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Original Article

Safe and effective dosage combination of purple sweet potato extract and red fruit oil extract as antioxidant and hypolipidemic in rats given high cholesterol feed

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ABSTRACT

The empirical use of combination herbal medicines is common among both traditional communities and the standardized herbal medicine industry. Scientific data on the advantages and disadvantages of using combination herbal medicines are still lacking; hence conducting experimental study using animal models is necessary. Single purple sweet potato extract has been shown to reduce blood cholesterol levels, act as an antioxidant and increase SOD-2 and SOD-3 in the blood vessel endothelium. Red fruit extract also has a strong antioxidant effect. This study aimed to identify the most effective and safe combination of these extracts for the liver and kidneys. The subjects were 21 adult male Wistar rats, divided into three groups consisting of a control group and two intervention groups with a randomized pre and post-test control group design. The first intervention group received 100 mg/day purple sweet potato tuber extract and 0.2 ml/day red fruit extract with high cholesterol feed for four weeks, while the second group received 200 mg/day purple sweet potato tuber extract and 0.4 ml/day red fruit extract with high cholesterol feed for the same duration. The lipid profile, MDA, and SOD were measured before and four weeks after the treatment began. In addition, post-test data included measurements of SGPT, BUN, and serum creatinine. The study found that the combination of 200 mg purple sweet potato extract and 0.4 ml red fruit extract led to a significant improvement in lipid profile, with lower levels of MDA and higher SOD ($p<0.05$). The results of SGPT and BUN tests and serum creatinine also showed lower values than the control group. Thus, it is concluded that this combination is a safe and effective antioxidant and hypolipidemic for the liver and kidneys.

KEYWORDS: purple sweet potato, red fruit, combination, effective.

INTRODUCTION

Using combination herbal medicines is a practice that has been carried out empirically by both the community in traditional herbal medicine and by the industry in standardized herbal medicine. Scientific data on the advantages and disadvantages of using combination herbal medicines are still lacking; hence it is necessary to conduct experimental studies using animal models [1-3]. The pharmacological effect of a combination of 2 or more herbal medicines will be beneficial if there is a synergistic effect of each drug component or detrimental if an antagonistic effect occurs. Single purple sweet potato extract has been shown to reduce blood cholesterol levels and act as an antioxidant in rats and rabbits fed high cholesterol feed [4]. Purple sweet potato tuber extract has also been shown to increase SOD-2

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and SOD-3 in the endothelium of blood vessels [5]. Red fruit extract also has a strong antioxidant effect reaching 81.02% because it contains flavonoids, beta carotene, and vitamin E [6, 7]. Based on preliminary research in mice given a high cholesterol diet, combining red fruit extract with purple sweet potato tuber extract at half dose improved lipid profiles and acted as an antioxidant. However, the results were not significantly different from a single dose of red fruit extract alone. It was found that administering 200 mg of purple sweet potato tuber extract was better. In this study, the safety of liver and kidney was not known because the metabolism of natural substances also occurs in the liver and kidneys. To determine the most effective and safe dose combination for the liver and kidneys, it is necessary to conduct an experimental research using animal models. It is important to identify the optimal doses of red fruit extract and purple sweet potato tuber extract that provide the maximum benefits without causing any harm to these organs.

In the present study, we will be investigating Wistar rats given high cholesterol feed and a combination of purple sweet potato extract and red fruit extract at two dosages, namely 200 mg and 0.4 ml; and 100 mg and 0.2 ml. The desired outcome is a fixed-dose combination of purple sweet potato extract and red fruit extract in the form of tablets or capsules ready for clinical trials.

MATERIALS AND METHODS

This research is an experimental laboratory design with a randomized pre and post-test control group design. The research subjects were male Wistar rats aged three months and weighing 225-250 grams. Rats that met the study requirements were grouped randomly into three groups of seven, namely the control group, treatment group 1, and 2. The control group was only given high cholesterol *ad libitum* for four weeks. Treatment group 1 was given high cholesterol feed *ad libitum* and purple sweet potato tuber extract at a dose of 100 mg/day and red fruit extract at 0.2 ml/day for four weeks. Treatment group 2 was given high cholesterol feed *ad libitum* and 200 mg/day purple sweet potato tuber extract and 0.4 ml/day red fruit extract for four weeks. Before treating all groups, blood was taken from the rats through the retro-orbital sinus for MDA, SOD, and lipid profile examination as pre-test data. After four weeks of treatment, blood was drawn through the retro-orbital sinus for pre-test and post-test analysis. The blood tests included SGPT, BUN, and serum creatinine to evaluate liver and kidney function. The procedures followed were in accordance with animal rights and as per the guidelines for the care and use of laboratory animals. Ethical clearance was obtained from Udayana University ethical committee.

