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ANTIOXIDANTS ACTIVITY OF EUCALYPTUS CAMALDULENSIS AGAINST ACETAMINOPHEN WHICH CAUSES OXIDATIVE STRESS IN MALES RATS

[1]*Wid Abass Mohammed and [2]Jawad Khadim Faris

[1]M.S.C. Department of Physiology, Al-Qasim Green University /College of Veterinary Medicine.

[2]Assistan Professor, Department of Physiology & Pharmacology, Al-Qasim Green University/ College of Veterinary Medicine.

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***Corresponding Author**
Wid Abass Mohammed
M.S.C. Department of Physiology, Al-Qasim Green University /College of Veterinary Medicine.

ABSTRACT

The present study has been designed to evaluate the beneficial effects of *Eucalyptus camaldulensis* extract upon acetaminophen-induced renal toxicity, The study has been conducted on adult male rats at the department of physiology, College of Veterinary Medicine, Al-Qasim Green University during the period extended from November, 2015 to February, 2016. Forty mature male Wistar rats (aged 90 days and weighted 190±10 g) were divided in to four randomly equal groups, the first served as negative control received only distilled water, the second as positive control received with Acetaminophen (2.5 g/kg b. w.) as a single dose. third and fourth groups received Acetaminophen (2.5 g/kg b. w.) as a single dose then treated after 2 hours with EUE (150,200 mg /kg b.w.) respectively orally through stomach tube during 42 days. males were anaesthetized (by injection IM of 0.3ml ketamine + 0.1 ml of xylazine /kg b. w. *ip*), blood samples were obtained from heart in non-heparinized tubes, Blood serum samples were separated for assessment of MDA, SOD, CAT, GSH concentration. Under our experimental conditions, acetaminophen poisoning resulted in an oxidative stress evidenced by statistically significant losses in the activities of catalase (CAT), superoxide-dismutase (SOD), Glutathione (GSH) and increase in lipids peroxidation(MDA) level in Blood serum of acetaminophen-treated group compared with the control group, and after the gavage *Eucalyptus camaldulensis* extract alcoholic has been results demonstrated statistically significant increase activity in catalase (CAT), superoxide-dismutase (SOD), Glutathione (GSH) and significant decrease in

lipids peroxidation(MDA) level in Blood serum of AE150.AE200 group compared with the Acetaminophen-treated group.

KEYWORD: Acetaminophen, Eucalyptus, Males rats, Superoxide dismutase (SOD), Glutathione (GSH), Catalase (CAT), and Malondialdehyde (MDA).

INTRODUCTION

Acetaminophen, also known as paracetamol, is a widely used pharmaceutical drug[1]. The popularity of this drug was based on its reputation for low toxicity[2]. Since its introduction in the 1950s, acetaminophen (N-acetyl p-aminophenol) has increased in use as an analgesic and antipyretic drug for the treatment of minor non inflammatory conditions and to alleviate the symptoms of more severe conditions such as chronic inflammatory arthritis and cancer[3][4]. However, acute overdose is fairly common and can lead to potentially fatal renal damages in humans and experimental animals[5]. Current evidence suggests that oxidative stress with increased generation of reactive oxygen species, depletion of reduced glutathione (GSH) and lipid peroxidation play a crucial role in the development of acetaminophen[6]. Indeed, several antioxidants were proved efficient in protecting the kidney against the deleterious effect of acetaminophen overdose[7]. *Eucalyptus camaldulensis* was proved successful in protecting different organs against oxidative in various experimental models[8]. Eucalyptus, a native plant of Australia, includes more than 800 species. Some of them, including *Eucalyptus camaldulensis*, were introduced in Europe and North Africa where they are well acclimated to the Mediterranean shores[9]. Eucalyptus is mainly cultivated for the paper and cosmetic industries, while some of them are used in traditional medicine, certain species of Eucalyptus are even used in modern medicine[10]. Many researches were conducted on the medicinal properties of *Eucalyptus camaldulensis*, The leaf extract or essential oil from the leaves of *Eucalyptus camaldulensis* were reported to possess antifungal, antibacterial, anti-inflammatory, antioxidant, and antihelmintic properties[10]. In addition, the beneficial effect of Eucalyptus was demonstrated in rats given toxic doses of acetaminophen. However, the therapeutic effect of *Eucalyptus camaldulensis* against acetaminophen induced oxidant–antioxidant status of blood serum was assessed by measuring level and activities of antioxidant enzymes SOD (Superoxide dismutase), GSH(Glutathione), CAT (Catalase) and MAD (Malonaldehyde).

