Impact of essential oils of clove *Syzygium aromaticum* in rats exposed to stress by nickel

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**Abstract**

The mechanisms that cause nickel (Ni) toxicity are multiple and potentially affect all cells in the body. For this purpose, we were interested in the oxidative stress induced by the Ni at the erythrocyte level at a dose of 2 g/l during the gestation and lactation period in the Wistar rats and the capacity of the clove essential oil, *Syzygium aromaticum*, (CEO) to restore or not this state of stress. Extraction of the CEO by hydro-distillation allowed us to obtain a CEO with a yield of 10.60% and the characterization of this essential oil by GC-MS indicates that the major components of this oil are: Eugenol (80.95%), eugenyl acetate (10.48%), β-caryophyllene (7.21%) and α-humulene (0.87%). The antioxidant activity of the CEO made in *vitro* showed a free radical scavenging capacity DPPH with an IC 50 of the order of 25.60 µg/ml. In addition, analysis of the erythrocyte antioxidant status indicated that Ni significantly increased the enzymatic activities of catalase (CAT) and superoxide dismutase (SOD) and significantly decreased the enzymatic activity of glutathione peroxidase (GPx), resulting in dysfunction of the antioxidant defense system. On the other hand, the administration of CEO by intraperitoneal (IP) over a period of 21 days to rats previously intoxicated with Ni, indicates that this CEO contributes significantly in improving defenses against free radical aggression, through a recovery. At the level of antioxidant enzyme activities by increasing their abilities to eliminate radical compounds.

**Keywords:** antioxidant activity; GC-MS; nickel; oxidative stress; *Syzygium aromaticum*

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**Introduction**

Nickel (Ni) is a natural element of the earth's crust and is widely distributed in our environment; therefore, it is present in food, water, earth, and even in the air (Bennett, 1984). The increase in the modern industrial application of this element has raised considerable concern, especially its adverse effects on human health (He Min-Di et al., 2011; Obone et al., 2012). (Ni) is a carcinogen responsible for lung cancer in the
workplace. In animals, it accumulates in the kidneys and induces lesions in the glomeruli and proteinuria. (Ni) exposure can also affect the immune system, neurological, renal, endocrine and fertility, as well as placental functions. Studies have shown that Ni leads to lipid peroxidation, which would be related to the production of hydroxyl radicals. It can thus alter the antioxidant status of the cell (Hfaiedh et al., 2011; Das et al., 2008).

Since ancient times clove (Syzygium aromaticum) has been used as a powerful stimulant of the nervous system, analgesic, carminative, antispasmodic, hypotensive and potentially neuroprotective (Goetz et al., 2010), antioxidant and hepatoprotective and immune stimulant (Lee et al., 2001; Shekhar et al., 2018; Radünz et al., 2019).

As a result, our work aims to evaluate the antioxidant effect of the essential oil of Syzygium aromaticum plant on chronic nickel poisoning in Wistar rats during the developmental period (gestation and lactation).

Materials and Methods

Extraction and determination of the chemical composition of the essential oil by GC-MS

Clove essential oil (CEO) was extracted by hydro distillation; 50 g of ground material was put in 500 ml of distilled water, the whole is brought to the boil for a period of 5 to 6 hours (Adli et al., 2014). The qualitative and quantitative analysis of this essential oil was carried out by Varian Chrompack-CP 3900 gas phase chromatography by injection of 0.2 µl of extract. The vector gas used is helium (He) with a flow rate of 0.3 ml/min. The column used is a capillary column of type VF5 (stationary phase nature: 5% phenylpolysiloxane and 95% methyl), 30 m long and 0.25 mm inside diameter. The thickness of the stationary phase is 0.25 µm; the programming of the temperature of the initial injection column is 70 °C for 2.50 minutes, and then proceeds in increments of 15 °C/min at 255 °C for 20 minutes; the detector used for this analysis is of mass spectrometry type (Saturn 20200), with a temperature of 250 °C. The device is driven by a computer with appropriate software for this kind of analysis and a NIST database (1A) (Version 2.0) that allows the identification of compounds.

Antioxidant activity

DPPH radical scavenging activity

DPPH• (2,2-diphenyl-1-picrylhydrasyl) is generally the most widely used substrate for rapid and direct evaluation of antioxidant activity due to its stability in free radical form and the simplicity of the analysis. At room temperature, the DPPH• radical exhibits, in alcoholic solution, an intense violet coloration which disappears on contact with a proton-donating substance. This discoloration highlights the antioxidant power of a sample by its ability to trap the free radical and results in a decrease in absorbance at 517 nm.

For the measurement of the activity, a test sample of 1 ml of extract at different concentrations is placed in the presence of 250 µl of a solution of DPPH• (0.2 mM, prepared in methanol). The mixture is placed for 30 min in the dark to react and the absorbance is measured at 517 nm against a negative control (without extract) (Prieto et al., 1999).

