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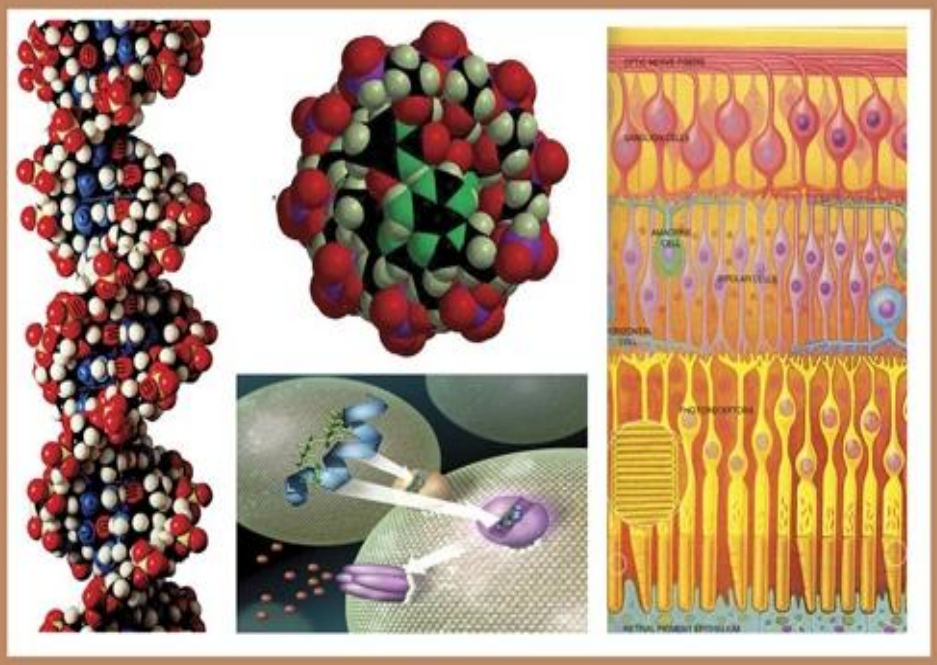

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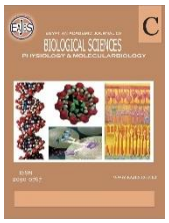

Ameliorative Effect of The Essential Oil of *Syzygium aromaticum* in Wistars Rats Exposed to Aluminum Chloride

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ABSTRACT

The objective of this study, on the one hand, is to evaluate the impact of aluminium intoxication, on the biochemical and hematological approaches of adult Wistar rats, on the other hand, to test the efficacy of *Syzygium aromaticum* essential oil (CEO) in restoring or not the harmful effects of the studied metal with a daily intraperitoneal injection of 0.1 ml CEO/kg. The extraction of the essential oil from *S.aromaticum* by hydro-distillation allowed us to obtain CEO with a yield of 10.18%. Chronic exposure to aluminum chloride at a concentration of 100mg/kg in rats decreased body and organ weights (Liver, Kidney) compared to controls. The intoxication also revealed a disruption of various biochemical parameters, including liver (TGO, TGP), and kidney (urea, creatinine) biochemical assays, Thus, the concentration of aluminium in the intoxicated group is high in the blood. Also, the results show a correction in the values of these parameters following the administration of CEO compared to those of exposed animals. CEO also corrected the shape of the affected blood cells after intoxication to aluminium.

INTRODUCTION

Aluminum is ubiquitous in our ecosystem and its toxicity induces deleterious effects in various living organisms (Tair, 2017). It is known for its toxic effects on different organs, such as the liver, brain, bone, kidneys and blood (Ozkaya *et al*., 2010). Al can only induce its effects once in the body, with oral bioaccessibility being the most important; it is represented by the soluble fraction of a substance in the gastrointestinal system available for absorption, elimination, or accumulation (Tair, 2017).