MATERIAL AND METHOD

Plant material collection

The leaves of *Euclayptus camaldulensis* plant were collected during August 2015, from Al-Hashmia city from park, by researcher Wid Abass.

Preparation of Ethanolic Extracts*(Eucalyptus camaldulensis)*

20gm of Eucalyptus camaldulensis powdered leaves were taken and extracted with soxhlet apparatus ethanol (70%) Within 24 hours, and then taking the extract and place it in a ptry dish and put in the oven at a temperature of (40°C) within 48 hours, The result of extract was stored at (4°C) until use[11].

Serum Preparation

Blood was collected in test tubes with cap and allowed to clot (for 20 minutes), then serum was separated by centrifugation at (4000 rpm, 0.894xg) for 10 minutes[12]. The separated serum of each animal was subdivided nearly into (6) samples using appendroff tubes (0.5ml) and kept at deep freezer until using for assessment of the biochemical parameters.

Determination of oxidant-antioxidant

Oxidant-antioxidant are determinate by kits of spectrophotometer to MDA, SOD, GSH and CAT, and this kits it's from US bio USA.

THE RESULT

Serum oxidant-antioxidant concentrations

Serum MDA concentration

The results illustrated in figure (4-1) showed significant differences between all experimental groups and control group. In group A with gavage acetaminophen showed significant increase ($p<0.05$) in serum concentration of MDA compared with control group, and in groups gavage with single dose of acetaminophen and continuous gavage Eucalyptus daily (AE150,AE200), showed significant decrease ($p>0.05$) in serum concentration of MDA compared with acetaminophen group.

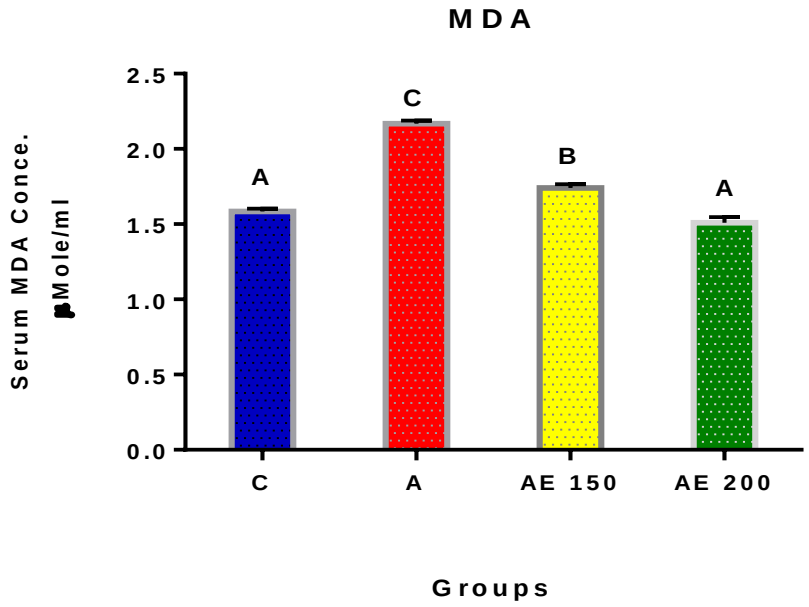

Figure (4-1): Effect of *Eucalyptus camaldulensis* treatment on serum MDA concentration (µMole/ml) in mature male rats gavaged Acetaminophen.

The results represented as mean ± SE.

Simillar capital letters denotes the absences of significant differences (P>0.05) between groups.

Different capital letters denotes the presence of significant differences (P>0.05) between groups.

