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Protective Effect of *Sauropus androgynus* (L) Merr against Toxicity of Copper Induced Oxidative Stress and Ovary Damage in Experimental Rats.

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ABSTRACT

The present study was investigated the protective effect of antidote *S.androgynus* L Merr in experimental rats which exposed by Cu(II). The administration of Cu(II) intraperitoneally lead to increase level of serum biochemical parameters including SGOT, SGPT, urea, creatinine and malondialdehyde (MDA) significantly. The pre treatment with antidote *S.androgynus* L Merr before exposure with Cu(II) could reduced the elevated level of the SGOT, SGPT, urea, creatinine and MDA as 16,11%; 64,38%; 28,51%; 50% and 38,7% respectively. Histopathological analysis of ovary tissue indicate that pre treatment with *S.androgynus* L Merr able to reduce the severity of tissue damage in the ovary which indicate that antidote of *S.androgynus* L Merr give protective effect against Cu(II) toxicacy

Keywords: *Sauropus androgynus* L Merr, Cu(II), oxidative stress, ovary

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INTRODUCTION

Copper is a reddish brown nonferrous mineral which has been used for thousand years by many cultures. This metal is highly conductive to electricity and heat and relatively resistant to corrosion so it is widely used in various industries [1]. Nowadays copper and its alloy are now used in large scale for cooking utensils, production of electrical wire, electroplating, photography and as a catalyst in chemical industry [2]. Although copper is an essential trace element and have lower toxicity compared to other heavy metals, it could be toxic once the safe dose exceeded. Copper in soil and water might enter the food chain and accumulate in humans and animals via ingestion of drinking water and contaminated food. Copper primarily stored in liver, but it might reach the kidney, the brain and other tissue and organs via the bloodstream [3]. Due to its potent emetic and strong oxidizing agent, copper may cause corrosive damage to mucous membrane. Copper sulphate in one of the powerful oxidizing agent that may cause hemolytic anemia and/or methemoglobinemia. A wide variety of clinical manifestations related to copper poisoning in organs such as in the liver, kidney, spleen and lungs have been reported using the experimental animals [4]. When the hepatic copper storage capacity is exceeded, it might lead to hepatocellular necrosis and consequently the release of copper from the liver into bloodstream, produces hemolysis, renal insuffiency and also generate highly reactive hydroxyl radicals that could lead to lipid peroxidation of mitochondrial membrane [5]. The most common way to alleviate intoxication of copper is chelation therapy, which can promote the excretion of copper. But chelators have some unavoidable side effect including kidney toxicity and loss of essential trace elements. *Sauropus androgynus* (L) Merr.,sweet leaf (Katuk) is a shrub belonging to the family Euphorbiaceae, that growing in the warm humid tropical regions. The plant is reported to have an upright stem reaching a height of 2,5cm and bears dark-green oval leaves 5-6cm long [6]. This plant was introduced to India from Malaysia in the 1950s for its nutritional and medicinal properties [7]. In the previous study has reported the ability of *S androgynus* L.Merr as low cost biosorbent to remove Cu(II) ions from aqueous solution. The optimum condition was achieved at pH 4, initial concentration 1500 mg/L, biosorbent mass 0,1 g and contact time 15 minutes. The optimum adsorption capacity of *S.androgynus* L.Merr was 30,18 mg/g [8]. Recently, the effect of copper poisoning on reproductive organs of male rats has been studied [9], but unfortunately there is few information about the effects of Cu toxicosis on female genital organs. This study aims to investigate the efficacy of *S androgynus* L Merr antidote against copper induced oxidative stress and ovary damage in experimental rats.

MATERIALS AND METHODS

Collection of S androgynus L Merr leaves

The leave of *S androgynus* L Merr was collected freshly from local garden in Padang, Indonesia

Antidote of *S androgynus* L Merr leaves

The leaves of *S androgynus L* Merr were washed using tap water, then air dried for a week under room temperature condition. The dried leaves then crushed using a crusher so it becomes a powder form. The leaves powder then weighed and 2 gram of the powder was dissolved on 120 mL boiling distilled water for a minute then filtered.

Experimental Rats

The experimental animals used in this study were adult white rats weighing approximately 140-160 g. The experimental rats were purchased by faculty of pharmacy, Andalas University, Indonesia. The experimental rats were placed in decent cage and maintained with ad libitum normal diet and drinking water during the study. All experimental procedures were approved by the animal ethics committee of the Andalas University.