For decades, the clove (*Syzygium aromaticum*), has been used for its culinary and medicinal virtues. Since then, other properties have been discovered, such as anti-inflammatory, antibacterial, neuroprotective, anesthetic, hepatoprotective, anti-anaphylactic and antioxidant and anti-anaphylactic and anticandidal properties (Dashti et Morshedi, 2009) (Adli *et al*.,2018) (Yassin *et al*.,2020)**.** Thus, the influential role of cloves as inhibitors of various degenerative diseases is attributed to diverse chemical constituents in high concentrations with antioxidant activity (Astuti *et al*.,2019) (Batiha *et al*.,2020).

Chromatographic analyses of Syzygium aromaticum essential oils identified 26 compounds which mainly represent approximately 80.83% eugenol, 10.48% eugenol acetate, 7.21% β caryophyllene (Adli *et al.*,2017)**.**

In light of these studies, our aim is to investigate the effect of the essential oil of the Syzygium aromaticum plant on aluminium chloride intoxication in adult Wistar rats, according to biochemical and hematological approaches.

MATERIALS AND METHODS

Extraction of the Essential Oil:

The researcher realized the present study using the *Syzygium aromaticum* (clove), imported from Indonesia (the Moluccan Island). This plant is widely used in Algeria because of its importance in the Algerian culinary tradition, and its use as a herbal medicine. The plant is available at the market all year. The dry and ripe seeds of *Syzygium aromaticum* were purchased from the local herb market in Saida (Algeria) and were identified and authenticated by an expert taxonomist. A voucher specimen code P-200676 was deposited at the herbarium of the Department of Biology at Saida University (Algeria). The part of the plant used to extract the essential oil is the flower buds of *S.aromaticum*. The extraction of essential oil has been done by hydro-distillation

Yield Calculation:

The yield of essential oil (EO) is the ratio between the weight of the extracted oil and the weight of the plant material used (Hadj Ammar et al.,2009; Adli et al., 2017). It is calculated as a percentage by the following formula: Y (%) = (M1 /M0) ×100

Y: essential oil yield.

M1: quantity of oil extracted expressed in grams (g); M0: quantity of dry matter used for extraction expressed in grams (g).

Experimental Animals:

Experiments were carried out on adult Wistar rats (obtained from the Department of Biology, Faculty of Sciences, University of Saida) weighing 380.00 ± 5.84 g. The animals were housed with free access to water and food in an animal room, with a 12/12-hour light/dark cycle, at 22 ± 2 °C.

Experimental Protocol:

The rats were divided into four groups (n=7 rats): control lot, intoxicated (Al), control treated with the essential oil (Control+CEO), and intoxicated treated with the essential oil (Al+CEO). The number and suffering of animals were minimized by the guidelines of the European Union Directive (2010/63/EU).

Group Al: The rats were intoxicated with an oral dose of 100mg/kg AlCl3 (El-Nahrery, 2015).

Group Al+CEO: intoxicated rats that received an intraperitoneal injection with 0.1 ml/kg for 21 days (Halder *et al.,* 2011; Adli *et al.,* 2018)

Group Control: Control rats received distilled water only.

Group Control+CEO: rats that received only intraperitoneal injection with 0.1 ml/kg for 21 days (Halder *et al.,* 2011; Adli *et al.,* 2018).

At the end of the experiment, the animals were sacrificed after 12 hours (morning fast), by IP injection with a solution of chloral (C2H3C13O2) to 10%. After the incision of the abdomen, blood is collected by cardiac puncture in heparin tubes for biochemical analysis and EDTA and tubes for hematological evaluation and dry tubes for blood smears.

Evolution of Body Weight and Organ Weights:

The follow-up of the adult rats required weekly weighing of body weight throughout the experimental period. Liver and kidney weights for the different groups are recorded after sacrifice.

Biochemical Assays:

Determination of the Level of Aluminium in The Blood:

The samples of total blood (100 µl) are recovered in a hemolysis tube of 5 ml containing 100 µl of triton at 0.1%. After using a vortex for 30 seconds, 600µl of HNO3 (1M) is added to deproteinize. The

agitation of the hemolysis tube in the vortex is followed by a 10-minute break at ambient temperature. After centrifugation for 10 minutes at 3000 turns/min,the samples are transferred into cups for the determination of the level of aluminium by using atomic absorption spectroscopy (SHIMADZU AA-7000).