- **C: male rats orally gavaged with drinking water (0.5 ml).**
- **A: male rats orally gavaged with Acetaminophen (2.5 g/kg.b.w) suspended in 1 ml of drinking water) for one time.**
- **AE150: male rats orally gavaged with Acetaminophen (2.5 g/kg b.w.) suspended in 1 ml of drinking water) for one time and gavaed with exctrated ethanol of *Eucalyptus Camaldulensis* (150 mg/ kg.b.w) for 42 days.**
- **AE200: male rats orally gavaged with Acetaminophen (2.5 g/kg b.w.) suspended in 1 ml of drinking water) for one time and gavaed with exctrated ethanol of *Eucalyptus Camaldulensis* (200 mg/ kg.b.w) for 42 days.**

4.1.4. Serum GSH concentration

The results illustrated in figure (4-2) showed significant difference between all experimental groups and control group. In group A with gavage acetaminophen showed significant decrease (p>0.05) in the serum concentration of GSH compared with the control group, and

in groups gavage with single dose of acetaminophen and continuous gavage Eucalyptus daily (AE150,AE200) is showed significant increase ($p<0.05$) in the serum concentration of GSH compared with the acetaminophen group.

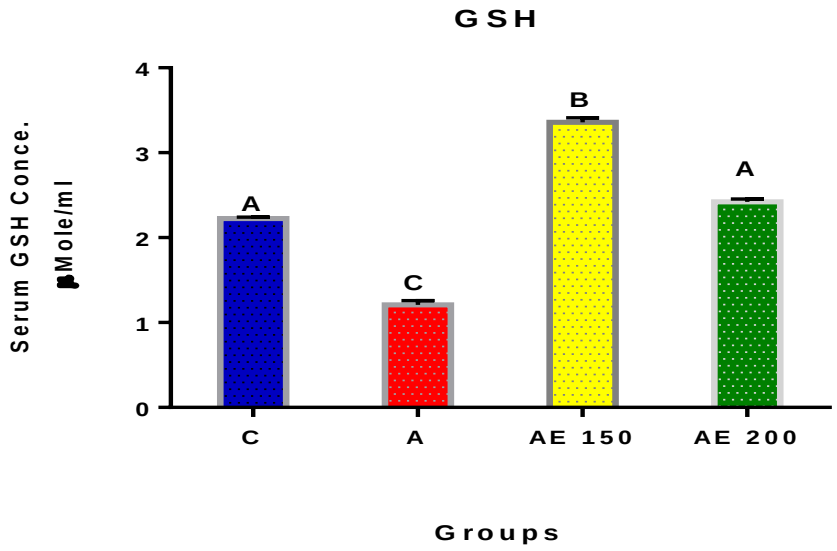

Figure (4-2): Effect of *Eucalyptus camaldulensis* treatment on serum GSH concentration (µMole/ml) in mature male rats gavaged acetaminophen.

The results represented as mean ± SE.

Simillar capital letters denotes the absences of significant differences (P>0.05) between groups.

Different capital letters denotes the presence of significant differences (P>0.05) between groups.

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4.1.2. Serum SOD concentration

The results illustrated in figure (4-3) showed significant difference between all experimental groups and control group. In group A with gavage acetaminophen showed significant decrease ($p>0.05$) in the serum concentration of SOD compared with the control group, and in groups gavage with single dose of acetaminophen and continuous gavage Eucalyptus daily (AE150,AE200) is showed significant increase ($p<0.05$) in the serum concentration of SOD compared with the acetaminophen group.