How to make the tablet? (Solid preparation)

One kg of purple sweet potato powder was steamed for 15 minutes, and then macerated with 3 L of 70% ethanol acidified with 3% citric acid for 24 hours. The product procured from the maceration process was collected and then concentrated with a vacuum rotary evaporator at a temperature of 40 °C and a pressure of ± 70-80 m bar until a concentrated extract was obtained with the volume of the extract reaching 1/10 of the initial filtrate volume. 10 mL of the thick extract was added with 25 grams of purple sweet potato starch powder adsorbent, and then crushed until it became homogeneous. The extract and adsorbent mixture were dried in an oven at 60 ^{o}C for 5 hours, and then printed with a single punch machine until the anthocyanin levels reached 200 mg per tablet.

Preparation of red fruit extract/oil

Red fruit (*P. conoideus*) oil was extracted using the Folch method. A total of 12 g of red fruit pulp was macerated with 80 ml of liquid consisting of chloroform and methanol (2: 1, v/v). The mixture was stirred with a magnetic stirrer for 1 hour at room temperature. The mixture was filtered with a vacuum pump, and 16 ml of 0.88% NaCl was added, and the next step is separation with a separating flask. The red fruit oil extract was then evaporated with a rotary evaporator at 40 °C, packed in dark bottles, dried with nitrogen gas, and stored at -20 °C until the study was carried out using Wistar rats.

RESULTS

This study was conducted in the Pharmacology and Therapy Laboratory for two months.

The subjects were adult male Wistar rats aged three months with a weight ranging from 225 mg to 250 mg. The rats were adapted to the cage for two weeks. The temperature of the cages and the lighting conditions were controlled, with 12 hours of light and 12 hours of darkness.

After adaptation for two weeks, we drew blood through the retro-orbital plexus for pre-test, including an examination of lipid profile, MDA, and SOD. The subjects were then divided into three groups, each consisting of seven rats: Control group, treatment group 1 and treatment group 2. Control group was given high cholesterol *ad libitum* feed for four weeks. Treatment group 1 was given high cholesterol feed *ad libitum* and purple sweet potato tuber extract at a dose of 100 mg/day and red fruit extract at 0.2 ml/day for four weeks; and Treatment group 2 was given high cholesterol feed *ad libitum* and 200 mg/day purple sweet potato tuber extract and 0.4 ml/day red fruit extract for four weeks.

The pre and post-test results showed a significant increase in cholesterol levels ($p<0.05$) in the control group. Both treatment groups showed an effect on blood cholesterol levels ($p<0.05$). However, treatment group 2 showed a lower cholesterol level (Figure 1). The control group also showed significant increases in triglyceride and LDL levels ($p<0.05$), whereas both treatment groups demonstrated a decrease of both triglyceride and LDL levels ($p<0.05$) as shown in Figures 2 and 3. Furthermore, we found that the HDL level in the post-test control group was lower than the pre-test. In treatment groups 1 and 2, we found

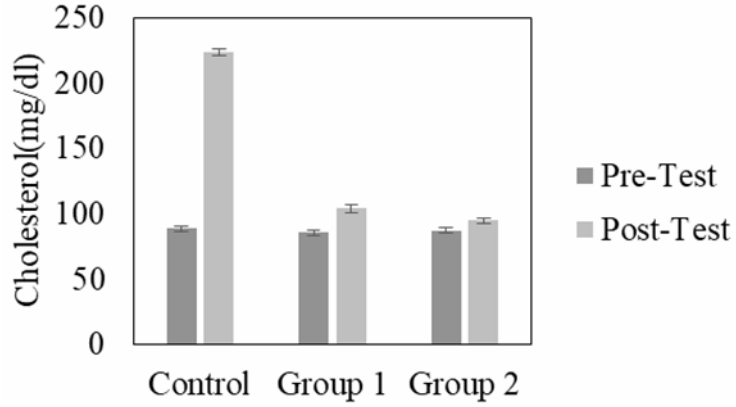

Figure 1. Comparison of pre-test and post-test cholesterol levels of each group of rats.