Determination of the Kidney and Liver Parameters:

The concentrations of kidney markers activities, urea, and creatinine were measured using kits (Chronolab, Spain) according to Kaplan, (1984) and creatinine according to Murray, (1984).

The serum samples were used to measure the activity of alanine aminotransferase (ALT: TGP), and aspartate aminotransferase (AST: TGO) according to the methods of Reitman *et al.* (1957)

Blood Glucose Determination:

Blood glucose testing was done according to the protocol included with the kit (SPINREACT).

Statistical Analysis:

Results were expressed as mean ± standard error of the mean (SEM). Data were analyzed by the two-way analyses of variance (ANOVAs). When a significant difference was found, the StudentNewman-Keulsposthoc test was conducted. For all analyses, a difference was considered significant at $p \leq 0.05$.

RESULTS

Yield of CEO:

The hydro-distillation method allowed us to obtain an essential oil with a yield of about 10.18%.

Evaluation of Body Weight and Organ Weights:

Body weight results show that animals exposed to $AlCl_3$ (Al) showed a significant ($p<0.001$) decrease in body weight compared to control animals. Animals exposed to $AlCl_3$ and treated with CEO (Al+CEO) showed a significant ($p<0.01$) increase in body weight compared to untreated intoxicated rats.

The results found in the poisoned animals also showed a significant ($p<0.001$) decrease in organ weights (liver and kidney) compared to controls (Table 1). On the other hand, animals treated with CEO showed a significant ($p<0.001$) increase in organ weights compared to the poisoned rats.

Table 1: Evaluation of weight parameters of control, Conrol+CEO, Al, Al+CEO rats.

lots	Body weight (g)	Liver weight (g)	Kidney weight(g)
Control	380.00 ± 5.84	13.98 ± 0.42	2.17 ± 0.06
Control+CEO	372.29 ± 3.60	13.77 ± 0.12	2.19 ± 0.05
Al	295.43 ± 5.13***	9.29 ± 0.35***	2.05 ± 0.07
Al+CEO	320.29± 5.93***	11.01 ± 0.23***	2.13 ± 0.06

The values are expressed in mean ± EMS (***: $p<0.001$).

Biochemical Assays:

Determination of the Level of Aluminium in The Blood:

The results obtained in the determination of blood aluminum using atomic absorption spectrophotometry (AAS) show that the serum $AlCl_3$ concentration was significantly elevated compared to control rats ($P<0.001$). In addition, intraperitoneal administration of EO of "*Syzygium aromaticum*" in intoxicated rats resulted in a significant decrease in blood aluminum levels compared to the intoxicated rats ($P<0.001$) (Table 2).

Table 2: Blood Aluminum Levels in Rats: Controls, Al and Al+CEO rats.

Group	Blood Aluminum (µg. L-1)
Control	1.54±0.025
Al	14.3±0.034 ***
Al+CEO	11.25±0.01***

The values are expressed in mean ± EMS (***: p<0.001).

Blood Glucose Determination:

The results (Fig.1) of the assay showed that glucose levels were significantly elevated in $AlCl_3$-intoxicated rats (P<0.001) compared to control rats. In addition, a significant decrease(P<0.01) in glucose levels was observed in intoxicated CEO-treated rats compared to the intoxicated rats.