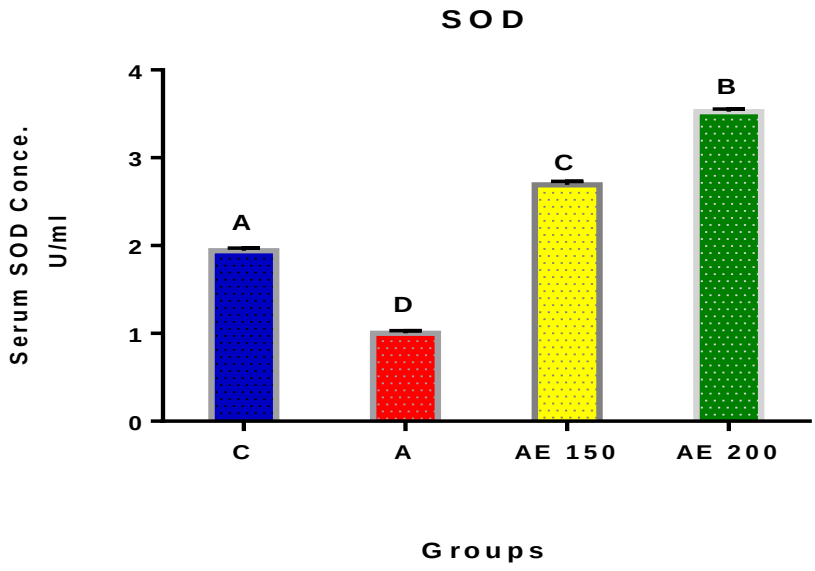

Figure (4-3): Effect of *Eucalyptus camaldulensis* treatment on serum SOD concentration (U/ml) in mature male rats gavaged acetaminophen.

The results represented as mean ± SE.

Different capital letters denotes the presence of significant differences (P>0.05) between groups.

- **C: male rats orally gavaged with drinking water (0.5 ml).**
- **A: male rats orally gavaged with Acetaminophen (2.5 g/kg.b.w) suspended in 1 ml of drinking water) for one time.**
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- **AE200: male rats orally gavaged with Acetaminophen (2.5 g/kg b.w.) suspended in 1 ml of drinking water) for one time and gavaed with exctrated ethanol of *Eucalyptus Camaldulensis* (200 mg/ kg.b.w) for 42 days.**

4.1.3. Serum CAT concentration

The results illustrated in figure (4-4) showed significant difference between all experimental groups and control group. In group A with gavage acetaminophen showed significant decrease ($p>0.05$) in the serum concentration of CAT compared with the control group, and in groups gavage with single dose of acetaminophen and continuous gavage Eucalyptus daily (AE150,AE200) is showed significant increase ($p<0.05$) in the serum concentration of CAT compared with the acetaminophen group.

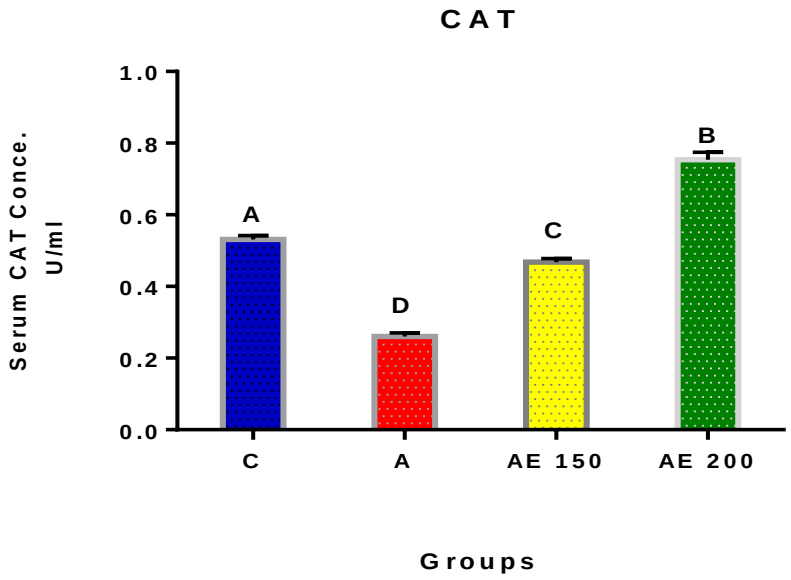

Figure (4-4): Effect of *Eucalyptus camaldulensis* treatment on serum CAT concentration (U/ml) in mature male rats gavaged acetaminophen.

The results represented as mean ± SE.

Different capital letters denotes the presence of significant differences (P>0.05) between groups.

