment with p value=<0,001 (p<0,05). The bar chart depicting the GSH level data can be seen in Figure 5 as follows.

FIGURE 5
BAR CHART OF GSH LEVELS BASED ON TREATMENT PREPARATIONS

Based on the ANOVA test, it was found that there was a significant difference in GSH levels between various preparations of antioxidant treatment with a p value of <0.05, then the posh hoc LSD test was carried out, and the following results were obtained.

Table 10
PARTIAL DIFFERENCE TEST (POST HOC) OF GSH VARIABLES BASED ON TREATMENT PREPARATIONS

Group	GSH	
	Diff Mean	p-value
K (-) *vs*. K (+)	6.016	<0.001*
K (-) *vs* P1	1.252	<0.001*
K (-) *vs* P2	0.266	<0.001*
K (-) *vs* P3	0.696	<0.001*
K (+) *vs* P1	-4.764	<0.001*
K (+) *vs* P2	-5,750	<0.001*
K (+) *vs* P3	-5,320	<0.001*
P1 *vs* P2	-0.986	<0.001*
P1 *vs* P3	-0.556	<0.001*
P2 *vs* P3	0.430	<0.001*

LSD post hoc test; * significant at α =5%; The sign (-) on the difference mean shows a lower value than the comparison

Table 10 shows the value of GSH levels significantly different from the K+ group (Cisplatin 20 mg/KgBB) were treatment P1, P2, P3, with p value <0.05, also had significantly different results with the K- group (normal) with p<0.05

Based on the description above, it can be seen that groups P1, P2, P3, had effective results in increasing GSH levels on Day 7 after treatment with cisplatin. The antioxidant treatment group that was most effective in increasing GSH was the P2 group, which had the widest range of different mean values with the K+group compared to other antioxidant treatments (P1 and P3), which was equal to -5,750, or it can be said that GSH levels in the K+group were lower by -5750 U/mg compared to P2 group. Thus administration of Cammelia Sinensis L Extract 75 mg/KgBW dan Curcuma Longa L extract 100 mg/KgBW is the most effective in increasing GSH.

DISCUSSION

Based on the mechanism of cisplatin cytotoxicity, the antioxidant group is the appropriate group to prevent cytotoxicity due to cisplatin. Non-enzymatic antioxidants are also called exogenous antioxidants that function in preventive defense systems, because there is no treatment for cytotoxicity, preventive measures are more important (Sheth et al., 2017). From the literature, antioxidants that can be used to prevent cisplatin cytotoxicity include polyphenols, Sodium Thiosulphate, D methionine, Vitamin E, N-acetylcysteine, Dexamethasone and Lycopene (Rybak, 2010; Esen et al., 2018). In recent years, flavonoids contained in polyphenols have become very important because of their potential use as prophylactic and therapeutic agents in many diseases related to oxidative stress and free radical damage (Banjarnahor & Artanti, 2014; Treml & Smejkal, 2016).

Ginkgo biloba is rich in flavoid which has long been used as an antioxidant. In the research of Amr Amin, Christeena Abraham's et al, 2012 study was to evaluate the protective effects of Ginkgo Biloba (GB) against testicular damage and oxidative stress as well as caudal sperm indices in a cisplatin- (CIS-) induced rodent model. Adult male Wistar rats were given vehicle, single i.p. dose of CIS alone (10 mg/kg), GB alone (200 mg g/kg every day for five days), or single dose of CIS followed by GB (50, 100, or 200 mg/kg every day for five days). On day 6, after the first drug treatment oxidative and apoptotic testicular toxicity was evaluated. This reproductive toxicity was accompanied with increased germ-cell degeneration in seminiferous tubules and increased germ-cell apoptosis, increased testicular MDA levels and decreased SOD and CAT activities in testes. This is in accordance with our study that the effect of Oral GB at doses of 144 mg/ KgBB effectively of the CIS-induced toxicity in blood serum. The present results provide further insights into the mechanisms of protection against CIS-induced toxicity and confirm the essential antioxidant potential of a GB extract. Unfortunately, Gingko biloba is not native to Indonesia and is very rare. Meanwhile, Indonesia itself is one of the countries with the largest biological wealth which has more than 30,000 high-level native plant species of Indonesia. To date, 7000 species of plants have been identified for their efficacy (Saifudin, 2011). Curcuma longa L in turmeric and Camellia sinensis L in green tea are both polyphenolic compounds that are widely grown in Indonesia (Saifudin, 2011). Indonesia is not the only one who uses the antioxidant effects of two plants rich in flavonoid as antioxidants. Noha Ibrahim Said Salem 1, Magda Mohammad Noshy 2, Azza Ali Said, 2017 investigate the possible protective role of the antioxidant curcumin (CMN) against genotoxicity, cytotoxicity and oxidative stress induced by cisplatin. Male mice were administered Curcumin orally in the dosages of 60, 90, and 120 mg/kg for three consecutive days before a single intraperitoneal injection of either cisplatin (6.5 mg/kg). Fengxian Wang, Wei Yang, Chunhua GAO, Shiti ZHANG, (2017). To investigate the improving effects of Epigallocatechin Gallate (EGCG) on rat kidney injury induced by cisplatin and its mechanism of action. Fifty male SD rats were randomly divided into blank control group, kidney injury group, low-, middle-and high-dose EGCG (25.50 and 100 mg/kg) groups. The kidney injury group and the drug administration