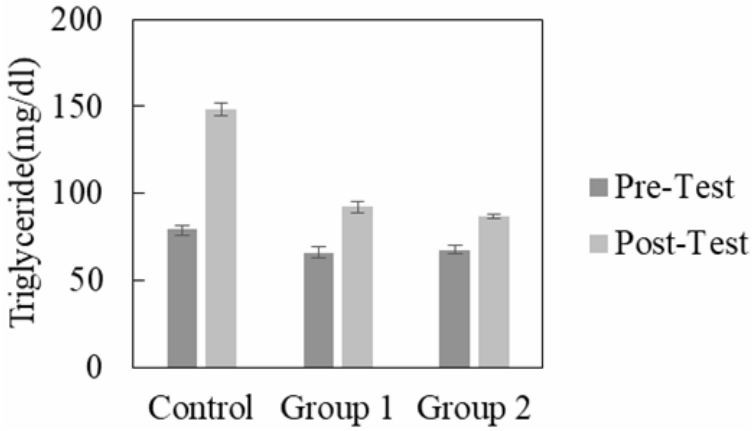

Figure 2. Comparison of triglyceride levels in pre-test and post-test of each group of rats.

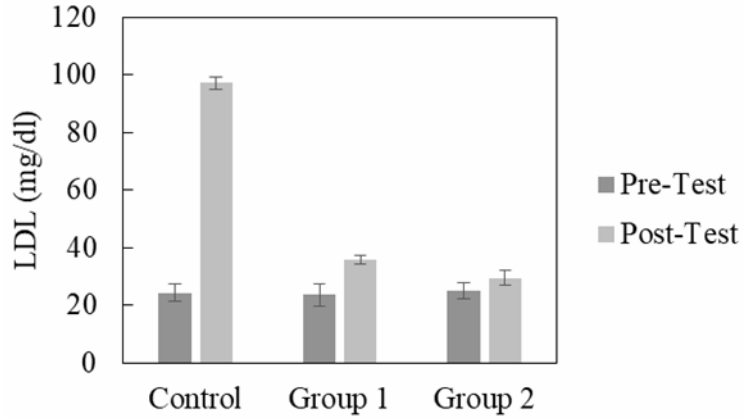

Figure 3. Comparison of pre-test and post-test LDL levels of each group of rats.

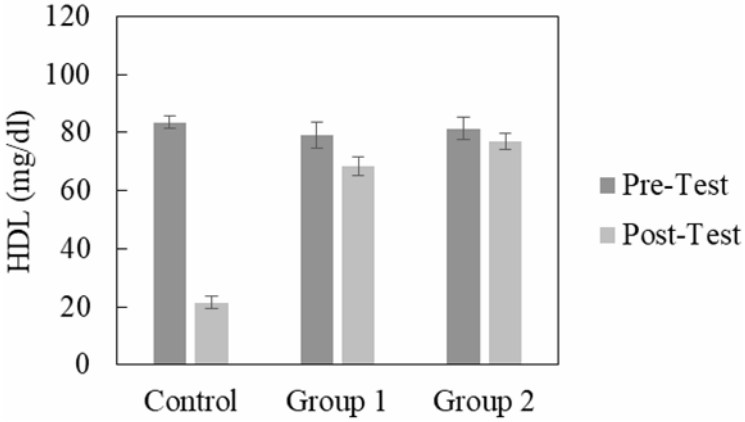

Figure 4. Comparison of pre-test and post-test HDL levels of each group of rats.

that HDL level was comparable to the pre-test ($p<0.05$) (Figure 4). For cholesterol, triglyceride, LDL, and HDL, treatment group 2 had a slightly superior effect than treatment group 1.

There was an increase in MDA levels and a significant decrease in SOD ($p<0.05$). Both treatment groups showed an effect on blood MDA and SOD levels ($p<0.05$) (Figures 5, 6). The effective combination for lowering MDA and increasing SOD was a dosage of 200 mg and 0.4 ml daily (group 2).