- **C: male rats orally gavaged with drinking water (0.5 ml).**
- **A: male rats orally gavaged with Acetaminophen (2.5 g/kg.b.w.) suspended in 1 ml of drinking water) for one time.**
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- **AE200: male rats orally gavaged with Acetaminophen (2.5 g/kg b.w.) suspended in 1 ml of drinking water) for one time and gavaed with exctrated ethanol of *Eucalyptus Camaldulensis* (200 mg/ kg.b.w) for 42 days.**

DISCUSSION

Clearly demonstrates that acute acetaminophen toxicity, increases lipid peroxidation(MDA) level, and decrease and weakens in the antioxidant defense(SOD, CAT and GSH) mechanisms leading to biochemical disturbances and deteriorates the renal architecture, and this result after gavage of acetaminophen is because the acetaminophen is causes damage in kidney (injuries), It has been reported that serum MDA concentration is widely used as a biomarker or an index for the degree of oxidative damage due to its capacity to interact with lipoprotein, as a compound containing the carbonyl group that originated from lipid peroxidation[13]. MDA is a good indicator of the degree of lipid peroxidation and this is agreement with the researcher[14][15]. With the dose of acetaminophen are ingested there is more severe GSH depletion as well as massive production of metabolites which compounds the toxicity leaving large amounts of reactive metabolite unbound. These intermediates then form covalent binding with macromolecules on cellular protein, This process disrupts homeostasis and initiates apoptosis or programmed cell death leading to tissue necrosis and ultimately to organ dysfunction, The concentration of intracellular, therefore is the key determination of the extent of acetaminophen-induced renal injury thus, interest has been focused on compounds that act as antioxidant and are capable of stimulating GSH synthesis depletion of renal GSH is on of the primary factors which permits lipid peroxidation, suggested to be closely related to acetaminophen tissue damage[16]. As for the enzymatic antioxidants SOD (superoxide dismutas) is consider first line defense and when the body is attach from any toxic is depletion of SOD, and this result in our study is agreement with the many researchers[17]. As it plays a crucial role in the antioxidant defense system of the hepatocytes by scavenging free superoxide radicals, removing the hydrogen and lipid peroxides and therefore preventing oxidation of different bimoleculs in the hepatocytes[18]. As for Catales CAT is the work with the SOD by degeneration of peroxide hydrogen (H2O2) is one result of damage of SOD to prevented of body from this toxic material this causes is decrease of SOD and CAT in group A comparable with the control group. And this agreement with many researchers like[19]. In our study, Eucalyptus treatment effectively protected against acetaminophen-induced nephrotoxicity by restoring almost normal activities of SOD, CAT and GSH. The attenuation of renal damage was confirmed by microscopic

examination. These findings are in agreement with those reported earlier by others[20]. Previous studies showed that nutraceutical benefit of the extract of our plant have been attributed to the flavonoids, flavonols, and phenolics compounds[21]. The localization of flavonoids in the membrane interiors and their resulting restrictions on the fluidity of membrane components could strictly hinder the diffusion of free radicals generated during acetaminophen oxidation, and there by decrease resulting damage effects[22]. Therefore, Eucalyptus extract might play a key role in protection against acetaminophen intoxication by modulating the cellular GSH pool[23]. In this regard, we suggest that *Eucalyptus camadulensis* exerts an in vivo antioxidant activity against harmful reactive oxygen species generated following acetaminophen oxidation, and could therefore prevent kidney damage.*Eucalyptus camadulensis*, as an antioxidant agent, may have inhibited the chain reactions of acetaminophen-generated free radicals or scavenged the reactive oxygen species before reaching its renal targets[24].

CONCLUSION

From the present observations, it could be postulated that EUCE is a herbal source of treatment with valuable pharmacological role when used by the present dose (150 and 200 mg/kg b. w.) and present duration (42 days) not only as an antioxidant by itself but also as an inducer of regeneration of kidney damage induce by acetaminophen its mean of increasing of superoxide dismutase, catalase, glutathione activity even in normal intact male rats not only in cases of oxidative stress.

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Praise be to Allah, who praise recited each book and mention the name of Allah, every speech begins. Grace of Allah, sumptuously folks bliss in paradise. Pray on the messenger, who is mercy to the world, prayer and peace, seeking with them a lot of rewards and intercession in the day of judgment. Thanks and great appreciation to the great person Jawad Kadhim faris (Assistant professor in the department of pharmacology, collage of veterinary medicine-university of Al-Qasim green)who included the researcher with his knowledge and his creation and sponsorship, his spared no effort in his guidance and support, since the research was an idea till it was completed in its final form. and that meant something but to demonstrate the greatness of father who cares with students and guide them to the path of survival and success. I wish to express my deepest thank to the deanery of the College of Veterinary Medicine for their assistances. and I extended my thanks to my family.

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