group were treated with 7.5 mg/kg cisplatin by intraperitoneal injection and the blank control group was intraperitoneally injected with normal saline. After fourteen days of administration, the general condition the renal cortex MDA concentration decreased, and renal cortex GSH concentration,T-SOD activity increased. EGCG plays a role in the improvement of rat kidney injury induced by cisplatin, this proves that these two plants have been widely used as anticarcinogenic, antioxidant, anti-inflammatory, antiproliferative and antiapoptotic (Alrawaiq, Nadia and Abdullah, 2014). This is in line with the results of our study which wanted to further increase the potential for the antioxidant effect of the two, by combining Cammelia Sinensis L Extract and Curcuma Longa L Extract with different doses, which is expected to provide a better antioxidant synergistic effect than the therapy that has been used so far, namely Ginkgo biloba, where the results obtained were 2 treatment groups after induction of Cisplatin 20 mg/KgBW and then given 2 doses of a combination of Cammelia Sinensis L Extract 50 mg and 75 mg/KgBW and Curcuma Longa L Extract: 75 mg and 100 mg/KgBW compared to the treatment group given the extract. Ginkgo biloba 1.44 mg/KgBW for 7 days showed the most effective results in reducing MDA and increasing antioxidant enzymes (SOD, CAT, GPx and GSH) after cisplatin induction was a combination treatment group with Cammelia Sinensis L Extract 75 mg/KgBW and Curcuma Longa L Extract: 100 mg/kgBW.

Conclusion: The synergism of the combination of Cammelia Sinensis L extract at a dose of 75 mg/KgBW and Curcuma Longa L extract: a dose of 100 mg/KgBW, was able to increase antioxidant enzymes (SOD, CAT, GPx and GSH) and reduce MDA better than ginkgo biloba at a dose of 1.44. mg/KgBW.

REFERENCES

Alrawaiq, Nadia S., & Abdullah A. (2014). A review of Antioxidant Polyphenol Kurkumin and its Role in Detoxification. *International Journal of PharmaTech Research*, 280-289.

Amin, A., Hamza, A.A., & Abraham, C. (2012). A standardized extract of ginkgo biloba neutralizes cisplatin-mediated reproductive toxicity in rats. *Journal of Biomedicine and Biotechnology, (5),* 362049.

Banjarnahor, S., & dan Artanti, N. (2015). Antioxidant properties of flavonoids. *Medical Journal of Indonesia, 23*(4), 239-44.

Biswas, S.K. (2016). Does the interdependence between oxidative stress and inflammation explain the Antioxidant Paradox? Oxidative Medicine and Celuller Longevity. Article 5698931

Callejo, A., Cabezon, L., Juan, I., & Liorens, J. (2015). Cisplatin-induced ototoxicity: Effects, mechanisms and protection strategies. *The Journal of Toxics,* (3), 268 293

Esen, E., Ozdogan, F., Gurgen, S., Gulsen S., Erdem H., Baser S., & Genc S. (2018). Ginkgo Biloba and Lycopene are Effective on Cisplatin Induced Ototoxicity. *The Journal of International Advanced Otology, 14*(1), 23-27

Karasawa, T., & dan Steyger, P. (2015). An integrated view of Cisplatin-induced nephrotoxicity and ototoxicity. *Toxicology Letters, 237*(3), 219-227.

Langer T., Dinnesen A., Radtke S., Radtke S., Meitert J., & Zolk O.(2013). Understanding platinum-induced Ototoxicity. *Trends in Pharmacological Sciences*, *34*(8), p458-469.

Molina-Jijon, E. (2011). Curcumin prevents (Cr(VI)-inducedrenal oxidant damage by a mitochondrial pathway. Free Radical. *Biology & Medicine, 5*(8), 1543-1557.

Rybak, L.P., Mukharjea, D., Jajoo, S., & Ramkumar, V. (2009). Cisplatin ototoxicity and protection: Clinical & experimental studies. *The Tohoku journal of experimental medicine, 219*(3), 177-186.

Rybak, L. (2010). Ototoxicity. In: Flint P, Haughey, eds.5 Cummings Head & Neck Surgery: Otolaryngology. Philadelphia: Mosby. (154): 2169-2173

Saifuddin, A., & Rahayu dan Yuda H., (2011). Standardization of natural medicinal ingredients. Graha Ilmu. Yogyakarta. hal. 1-22

Salem, N.I.S., Noshy, M.M., & Said, A.A., (2017). Modulatory effect of curcumin against genotoxicity & oxidative stress induced by cisplatin and methotrexate in male mice. Food and chemical toxicology : An *international Journal published for the British Industrial Biological Research Association*, *105*: 370–376.

Sampaio A., Dias M., & Venosa A., (2016). The effect of Gingko Biloba (EGB-761) as an Otoprotector against Cisplatin Ototoxicity. *Otolaryngology-Head and Neck Surgery. 149* (25): 222-223

Sheth, S., Mukherjea, D., Rybak, L., & Ramkumar, V. (2017). Mechanisms of Cisplatin-Induced Ototoxicity& Otoprotection. *Frontiers in Cellular Neuroscience, 11*(338) 1-12

Treml, J., & dan Smejkal, K., (2016). Flavonoids as potent scavengers of hydroxyl radicals. *Comprehensive Reviews in Food Science and Food Safety, 15*, 720-738.

Wang, F., Yang, W., Gao, C., & Zhang, S., (2017). Improving effects of epigallocatechin gallate on rat kidney injury induced by cisplatin. *Chinese Traditional Patent Medicine, 12*, 1336-1341.