Assessing the Antioxidant Potential of Ginger Aqueous Extract on H2O2 Induced Oxidative Stress in Local Rabbits: A Comprehensive Study of Hematological Parameters

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Abstract

This research, that was carried out at the Technical Institute in Bakraju/ Sulaimaniyah Technical University, the aim was to investigate the impact of administering 0.5% hydrogen peroxide via drinking water as an oxidative stress inducer, as well as the effect of an antioxidant in the form of an aqueous extract of ginger tubers (at a dosage of 200 mg/kg), on the physiological characteristics of male domestic rabbits. A total of 48 rabbits distributed into four groups, each group consisting of 12 rabbits. The study lasted for 42 days, the groups were: a control group, a group receiving only hydrogen peroxide (0.05%), a group receiving only the ginger extract (200 mg/kg), and a group receiving both hydrogen peroxide (0.05%) and the ginger extract (200 mg/kg). The second group resulted in negative effects on blood indicators, including a decrease in the total number of RBCs, hemoglobin concentration, PCV, and platelets compared to the other groups. These effects were significant at a level of P≤0.05. However, the third group (200 mg/kg) of aqueous ginger tubers extract had a positive effect, improving all the previously mentioned indicators and significantly surpassing the control and second groups at a probability level of P≤0.05. Additionally, the use of the aqueous extract of ginger tubers in conjunction with hydrogen peroxide in the fourth group prevented or minimized the negative effects of oxidative stress caused by hydrogen peroxide on most of the blood indicators mentioned in the study.

Key words: Oxidative stress, Ginger Roots, Rabbits.


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Introduction

The imbalance between the concentration of naturally occurring antioxidants and the concentration of free radicals and oxidants within the body leads to oxidative stress, which is a significant contributor to the development and progression of various diseases. These diseases include but are not limited to heart disease, diabetes, liver disease, and arthritis, as well as cancer, as noted by [1]. Furthermore, oxidative stress has been identified as a key factor in the pathogenesis of neurodegenerative diseases, as observed by [2], and immune diseases, as indicated by [3], among other conditions.

In light of the significant role played by oxidative stress in the development of various diseases, recent research has focused on investigating the potential of antioxidants, particularly those derived from plants and medicinal herbs, in enhancing cellular function and protecting cells from oxidative stress. This is a result of the imbalance between the generation of oxidative species and the cellular antioxidant defense mechanism, as noted by [4], and has led to a growing interest in the beneficial effects of plant-derived antioxidants in preserving cellular integrity and function, as highlighted by [5].

Ginger, scientifically known as Zingiber officinale, has been utilized for centuries as a medicinal plant and a popular spice for culinary purposes due to its unique taste. Its various therapeutic properties have been recognized by ancient Chinese and Indian cultures, which used it to alleviate ailments such as nausea, headaches, rheumatism, and colds, as noted in [6]. Nowadays, ginger is widely recognized for its anti-inflammatory and allergy-reducing properties, as well as its ability to lower blood sugar levels, as studied by [7]. It is also used as an antidiarrheal agent, according to [8], and as an antulcer agent, according to [9]. [10] found that ginger was traditionally used as a folk remedy for weight loss, fat burning, and reducing triglycerides and cholesterol in human blood, as well as having antioxidant properties, as reported by [11] and [7]. Additionally, ginger has been utilized as an antioxidant and growth stimulant in animal and poultry feed, as stated in studies by [12] and [13]. Furthermore, some research suggests that ginger may improve growth performance, immune response, physiological state, and resistance to bacterial diseases and pH stress, as reported by [14] and [15].

Materials and methods

In this study, 48 male rabbits of local origin aged 5-6 months, were obtained from local markets and randomly allocated to four groups of 12 rabbits each. The rabbits were housed in cages of various sizes, with the breeding rabbits being housed in cages measuring 2×2 meters. All rabbits were kept under controlled environmental conditions with a temperature of 20-25 degrees Celsius and a lighting period of 12 hours light and 12 hours dark.