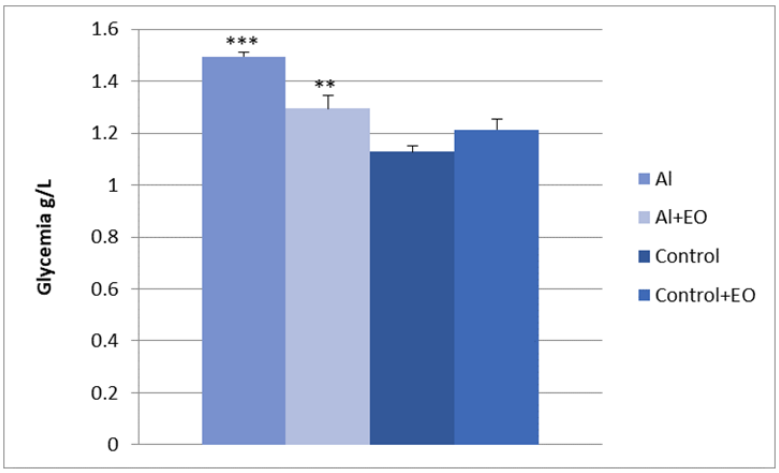
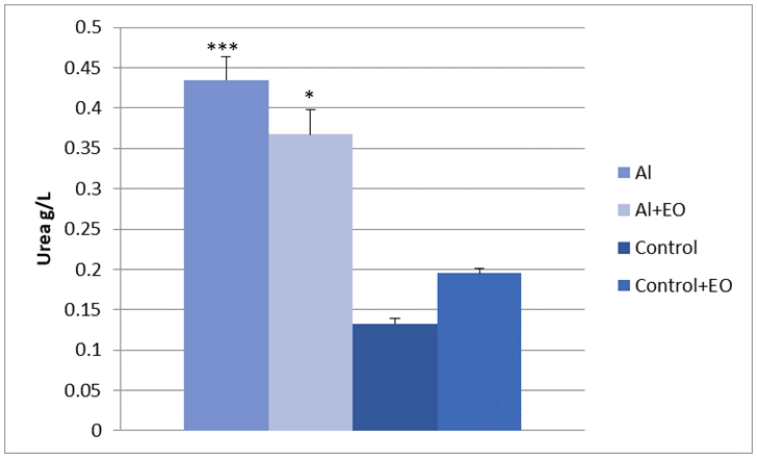


Fig. 1: Blood glucose levels in control, treated control, AlCl3 intoxicated and CEO-treated rats. Values are expressed as mean ± EMS: ***: P<0.001, **: P<0.01.

Exploration of Renal Function:
Determination of Uremia:

The results (Fig.2) indicate a significant increase in urea values in the group of animals that were exposed to AlCl3 compared to the control group (P < 0.001). However, the intoxicated and treated lot (Al+CEO) showed a significant decrease in urea values (P < 0.05).



Fig; 2: Plasma urea levels in rats: controls, treated controls, $AlCl_3$ intoxicated, intoxicated CEO-treated rats. Values are expressed as mean ± EMS: ***: P<0.001, *: P<0.05.

Determination of Creatinine:

Creatinine values (Fig.3) were significantly elevated in rats exposed to $AlCl_3$ compared to control rats ($P < 0.001$). There was a non-significant decrease in creatinine levels between intoxicated CEO-treated (Al+CEO) and intoxicated untreated (Al) batches.