The results are expressed as a percentage of inhibition, calculated following the decrease in the intensity of the coloring of the mixture, according to the formula:

\[ PI = \left( \frac{A \text{ control} - A \text{ extract}}{A \text{ control}} \right) \times 100. \]

PI: percent inhibition; A control: negative control absorbance; A extract: absorbance of the extract. The study of the variation in anti-free radical activity as a function of the concentration of the extracts makes it possible to determine the concentration which corresponds to 50% inhibition (IC50). A low value of IC50 corresponding to a high efficiency of the extract.
**Distribution of groups**

The experiments are performed on Wistar rats, weighing 200 to 400 g. The rats are grouped in cages with 2 females and one male. They are placed in a ventilated animal house at a temperature of 21 °C ± 1 °C with artificial lighting that establishes a day/night cycle (day between 7 and 19 h). On the first day of gestation, females are divided into two lots:

- **Lot Ni**: consisting of animals receiving nickel sulphate at a rate of 2 g / l in distilled water orally from the first day of pregnancy until weaning. (n = 10 male rats) (Kahloul et al., 2009).
- **Lot T**: consisting of animals receiving distilled water orally without nickel sulphate. (n = 10 male rats). The poisoning of females begins on the first day of cohabitation with the male rats which is represented by d 0 and continues during the period of gestation. Newborns are also exposed to Ni until weaning (21 days after birth). The offspring is subjected to the same conditions of experimentation;
- **Lot Ni-C:EO**: 24 hours after weaning the animals are treated with CEO (0.1 ml/kg) (Ni: n = 10) with one intra-peritoneal injection per day for 21 days.
- **Lot T**: weaned animals receive distilled water orally and intra-peritoneal injection (IP) of CEO (0.1 ml / kg) daily for 21 days.

**Measurement of the activity of antioxidant enzymes**

Blood samples were centrifuged at 3000 rev/min for 15 min. The serum was carefully removed and the remaining base containing the erythrocytes was washed three times with 0.9 g/l saline solution and then lysis of the cells was performed by adding ice-cold distilled water and incubating for 15 min in the ice. The lyate is then recovered for the determination of antioxidant enzymes erythrocyte markers of the oxidizing status after centrifugation at 5000 rev/min for 5 min (Rotruck et al., 1973).

Activities and levels of erythrocyte antioxidants at the erythrocyte level such as superoxide-dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were analyzed by the method of Sinha (1972), and Rotruck et al. (1973) and Kakkar et al. (1984).

**Expression and statistical analysis of the results**

The results are expressed as the mean (M) of the individual values, assigned from the standard error to the mean (SEM). The comparison of several means is carried out by an analysis of variance (one-way ANOVA) with the factor intoxication (Ni, T). Repeated measures ANOVA were used for time factor analysis. A probability p less than 0.05 is considered significant. Statistical analyzes were performed with Sigma Stat software (SPSS Inc., Chicago, IL, USA).

**Results and Discussion**

**Yield of essential oil and main compounds**

The EO of *Syzygium aromaticum* was obtained after several hydro distillation cycles yield of 7.49%, which is in disagreement with the work of (Wenqian et al., 2006) which gave a yield of 11.50%. This difference in yield can be attributed to several factors, mainly the origin, species, harvesting period, drying time and the technique used to extract essential oils (Loziene et al., 2005).