Preparation of Ginger Extract

The resulting powder was ground using an electric grinder. 100 grams of this powder was mixed in one liter of distilled water at a temperature of 20-25 °C. The mixture was then left to settle for an hour in a horizontal vibrator at medium speed before being allowed to settle for an additional hour. It was then filtered using WattMan Filter Paper No. 1 with a centrifuge operating at 3000 cycles per minute for 15 minutes. The resulting extract was dried in an incubator at 40 degrees Celsius to obtain a dry powder of...
ginger extract. For the experiment, 2 gm of this powder was dissolved in 10 milliliters of distilled water to make a stock solution with a concentration of 0.1 gm per milliliter. This concentration was then used to prepare the required concentrations of 100 mg/kg, 200 mg/kg, and 300 mg/kg.

**Feeding Experiment Rabbits**
Throughout the experiment, the rabbits were provided with an open supply of food and water. The rabbits were fed a specially formulated diet intended for breeding rabbits to meet their maintenance and growth requirements, as detailed in Table 1.

**Table 1. The components of the diet used in the experiment.**

<table>
<thead>
<tr>
<th>Primary Feed Material</th>
<th>%</th>
<th>Raw protein %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran</td>
<td>47</td>
<td>7.50</td>
</tr>
<tr>
<td>Local barley</td>
<td>38</td>
<td>3.60</td>
</tr>
<tr>
<td>Soybean meal (44% protein)</td>
<td>10</td>
<td>4.40</td>
</tr>
<tr>
<td>Animal protein (50% protein)</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Limestone powder</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Salt</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin and mineral mixture</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>%100</td>
<td>%16.5</td>
</tr>
</tbody>
</table>

The rabbits were fed using a typical grain that was intended for rabbit breeding to meet the growth and maintenance needs of the animal, as shown in Table 1. The feed and water were provided *ad libitum* for the duration of the experiment.

**Study Plan and Design of Experiments**
An experiment was conducted to investigate the impact of ginger tubers' aqueous extract on reducing oxidative stress by subjecting 48 adult male domestic rabbits to hydrogen peroxide in their drinking water. The rabbits were randomly divided into four groups, with each group consisting of 12 rabbits. The study focused on various physiological blood parameters, including total number of erythrocytes, total white blood cell count, hemoglobin, packed cell volume, platelets.

The group of animals used in this experiment was divided into four groups, as follows:
- The first group: (Control) negative control; this group was treated with 1 ml of distilled water.
- The second group: rabbits were treated with water with H2O2 added to it at a concentration of 0.5%.
- The third group: rabbits (200 mg/kg rabbit/day) was dosed with the aqueous extract of the tubers of the ginger plant.
- The fourth group: rabbits were dosed (200 mg/kg rabbit/day) with an aqueous extract of ginger tubers in addition to 0.5% of hydrogen peroxide by drinking water.

**Blood Collection**
At the conclusion of the experiment, blood samples were obtained from the rabbits. The animals were distributed evenly among the experimental groups prior to conducting the tests. Blood collection was performed by taking samples from the ear of each experimental animal, following a 12-hour fasting period [16].

**Result and Discussion**
Table 2 demonstrated that the total number of erythrocytes was significantly lower (P≤0.05) in the second group than in the third treatment, as well as in the control
treatment and fourth treatment. Conversely, all coefficients displayed a significant increase (P≤0.05) in comparison to the control treatment regarding the quality of the total number of leukocytes.

Regarding the hemoglobin characteristic, statistical analysis indicated that the third treatment, utilizing an aqueous extract of ginger at a dose of 200 mg/kg, exhibited a significantly higher (P≤0.05) level of superiority compared to all other treatments, including the control treatment and the fourth treatment using an aqueous extract of ginger with hydrogen peroxide (0.05%). Moreover, the second treatment showed significant inferiority (P≤0.05) compared to the fourth treatment in terms of hemoglobin characteristics. The statistical findings indicated a significant reduction (P≤0.05) in the volume of accumulated blood cells in the second treatment, as compared to the third and fourth treatments, and also to the first treatment when analyzed mathematically. In terms of platelets, the second treatment exhibited a significant decrease (P≤0.05) when compared to all other treatments.