Experimental Design

As many as 9 experimental rats were randomly divided into 3 groups with 3 rats in each groups. The first group (Group I) were control group which only given distilled water. The second group (Group II) were treated with 1 mL x bw Cu(II) 1000 mg/L intraperitoneally.The third group (Group III) were given pre-treatment

with antidote of *S.androgynus* L Merr 1 mL x bw/200 g bw orally for 7 days, followed by administration of 1 mL Cu(II) 1000 mg/L on the 8th day. After 5 hours of administration of Cu(II), the rats were sacrificed using anesthesia. The blood were drawn for biochemical serum analysis. The ovary organs of the rats were removed and preserved in Bouin solution for histopathology analysis

Biochemical Serum Analysis

The serum biochemical parameters were analyzed include the levels of malondiadehyde (MDA), urea, creatinin, SGOT and SGPT

Statistical analysis

The data was statistically analyzed using Statistical Package for Socal Science Program ver. 16 (SPSS ver. 16). The analysis was conducted using analysis of variance (ANOVA) followed by Tukey Test.

RESULT AND DISCUSSION

Biochemical Serum Analysis

The result for biochemical serum analysis were showed in table.1

Table 1

No	Parameters	Group 1 (Control)	Group 2 (Cu(II) treatment)	Group 3 (pre treatment with antidote)
1	Malondialdehyde (MDA) (mg/dl)	3,61	8,06	4,94*
2	Urea (mg/dl)	20,36	38,58	27,58*
3	creatinine (mg/dl)	0,21	0,6	0,3
4	SGOT (U/L)	91,98	123,97	103,99*
5	SGPT (U/L)	25,88	92,5	32,94*

*P<0,05 compare to Group 2

Based on the table above there are increased levels of biochemical parameters in Group 2 compared with Group 1. The percentage of elevated levels of the parameters for MDA, urea, creatinine, SGOT and SGPT were 55,21%; 47,22%; 65%; 25,8%; 72,02% respectively. Pre treatment with antidote *S.androgynus* L Merr could reduce the levels of biochemical serum parameters compared to Group 2. The percentage of reduced levels of MDA, urea, creatinin, SGOT and SGPT were 38,7%; 28,51%; 50%; 16,11% and 64,38% respectively.

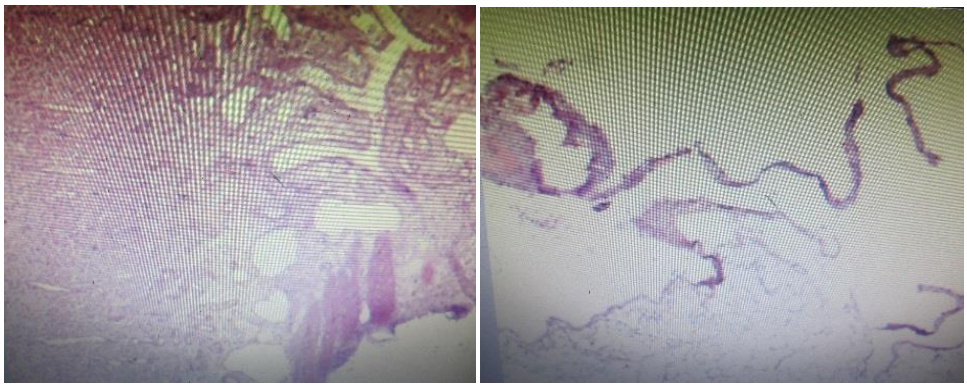
Copper is an essential cofactor for enzymes associated with oxidative stress including catalase, superoxide dismutase, peroxidase, cytochrome c oxidase, ferroxidases, monoamine oxidase and dopamine-β monooxygenase. The ability of copper to cycle between oxidized state, Cu(II) and reduced state, Cu(I) is used by cuproenzymes involved in redox reactions. However the transitions between Cu(II) and Cu(I) could result in generation of superoxide and hydroxyl radicals which potentially toxic [10]. Reactive oxygen species such as hydroxyl radicals and superoxide would enhance the lipid peroxidation, and malondialdehyde (MDA) is one of the product to measure the level of lipid peroxidation. Based on the result, there is a significant increase of MDA after administration of Cu(II). The similar result was reported by Jing et al [11]. Jing et al reported that copper can disturb the intracellular redox balance and induce oxidative stress. There are significant increase of MDA level in mice which exposed with high dose of copper in short term. The similar result also reported by Becaria et al [12]. Becaria et al reported that there was significantly elevated level of MDA after exposure of experimental mice with Cu 8µM which indicated lipid peroxidation was occur. The protection effect of *S.androgynus* L Merr against copper induced oxidative stress might be due to antioxidant activity and scavenging of reactive hydroxyl in this plant. Nahak and Sahu [13] reported that *S.androgynus* L Merr has a promising antioxidant activity. Mentioned that the antioxidant activity of aqueous extract *S.androgynus* L Merr 200µg/mL was 48,48±0,03%. The mechanism of antioxidant in *S.androgynus* L Merr was by inactivating lipid free radicals of preventing decomposition of hydroperoxides into free radicals.