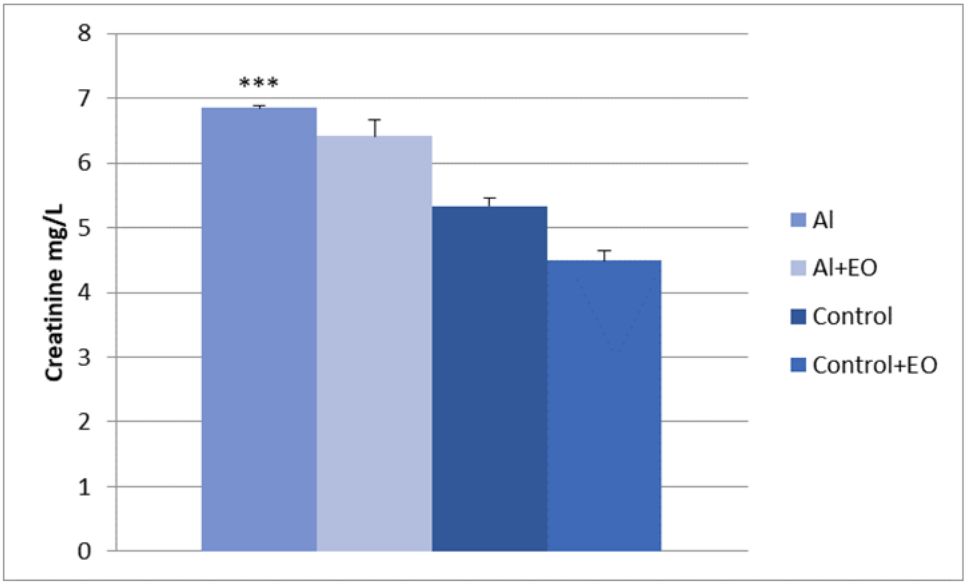


Fig. 3: Comparison of creatinemia concentration between rats: controls, treated controls, AlCl3 intoxicated, intoxicated CEO-treated rats. Values are expressed as mean ± EMS : ***: P<0.001.

Exploration of Liver Function:

Determination of Transaminases (ASAT-ALAT):

The transaminase assay (Figs.4 &5) revealed a significant increase in the levels of ASAT and ALAT in AlCl3 intoxicated animals compared to control rats (P<0.001); however, serum levels of ASAT and ALAT in intoxicated animals treated with CEO showed a significant decrease compared to those in intoxicated animals (P <0.001).

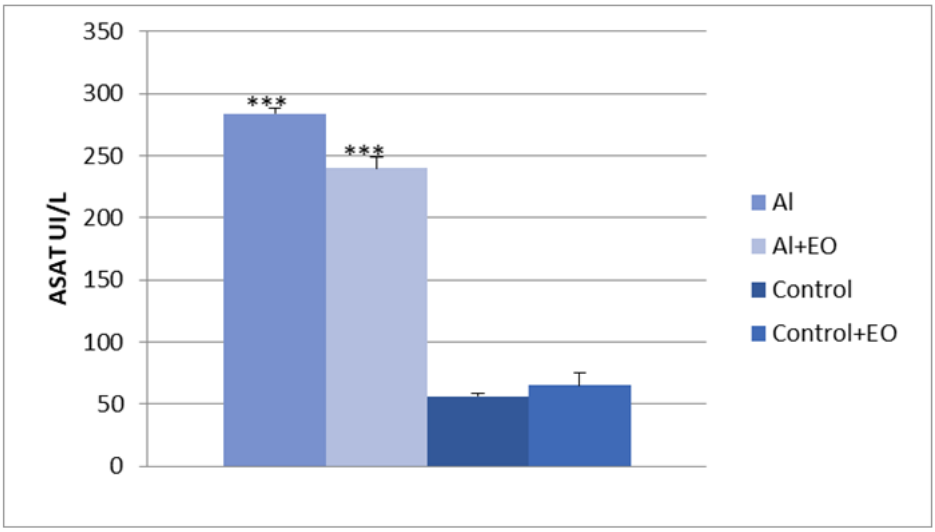


Fig. 4: Transaminase levels (ASAT) in rats: controls, treated controls, $AlCl_3$ intoxicated, intoxicated CEO-treated rats. Values are expressed as mean ± EMS: ***: P<0.001.