Table 2. The effect of the aqueous extract of the tubers of the ginger plant (Zingiber officinale) and hydrogen peroxide (0.05%) on physiological blood parameters.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>Erythrocyte</th>
<th>White blood cell count</th>
<th>Hemoglobin (Hb)</th>
<th>Packed cell volume (PCV)</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Negative control (without treatment)</td>
<td></td>
<td>6.83±0.07</td>
<td>5.83±0.73</td>
<td>12.50±1.16</td>
<td>41.8±1.46</td>
<td>385.00±11.51</td>
</tr>
<tr>
<td>T2 H$_2$O$_2$ (0.05%)</td>
<td></td>
<td>6.10±0.06</td>
<td>10.37±3.30</td>
<td>10.30±0.76</td>
<td>37.77±4.30</td>
<td>333.25±17.05</td>
</tr>
<tr>
<td>T3 ginger 200 mg/kg</td>
<td></td>
<td>6.74±0.84</td>
<td>10.11±2.03</td>
<td>15.62±0.52</td>
<td>44.17±1.95</td>
<td>397±24.59</td>
</tr>
<tr>
<td>T4 H$_2$O$_2$ (0.05%) + ginger 200 mg/kg</td>
<td></td>
<td>6.48±0.10</td>
<td>9.65±0.59</td>
<td>13.90±1.37</td>
<td>42.12±1.04</td>
<td>401.25±10.78</td>
</tr>
</tbody>
</table>

* Different letters in the same column indicate that there are significant differences (P≤0.05) between the coefficients

The findings presented in Table 2. Were in agreement with a study conducted by [17] on New Zealand White Rabbits over a two-month period, which was divided into two stages. Owain observed that administering hydrogen peroxide resulted in a significant reduction (p≤0.05) in the total count of erythrocytes and the volume of the total count of white blood cells compared to the control group.

The significant decrease in the total count of erythrocytes, packed blood cells, hemoglobin, and platelets observed in Group II rabbits treated with hydrogen peroxide may be attributed to the oxidative stress induced by hydrogen peroxide. When ingested with water, hydrogen peroxide (H2O2) increases the partial pressure of oxygen in the stomach, which subsequently affects the partial pressure of oxygen in the tissues [18]. The elevated molecular
pressure of oxygen can negatively impact the production of red blood cells and heme, leading to reduced hemoglobin values.

The observed decrease in erythrocyte count, hemoglobin, and packed blood cells in the group treated with hydrogen peroxide may be attributed to various factors. One possible explanation is that free radicals attack erythrocyte membranes and oxidize the fats present in these membranes, as noted by [19]. Another possibility is that the oxidative damage caused by free radicals to the kidneys can lead to nephropathy, which impairs their function and reduces the secretion of the hormone erythropoietin. This hormone stimulates the formation of red blood cells in the bone marrow, and a decrease in its secretion can lead to a drop in hemoglobin concentration, as noted by [20]. This decrease in hemoglobin concentration may also coincide with a decrease in the volume of packed blood cells, as there is a positive correlation between these indicators, as noted by [21].

These factors may contribute to the observed decrease in these indicators in the hydrogen peroxide group compared to the control group. Table (2) also shows that administering ginger aqueous extract had a positive impact on the blood parameters of rabbits. Hemoglobin, packed blood cell volume, and platelets all reached their highest values when ginger was dosed at a concentration of 200 mg/kg. This improvement was particularly notable when compared to the group treated with hydrogen peroxide.