This study showed that SGPT, BUN, and serum creatinine levels are the highest ($p<0.05$). Both treatment groups showed significantly lower ALT levels, BUN, and serum creatinine than the control (Figure 7). The effective combination that caused the lowest SGPT, BUN, and serum creatinine was a dose of 200 mg and 0.4 ml per day.

DISCUSSION

This study indicates that giving a combination of 200 mg of purple sweet potato tuber extract and 0.4 ml of red fruit oil can maintain the lipid profile in rats fed high cholesterol feed. The dose in group 1 which is half, contributed to maintaining of the lipid profile and reducing oxidative stress; however the effect was weaker than the 200 mg and 0.4 ml doses. The administration of this combination dose also caused lower SGPT, BUN, and serum creatinine levels than the group given high cholesterol feed.

Feeding high cholesterol caused dyslipidemia, accompanied by a significant increase in SGPT,

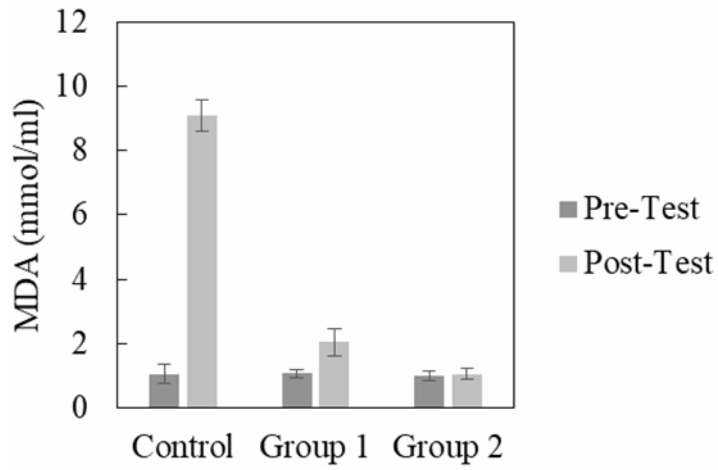

Figure 5. Comparison of pre-test and post-test MDA levels of each group of rats.

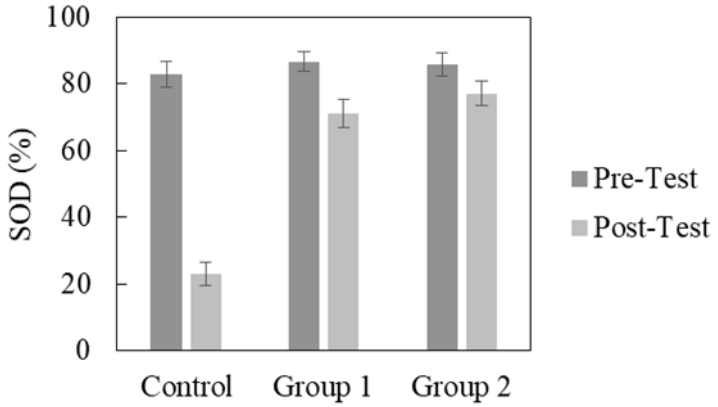

Figure 6. Comparison of pre-test and post-test SOD percentage of each rat group.

BUN, and serum creatinine ($p<0.05$). In this study, the obese patients had an increase in liver transaminase [8]. In this study, feeding high cholesterol *ad libitum* rats for one month (control) caused a very significant increase in cholesterol, triglyceride, and LDL ($p<0.05$). Feeding high cholesterol also caused a very significant HDL reduction ($p<0.05$). Moreover, hypercholesterolemia can cause oxidative stress which is indicative of increase in MDA and decrease in SOD. Oxidative stress is due to disproportion of antioxidants such as superoxide ion, hydrogen peroxide, hydroxyl radical, peroxynitrite, catalase, glutathione peroxidase, glutathione reductase, and heme oxygenase. The increase in oxidative stress in hypercholesterolemia results from increased activity of the NADPH enzyme, which will increase the production of superoxide ions and decrease the endogenous antioxidant enzymes [9]. Oxidative stress in hypercholesterolemic conditions is also due to increase in the total cholesterol pool in cells, resulting in increased lipid peroxidation in cell membranes, and an increase in MDA (as a marker of MDA oxidative stress), and a decrease in SOD. Hence it led to the increase of free radicals due to the hypercholesterolemia [10].