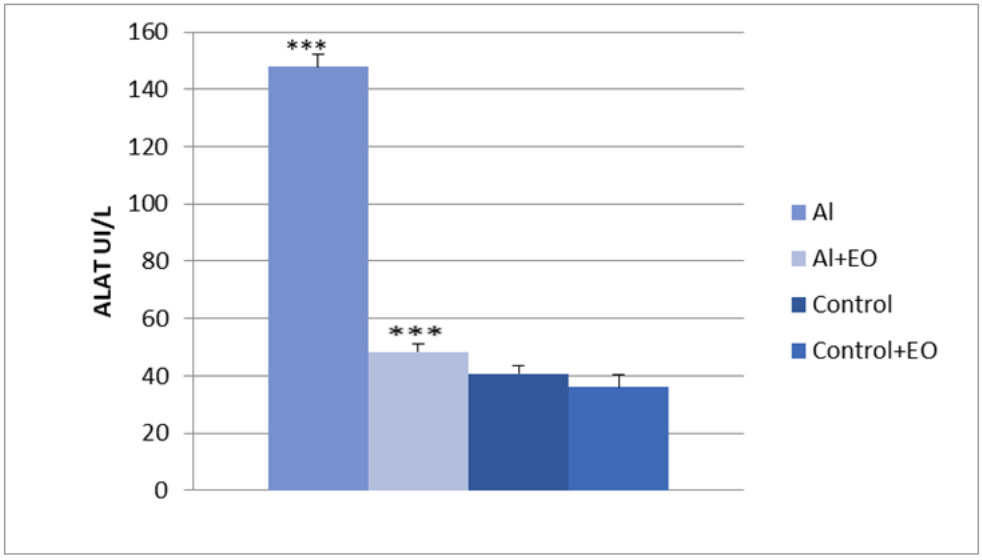


Fig. 5: Transaminase levels (ALAT) in rats: controls, treated controls, $AlCl_3$ intoxicated, intoxicated CEO-treated rats. Values are expressed as mean ± EMS: ***: P<0.001

DISCUSSION

Body and organ weight results showed that AlCl 3 intoxication significantly reduced body weight and liver and kidney weights in rats compared to control rats. These results are similar to the studies of Arokiasamy *et al*, (2015); Kaddour *et al*, (2016).

Azzaoui *et al*. (2008) worked on female rats intoxicated with aluminum nitrate (80 mg/l in the drink) for 3 months. They found that aluminum decreased the total weight of the rats, which was accompanied by a reduction in water consumption. On the other hand, this decrease in weight can be explained by the effect of aluminium on the brain and kidneys, which control water consumption behavior. On the other hand, attenuation of serum triglycerides and mitochondrial energy metabolism following exposure to different aluminum salts may result in decreased body weight in rats (Buraimoh et Ojo, 2014). The majority of studies that used chronic doses of aluminum reported significant weight reduction (Kaizer *et al*., 2005). The decrease in total and relative organ weights is due to the toxicity of the aluminum that accumulated in the organs (Anand *et al*., 2012). Aluminum exposures may influence food intake, gastrointestinal tract and intestinal food absorption in rats, leading to growth retardation (Lukyanenko *et al*., 2013; Allagui *et al*., 2014).

Administration of *S. aromatic* essential oil to rats exposed to AlCl3 resulted in a marked increase in body gain compared to animals exposed to AlCl3 and untreated. This recorded weight gain could be due to the presence of terpenoid compounds that act by stimulating glucose transport in cells (Vinay-Dwivedi *et al*., 2011). Our results are in agreement with those of Adli *et al*, (2018) who noted an increase in body weight in rats exposed to lead and treated with clove essential oil compared to untreated exposed rats, and with those of Anbu et Anuradha, (2012) who indicated that eugenol may suggest a beneficial effect to correct weight loss in animals. Lobstein *et al*., (2017) indicated that Clove and its EO can be used in cases of difficult digestion because they increase gastric emptying. Clove facilitates digestion by acting on peristalsis and rebalancing the intestinal flora (Tajuddin *et al*.,2004).

After entering the body, aluminum is carried through the bloodstream to the organs. Our results showed that AlCl 3 intoxication of rats for 90 days shows the presence of aluminum in the blood compartment. It is established that the majority of the aluminium is found in the plasma (90%) and the rest is located in the

erythrocytes (10%) (CUNAT, 1999). 80% of aluminium is non-dialysable, which means it is bound to plasma proteins such as transferrin and albumin. The remaining 20% is soluble and ultra-filterable and forms low molecular weight chemical species (Savory et Wills, 1984; Harris, 1992; Gardiner *et al*., 1984).