The possible reason for the betterment in blood indicators could be attributed to the presence of abundant nutrients and minerals in ginger. Several studies have shown that ginger is rich in antioxidants, such as camphene, zingiberin, zingiberol, chagol, and zingerone, which have a more potent effect than vitamin C and E in neutralizing free oxygen radicals, as per [22]. So for this reason the cellular membranes of red blood cells become more stable, and unsaturated fatty acids are less susceptible to oxidation. Apart from the beneficial impact of ginger's antioxidants on bone marrow activity and liver function, ginger is also a rich source of essential nutrients like proteins, carbohydrates, fats, vitamins, minerals, and trace elements, which can enhance the strength of red blood cells and bolster the immune system of the body, according to [23].

**Conclusion**

Based on the results which obtained the following conclusions:

- Oxidative stress with hydrogen peroxide in male rabbits led to a decrease in the level of Erythrocyte, Hemoglobin, and Platelets significantly in the blood serum of rabbits exposed to oxidative stress compared to normal rabbits, as well as an increase in the level of total white blood cell count.

- Administering ginger aqueous extract had a positive impact on the blood parameters of rabbits. Haemoglobin, packed blood cell volume, and platelets all reached their highest values when ginger was dosed at a concentration of 200 mg/kg.

**References**


تقييم القدرة المضادة للأكسدة لمستخمص الزنجبيل المائي على تأثير الأكسدة الناتج عن H2O2 في الأرانب المحمية: دراسة شاملة للمعايير الدموية

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الخلاصة
أجري هذا البحث في المعهد التقني في بكرجو / جامعة السليمانية التقنية، هدف لتحقيق تأثير إعطاء 0.5% بيروكسيد الهيدروجين عن طريق مياه الشرب كمحفز للإجهاد التأكسدي، وكذلك تأثير مضادات الأكسدة في شكل مستخمص مائي من درنات الزنجبيل (بجرعة 200 مجم/كجم) على الخصائص الفسيولوجية والأداء للأرانب الأليفة الذكور. شارك مجموعه 48 أرنبًا أرشادًا في 6-6 أشهر، وُضعت في أربع مجموعات كل مجموعة تحتوي 12 أرنبًا. استمرت الدراسة لمدة 42 يومًا، وكانت المجموعات: مجموعة ضابطة، ومجموعة تلقى فقط بيروكسيد الهيدروجين (0.05%), ومجموعة تلقى فقط مستخلص الزنجبيل (200 مجم/كجم)، ومجموعة تلقى كل من بيروكسيد الهيدروجين (0.05%) ومستخلص الزنجبيل (200 مجم/كجم). وفقًا للتحليل الإحصائي، أدى استخدام بيروكسيد الهيدروجين في المجموعة الثانية إلى آثار سلبية على مؤشرات الدم، بما في ذلك انخفاض في العدد الإجمالي لخلايا الدم الحمراء، وتركيز هيموجلوبين، وحجم خلايا الدم المكملة، والصفائح الدمويةمقارنة بالجموعات الأخرى. كانت هذه التأثيرات مهمة عند مستوى احتمالية 0.05 دولار ص. ومع ذلك، فإن إعطاء المستخلص المائي لدرنات الزنجبيل (200 مجم/كجم) للأرانب في المجموعة الثالثة كان له تأثير إيجابي، حيث أدى إلى تحسين جميع المؤشرات المذكورة سابقاً وتجاوز بشكل كبير التحكم والمجموعات الثانية عند مستوى احتمال 0.05.

بالإضافة إلى ذلك، فإن استخدام المستخلص المائي لدرنات الزنجبيل بالتزامن مع بيروكسيد الهيدروجين في المجموعة الرابعة منع أو قلل من الآثار السلبية للإجهاد التأكسدي الناجم عن بيروكسيد الهيدروجين على معظم مؤشرات الدم المذكورة في الدراسة. أظهرت المجموعة الرابعة ميزة كبيرة مقارنة بالمجموعة الثانية التي تلقى فقط بيروكسيد الهيدروجين (0.05%). تم تمثيل هذه التأثيرات بالمجموعة الرابعة في الغالب إلى قيم المجموعة المضابطة أو كانت أفضل قليلاً.

الكلمات المفتاحية: الإجهاد التأكسدي، جذور الزنجبيل، الأرانب.