The chromatographic analyses of the essential oils of *Syzygium aromaticum* made it possible to identify 26 compounds which mainly represent approximately 80.95% for Eugenol and 10.48% for eugenyl acetate 7.21% β-caryophyllene. These results corroborate with those of (Chaib et al., 2007) which show that eugenol, acetate-eugenyl and β-caryophyllene are the major components of this oil (Table 1).
Table 1. Concentration in % and retention time of the different compounds obtained by gas chromatographic analysis of the essential oil of *S. aromatcum*.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>RT*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eugenyl acetate</td>
<td>89.6</td>
<td>10.48</td>
</tr>
<tr>
<td>2</td>
<td>Eugenol</td>
<td>85.3</td>
<td>80.83</td>
</tr>
<tr>
<td>3</td>
<td>β-Caryophyllene</td>
<td>53.4</td>
<td>7.21</td>
</tr>
<tr>
<td>4</td>
<td>α-Humulene</td>
<td>57.8</td>
<td>0.87</td>
</tr>
<tr>
<td>5</td>
<td>Caryophyllene oxide</td>
<td>76.7</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>α-Cubebene</td>
<td>43.4</td>
<td>0.02</td>
</tr>
<tr>
<td>7</td>
<td>α-Copaene</td>
<td>46.0</td>
<td>0.03</td>
</tr>
<tr>
<td>8</td>
<td>Isocaryophyllene</td>
<td>51.6</td>
<td>0.02</td>
</tr>
<tr>
<td>9</td>
<td>Aromadendrene</td>
<td>54.2</td>
<td>0.01</td>
</tr>
<tr>
<td>10</td>
<td>α-himachallene</td>
<td>58.8</td>
<td>0.02</td>
</tr>
<tr>
<td>11</td>
<td>Zonarene</td>
<td>57.0</td>
<td>0.02</td>
</tr>
<tr>
<td>12</td>
<td>γ-himachallene</td>
<td>58.8</td>
<td>0.01</td>
</tr>
<tr>
<td>13</td>
<td>Germacrend</td>
<td>50.4</td>
<td>0.01</td>
</tr>
<tr>
<td>14</td>
<td>β-himachallene</td>
<td>60.9</td>
<td>0.01</td>
</tr>
<tr>
<td>15</td>
<td>Isomeric selinadiene</td>
<td>61.2</td>
<td>0.01</td>
</tr>
<tr>
<td>16</td>
<td>α-Farnesene</td>
<td>61.9</td>
<td>0.02</td>
</tr>
<tr>
<td>17</td>
<td>Geraniol</td>
<td>67.5</td>
<td>0.03</td>
</tr>
<tr>
<td>18</td>
<td>Calamene</td>
<td>67.6</td>
<td>0.01</td>
</tr>
<tr>
<td>19</td>
<td>Sesquiterpene epoxide</td>
<td>72.8</td>
<td>0.01</td>
</tr>
<tr>
<td>20</td>
<td>Isomeric epoxide</td>
<td>73.9</td>
<td>0.01</td>
</tr>
<tr>
<td>21</td>
<td>Caryophyllene oxide</td>
<td>76.7</td>
<td>0.08</td>
</tr>
<tr>
<td>22</td>
<td>Methyl eugenol</td>
<td>76.9</td>
<td>0.01</td>
</tr>
<tr>
<td>23</td>
<td>Epoxy-6.7 Humulene</td>
<td>79.7</td>
<td>0.01</td>
</tr>
<tr>
<td>24</td>
<td>Sesquiterpenol</td>
<td>79.9</td>
<td>0.01</td>
</tr>
<tr>
<td>25</td>
<td>Cubenol</td>
<td>80.6</td>
<td>0.01</td>
</tr>
<tr>
<td>26</td>
<td>Chavicol</td>
<td>92.7</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**Group of constituents (%)**

| Phenols                        | 80.93 |
| Sesquiterpene hydrocarbons     | 8.56  |
| Esters                         | 10.48 |
| Terpenic alcohol               | 0.03  |

**Total identified (%)** 100

The antioxidant activity of the essential oil

The antioxidant activity of CEO with respect to the DPPH radical was evaluated spectrophotometrically in function of its reduction by a shift from the violet colour to the yellow colour measurable at 517 nm. (Figure 1), which indicates a proportional relation between the percentage of reduction of the free radical and the concentration of EO in the reaction medium.

Results on the antioxidant power of CEO showed a potential antioxidant activity that could reach 87.03%. Gülcin *et al.* (2004), achieved antioxidant potency that was close to 83.6% for CEO. In the same context, the IC50 value of this CEO is 25.60 µg/ml, these results are consistent with those of Gülçin *et al.* (2011) which reported a value of 21.50 µg/ml.

The antioxidant activity of CEO is mainly due to the presence of bioactive components in the tested EO (Vermerris *et al.*, 2006). It also seems that this activity is related to the presence of phenolic compounds in the Essential oil. The main role of compounds as reducing free radicals is emphasized in several reports (Villano *et al.*, 2007). Eugenol, which is a major component of our essential oil with a concentration of 80.83%, has a
strong antioxidant activity (Ogata et al., 2000). However, other studies show that the anti-radical activity is correlated with the rate of polyphenols and flavonoids in extracts of medicinal plants (Mariod et al., 2009). The mechanism of the reaction between the antioxidant and the DPPH radical depends on the structural conformation of the antioxidant (Kouri et al., 2007). The latter reacts with the DPPH radical by reducing a number equal to the hydroxyl groups carried by the antioxidant molecule (Bondet et al., 1997). Furthermore, it is not only the major components of CEO that are responsible for this antioxidant activity, but there may also be other minority compounds that can interact in a synergistic or antagonistic way to create an effective system for free radicals (Gulcin et al., 2004).