Most studies suggest that the removal of toxic metals from the human body can be a useful tool to prevent the onset or progression of many diseases related to metal poisoning (Fulgenzi *et al*., 2014). Flavonoids are effective metal ion chelators and form stable products with beryllium, aluminum, iron and zinc ions (Pavun *et al*., 2012). Treatment with clove essential oil from AlCl 3 intoxicated rats decreased blood aluminum levels, which may be due to its chelating property.

Exposure of adult rats to aluminum chloride-induced a significant increase in blood glucose levels in comparison to the blood glucose levels of control rats. These results are in agreement with the results of Belaïd-Nouira *et al* (2012); Taïr (2017) where blood glucose levels were higher in aluminum-intoxicated rats than in control rats. This means that chronic administration of aluminum leads to dysfunction of energy metabolism, this hyperglycemia is probably the consequence of hepatic and muscle glycogenolysis to cover energy requirements (Kasdallah *et al*., 2005). These results are not consistent with those of Kowalczyk *et al*. (2004) who found that aluminum caused a decrease in blood glucose levels in poisoned rats compared to control rats. On the other hand, the observations obtained in terms of blood glucose levels in animals exposed to Al and treated with CEO indicate that the glucose level is similarly decreased in the intoxicated rats. This can be explained by the effect of S.aromaticum essential oil which reduces the blood glucose level. These results are corroborated by the work of Minpei *et al*, (2012) who demonstrated that CEO has beneficial effects against diabetes. Akila *et al* (2018) showed that clove essential oil could reduce the diabetes-mediated increase in serum glucose and glycated hemoglobin (HbA1c) levels.

Results showed elevated serum urea and creatinine levels in $AlCl_3$-exposed rats compared to control rats. Several authors report similar results (shilpi *et al*., 2009) (Mohammadirad *et* Abdoallahi, 2011) (Silpa, 2014) (Al-Qayim et Mashi, 2014) (Kalaiselvi *et al*., 2015)**.**, According to Soudani *et al***.,** the increase in creatinine and urea concentrations indicates that the glomeruli and tubules are damaged. The explanation for these results may lie in the notion of bioaccumulation of aluminum at the level of different organs (Afifi,2002) (Chen *et al*., 2002) (Platt, 2001) (Exley *et al*.,1996)**.** Particularly in the kidneys (Kowalczyk *et al*.,2004).

On the other hand, after treatment with CEO in intoxicated rats, there was a decrease in urea and creatinine, our results are in accordance with the work of Adli *et al*, (2018) who evaluated that CEO has a protective effect against hepato-nephrotoxicity.

Bakour *et al***, (**2018**)** also found that urea and creatinine levels were decreased in H2O 2intoxicated rats treated with *Syzygium aromaticum* essential oil.

The efficacy of CEO in the kidney may have been due to the presence of chelating compounds such as eugenol (Nassar *et al*., 2007; Said, 2011). Said, 2011 also showed that eugenol reduces gentamicin-induced tubular necrosis in the kidneys and offers protection against renal failure by acting as an antioxidant.

The results showed that exposure of rats to AlCl 3 induced a significant increase in transaminase enzyme activity in the poisoned group compared to the control group. Oral administration of aluminum in the studies by Yeh *et al*. (2009); Shrivastavas (2012); Kalaiselvi *et al*. (2015); Tahari *et al*. (2016) induced an increase in transaminase activity (ALAT and ASAT) in rat plasma.

In addition, there was a significant decrease in hepatic parameters following CEO administration in intoxicated rats compared to those of the intoxicated. These

results are consistent with those of Anbu et Anuradha, (2012) who reported that eugenol decreases plasma transaminases, and with Bakour *et al*, (2018) who reported a decrease in AST and ALT in H2O2 intoxicated rats. In rats with hepatic dysfunction, daily intake of EO or an EO- containing emulsion of cloves or a microemulsion of eugenol improved these symptoms in all cases (Al-okbi *et al*., 2014).

Conclusion:

Our study revealed that infection of adult Wistar rats to aluminium chloride caused biochemical changes. In addition, our data showed that *Syzygium aromaticum*essential oil could restore these damages.

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