SGOT and SGPT are the marker for liver activity [14]. Amino transferases SGT and SGOT catalyze the interconversion of amino acids and α-keto acids by the transfer of an amino group. These enzymes are very sensitive and reliable indicies for hepatoprotective or curative effects of various compounds . The elevated levels of these enzyme indicate there are leakage and loss of integrity of cell membrane [15]. Garcia-Nino and Pedraza-Chaverri [16] reported that the hepatotoxic effects of a medicinal plant might be due to its intrinsic antioxidant properties, anti-inflammatory, anti-cholestetic, anti-carcinogenic and anti fibrogenic. Madhu et al [17] repoted that the leaves of *S.androgynus* has potential to be applied as a therapeutic product due to the high antioxidant content or the presence of a functional protein and phenolic compound. Antioxidant activity of *S.androgynus* against DPPH reported as 50% compared with standard curcumin, 62,31%, while in vitro anti inflammatory activity by hypotonic induced model showed maximum protection (74,17%) compared to standard acetylsalicylic acid (86,88%).

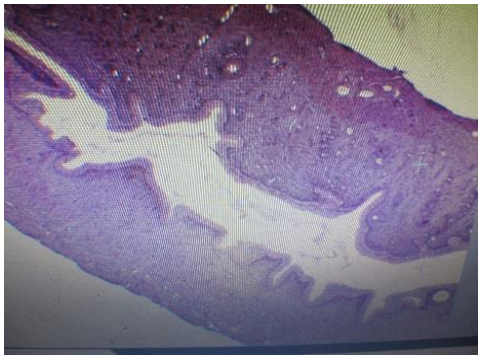
Oxidative stress is a causative factor in many diseases, including disorders of the kidneys. The protective effect of the kidney by a medicinal plant might be due to its high content of antioxidant such as polyphenol compound that could prevent lipid peroxidation in kidney [18]. Polyphenols have demonstrated a wide range of pharmacological effects and offer substantial protective effects on human carcinogenesis, cardiovascular and renal disorders. Petrus [19] reported that *S.androgynus* L Merr leaves contain high content of polyphenolic metabolites including anthocyanins (1,53 mg/100 g), flavonoids (142,64 mg/100g) and gallic acid equivalent (138,01 mg GAE/100g).

Histopathology Analysis

The protective effect of *S.androgynus* L Merr antidote against ovary damage histopathologically described in Figure.1



(a) **(b)**



(c)

Figure 1 : (a) photomicrograph of rat's ovary in group control; (b) photomicrograph of rat's ovary in group II (Cu(II) 1000 mg/L), there are cavities coated with columnar epithelial cells (c) photomicrograph of rat's ovary in group III (pre treatment with *S.androgynus* L Merr antidote), dilated lumen endometrium

Copper is a strong oxidant, in which it could bind to cell molecules during the high load. Indirectly they can catalyze through Fenton/Haber-Weiss that lead cell damage as a consequence of metal-diven formation of reactive oxygen species [20]. Babaei et al [21] reported that the administration of 100 mg/kg to the experimental rats will produce some detrimental effects such as the decrease in number of antral follicles, and also significantly reduced different classes of follicles and corpora lutea in ovaries. Sakhaee et al [22] reported administration of copper sulphate 100 mg/kg for will lead to ovarian lesion. In group which administered with 200mg/kg copper sulphate will induce degenerating antral follicle with desquamation of pyknotic granulose cells and degenerated oocyte. In the present study, we administered *S.androgynus* (L) Merr as natural antioxidant to prevent the adverse effect of copper toxicosis on ovary of experimental rats. Murti et al [23] reported that crude water extract of *S.androgynus* (L) has a strong antioxidant activity determined by DPPH free radical scavenging activity. The values of IC_{50} (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of *S.androgynus* (L) was 307.5 µg/mL. Bunawan et al [23] reported that *S.androgynus* leaves were found to have highest content of flavonoids and bioactive compound among 11 vegetables from Indonesia with 142.64 mg per 100 gram of fresh weight with quercetin, myricatin, luteolin, apigenin and kaempferol which has detected by HPLC analysis, and it also contain a high polyphenol content, cupric ion chelating activities, free radical scavenging and reducing ferric ion antioxidant properties.

CONCLUSION

The experimental rats that received Cu(II) will lead to changes in serum biochemical parameters including SGOT, SGPT, urea, creatinin and malondialdehyde (MDA). Pre treatment with antidote *S.androgynus* L Merr could reduce the elevated levels of serum biochemical parameters by indicates that *S.androgynus* L Merr have protective effect against oxidative stress induced by Cu(II) exposure. The analysis of ovary tissue histopathologically revealed that pre treatment with antidote *S.androgynus* L Merr could reduce the damage of the ovary. This indicates that *S.androgynus* L Merr have protective activity due to its antioxidant content

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