The group of rats given a combination of 100 mg purple sweet potato extract and 0.2 ml red fruit oil (treatment 1) showed significant changes in all study variables ($p<0.05$) compared to controls. In this group, there was an increase in the lipid profile, but it was lower than that of the control ($p<0.05$). The same effect was also observed in

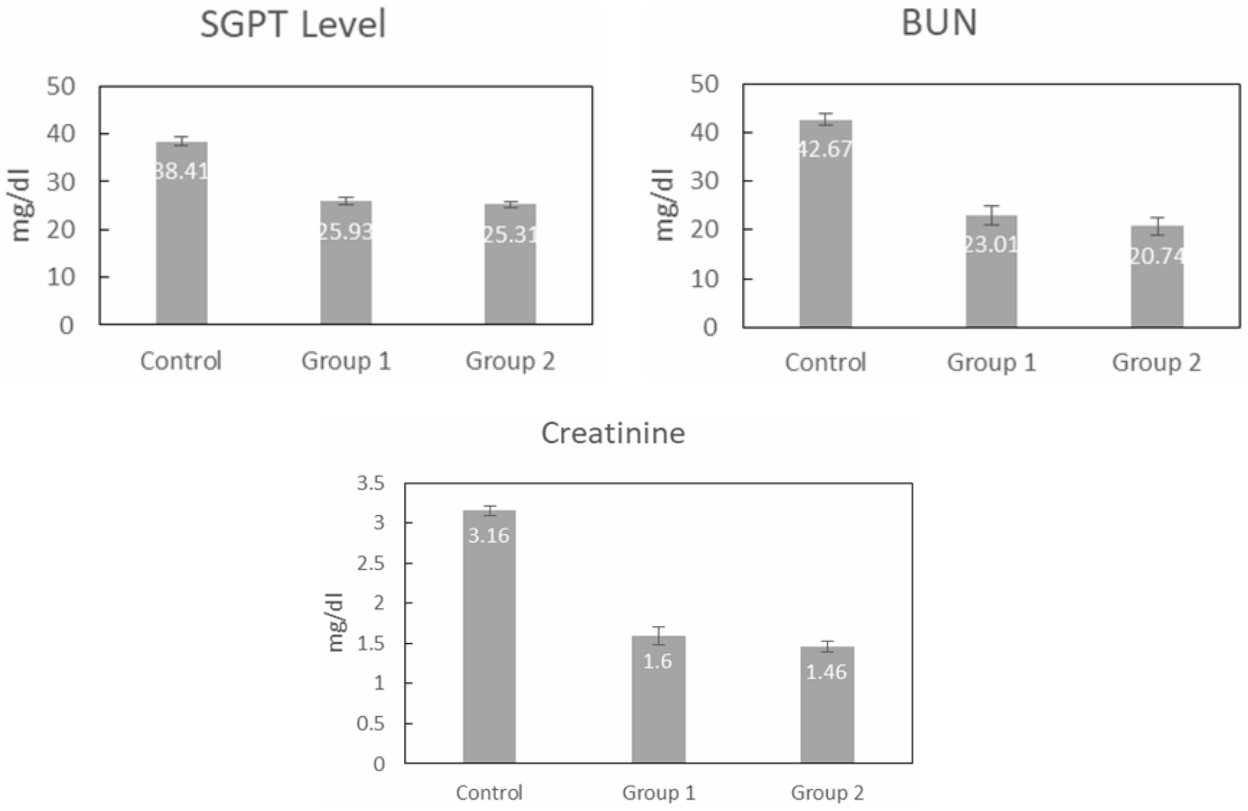

Figure 7. Comparison of post-test serum SGPT, BUN, and creatinine levels of each group of rats.

the group of mice given high cholesterol feed and a combination of 200 mg purple sweet potato tuber extract and 0.4 ml red fruit oil/head every day for one month (treatment 2). Changes in lipid profile and oxidative stress in treatment 2 showed better results compared to the pretest. HDL levels in this group tend to show no difference compared to the pretest ($p>0.05$).