![Graph showing percentage of inhibition vs concentration (μg/ml)](image)

**Figure 1.** Percentages of reduction of the free radical DPPH by CEO

*Activity of enzymes with oxidative status at the erythrocyte level*

After (Ni) exposure, the activity of SOD and CAT was significantly higher (p < 0.05), whereas GPx was significantly decreased in intoxicated rats compared to erythrocyte control rats. After 21 days of treatment with CEO, there was a marked improvement in the activity of these enzymes in treated rats compared to intoxicated rats (Table 2).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>T</th>
<th>CEO</th>
<th>Ni</th>
<th>Ni-CEO</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT (U/mg protein)</td>
<td>10.51±0.58</td>
<td>10.66±0.7</td>
<td>13.84±1.02*</td>
<td>11.61±0.79*</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>15.89±0.067</td>
<td>15.94±0.02</td>
<td>10.43±0.57*</td>
<td>7.46±0.62*</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>18.70±0.37</td>
<td>19.73±0.8</td>
<td>24.73±1.85*</td>
<td>21.73±0.51*</td>
</tr>
</tbody>
</table>

*Values are expressed as an average ± SEM (*; p<0.05).*

Oxidative stress is defined as a pronounced imbalance between antioxidant and oxidative elements in favour of the latter and their harmful effects. The origins of oxidative stress are multiple and result from the formation of reactive oxygen species (ROS) within the body (De Moffarts et al., 2005). Moreover, the evaluation of the toxicity of nickel sulfates was expressed by the determination of erythrocyte antioxidant enzymes namely CAT, GPx and SOD. Knowing that these antioxidant enzymes make it possible to maintain the homeostasis of the redox potential (Chou et al., 2013).

At the erythrocyte level, an experiment focusing on the demonstration of the oxidative status following (Ni) intoxication during pregnancy and lactation was developed. The data provided by the literature allowed us to focus on the first dose of the activity of the CAT which after having determined its concentrations at the
erythrocyte level, it was noted that the (Ni) intoxication significantly increased the concentration of this enzyme. This result is clearly in agreement with the work of Martin-Mateo et al. (1997) which attributed to this increase in activity following an increase in the blood Ni level accompanied by overexpression of CAT. In addition, Das et al. (2007) reported similar erythrocyte level results in adult rats given the intraperitoneal (IP) NiSO₄ solution.

On the other hand, CAT can partially reduce the oxidants induced by the elevation of (Ni). It also prevents the generation of hydroxyl radicals (Das et al., 2008).

In the same context, it was found that oral administration of (Ni) to developing rats caused a decrease in glutathione peroxidase (GPx) activity. This work is consistent with that of (Das et al., 2008; Wu et al., 2013) who reported that the (Ni) administration is causing a decline in GPx activity.

Nevertheless, (Saka et al., 2011) revealed that the decrease of this activity can lead to a state of oxidative stress, particularly in red blood cells.

Regarding the SOD assay, the results showed that Ni intoxication significantly increased the concentration of this enzyme at the erythrocyte level. This is on the one hand consistent with the work of (De Moffarts et al., 2005) who suggested that (Ni) plays an important role as a catalyst for SOD, with those of (Das et al., 2007) who have achieved similar erythrocyte adult rats that received the NiSO₄ solution by intraperitoneal (IP).

Our results showed that chronic (Ni) treatment caused significant erythrocyte oxidative damage, leading in the first place to lipid peroxidation, which is usually accompanied by a significant increase in the level of free radicals, which subsequently gives birth to a series of changes at the biochemical scale particularly affecting antioxidant enzymatic activity. However, these cellular disturbances were at the origin of the loss of the capacity to fight against the damages resulting from the radical aggressions. This has been confirmed by (Saka et al., 2011).

In other works, (Rioux, 2009) suggested that (Ni), being one of the heavy metals, has a high affinity with sulphydryl (-SH) groups, which allows it to easily activate sulfur-containing antioxidants.

In addition, administration of intraperitoneal CEO at the 0.1 ml/kg dose showed a significant increase in CAT content and GPx activity in rats exposed to Ni. Our results are in agreement with (Shyamala et al., 2003; Ravi et al., 2004; Kouri et al., 2007) which indicate that gavage of the aqueous extract of clove for 14 consecutive days resulted in a significant increase in GSH content and GPx activity in rats previously intoxicated with aluminium.

Tasleem et al. (2012) show that catalase activity increases following the administration of the aqueous extract of clove flower buds.

In fact, the administration of a dose of 10.7 mg/kg of clove for 30 days in rats allowed a significant increase of the enzymatic activity of GPx, CAT and non-enzymatic plasma GSH compared to those treated with ethanol (Anbuet et al., 2012).

Conclusions

Exposure of Wistar rats during gestation and lactation to (Ni) revealed a dysfunction of the kidney and its antioxidant defense system, which results in significant alteration of the anti-free radical system represented by the different enzymes. Treatment with CEO in previously intoxicated rats leads to rehabilitation of this system and correction of these disorders.
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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References