The red fruit extract is a natural medicine that contains high antioxidants, including carotenoids, unsaturated fatty acids, which include oleic acid, linoleic acid, linolenic acid, and decanoic, tocopherol, and also beta-carotene. Red fruit has been researched and established as a non-toxic medicinal ingredient [11] that is safe for long term usage. In this research, a dose of 0.2-0.4 ml per day red fruit extract was given to the rats according to the 2016 Sugiritama study [12], which was proven to be an antioxidant in an animal model of preeclampsia.

Several animal studies stated that purple sweet potato tuber extract is also relatively safe at appropriate doses [4, 5, 13]. This study proved that the treatment combination dose 2 was more effective than the treatment combination dose 1. Red fruit contains high levels of tocopherol and β-carotene as antioxidants [14]. In contrast, purple sweet potato tuber extract contains anthocyanin flavonoids and antioxidants that work on different targets causing synergistic antioxidant effects. One indication of the antioxidant effect of red fruit extract and purple sweet potato tuber extract is decreased blood MDA in all treatment groups. This study's results are consistent with the research conducted on the testes of mice; there was a decrease in testicular MDA, which was protected by red fruit because of the presence of beta-carotene and tocopherol as antioxidants [15]. The reduction of LDL and cholesterol levels in this study could be attributed to the antioxidant properties which inhibited the synthesis of cholesterol in the liver.

Moreover, another study found a significant reduction in LDL in diabetic rats given red fruit

extract [7]. Purple sweet potato tuber extract reduces blood cholesterol, by regulating cholesterol synthesis and adipogenic enzymes' expression through an inhibition of transcription factors. High anthocyanin levels in those extracts also reduced the expression of acetyl-coenzyme A (CoA) synthetase. Furthermore, other than the lipid-lowering and antioxidant effect, anthocyanin found in purple sweet potato may have potential action in the neurogenic pain pathway, specifically inhibiting the microglia activations and lowering oxidative stress [16, 17].

Among the several anthocyanins contained in purple sweet potato tubers, cyanidin and peonidin are more commonly studied [18, 19]. The purple sweet potato found in Bali also contains cyanidin and peonidin, which are quite strong antioxidants [20]. Anthocyanins have stronger effect on the electrons than L-AA (L-Ascorbic acid) and BHT (butylated hydroxytoluene), which are standard antioxidants commonly used in laboratory experiments. Therefore, anthocyanin is a potential antioxidant [21]. Anthocyanins are also able to increase SOD mRNA in mice [22]. Thus, purple sweet potato tuber extract in tablet form reduces MDA by eliminating electrons and increasing SOD [21, 22].

Additionally, purple sweet potato tuber anthocyanins also reduce oxidative stress by lowering mitochondrial lipid peroxidation and eliminating mitochondrial transmembranous electrical potential [23]. The protective effect of anthocyanins on the liver is through AF (anthocyanin fraction)-up-regulated heme oxygenase-1 (HO-1), NAD (P) H: quinone reductase, and glutathione S-transferase. Nrf2 nuclear translocation, and Akt and ERK1/2 activation pathways involved in Nrf2 nuclear translocation is also induced by AF. AF is suggested to reduce t-BHP-induced hepatotoxicity by scavenging ROS and regulating the antioxidant enzyme HO-1 through the Akt and ERK1/2 / Nrf2 signaling pathways [24].

CONCLUSIONS

The combination of 200 mg purple sweet potato tuber extract with red oil at a dose of 0.4 ml is the best dose to improve the lipid profile and reduce oxidative stress in rats given high cholesterol feed and it is safe for the liver and kidneys.

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interests.

ABBREVIATIONS

AF : Anthocyanin Fraction
Akt : Protein Kinase B
BUN : Blood Urea Nitrogen
BHT : butylated hydroxytoluene
CoA : Acetyl-coenzyme A
ERK : Extracellular Signal- regulated Kinase
HDL : High Density Lipoprotein
HO : Heme Oxigenase
L-AA : L-Ascorbic acid
LDL : Low Density Lipoprotein
Nrf : Nuclear Respiratory Factor
MDA : Melondialdehyde
mRNA : Messenger Ribonucleic Acid
SGPT : Serum Glutamic Pyruvic Transaminase
SOD : Super Oxide Dismutase
ROS : Reactive Oxygen Species

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