**The antioxidant and analgesic effect of red and white ginseng**

 **on Wistar rats**

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**abstract**

Ginseng is a perennial herbaceous plant from the Araliaceae family which belongs to the genus Panax, especially cultivated in Korea, ginseng is recognized for its general tonic effect, useful for invigorating the body of tired or weakened people, in particular due to its anti-inflammatory and antioxidant effects.

The present work is conducted to assess the importance of panax ginseng by evaluating its use in a targeted population, then determining the pharmacological and therapeutic properties of panax ginseng to study the antioxidant activity of ginseng and its impact on analgesic activity in male Wistar rats.

First, information on the nutritional, therapeutic and cosmetic uses of ginseng was collected from 66 questionnaires. The second step is to evaluate the biochemical potential of the hydro-ethanolic extract of red ginseng and the aqueous extract of white ginseng in vitro and their impact on the antioxidant activity by the DPPH method, then the evaluation of the effect of two variations of panax ginseng on peripheral analgesic activity in male Wistar rats that were injected with acetic acid to induce abdominal pain.

 The investigation proved on the one hand, that ginseng is used as a treatment against: Depression, cardiovascular diseases and Diabetes.

The antioxidant activity of the extract, by the DPPH method, showed that the red extract tested has a very high antioxidant potential, with an IC50 of 0.75 mg/ml. Comparison of white extract (IC50 of 6.03 mg/ml). The extract revealed a remarkable peripheral analgesic effect, Red Ginseng studied reduced the number of writhing from (46.7±10.0) to (12.3±5.0) with an inhibition rate of 73.66%. In addition, about the White Ginseng extract which has an inhibition rate of 66.8% with number of contortions (15.5±6.6).

To conclude, we can say that there is a significant difference between red and white panax ginseng, moreover it is mainly used for therapeutic purposes. Although the benefits of this product are well known, it is not yet widely used. The biochemical potential, without toxic effect, shows a good content in phenolic compounds due to the variability of these botanical origins which explains its considerable antioxidant capacity.

**Keywords:** white ginseng\_ red ginseng\_ analgesic activity\_ antioxidant activity\_ pharmacological\_ therapeutic.

**INTRODUCTION**

Chronic pain is challenging to treat due to the limited efficacy and adverse side effects of therapies. Commonly used analgesics are classified as opioids (cocaine, morphine) and nonsteroidal anti-inflammatory drugs (NSAIDs), which have a good clinical therapeutic effect.

Pain is one of the most important health problems due to its prevalence and the disability it can induce. Chronic pain can be the consequence of inflammatory diseases or tissue damage such as nerve damage in the case of neuropathic pain. In Europe, chronic pain affects around 17 to 45% of the population (Elliott et al., 2002). In developing countries, medicinal plants are widely used by populations and are a source of new active components, especially treating pain and inflammatory processes (***Calixto et al., 2004; Télesphore et al., 2010)***. In addition, chronic pain is often resistant to existing therapy, a great need for research into new drugs is felt.

Ginseng (Panax ginseng C.A. Meyer) is an herbal plant within the Araliaceae family and belongs to the Panax Genus ***(Guo et al, 2021).*** The roots of ginseng contain ginsenoside, a class of steroid glycoside that is responsible for its pharmacological activity. Ginseng has many bio properties such as antioxidant (Chen & Huang, 2019), anti-inflammatory (Im, 2020), analgesia antipruritic ***(Lee et al., 2018; Luo et al., 2018),*** and anticancer activities ***(Zhang J. et al, 2019).***

In this work, we provide an ethnobotanical study of ginseng and its active constituents and its major therapeutic applications for the analgesia effect (abdominal pain) and the antioxidant effect. We discuss the pharmacological mechanisms of action of ginseng and ginsenoside’s analgesic effects on abdominal pain.

***Methods:***

At the beginning a survey on the use of ginseng by interview, then the ginseng obtained from a local collector is identified, the latter has undergone a hydroalcoholic extraction, then a rotary evaporation is elaborated. Pharmacognostic and therapeutic properties are determined. In the end, an experimental study is carried out to study the analgesic effect of panax ginseng in Wistar rates.

The survey is carried out among scientists, traditional therapists, herbalists and old connoisseurs of the Wilaya of Sidi Bel Abbes. To this end, a survey form is established beforehand, modified by the method described by (Martin, 1995) and carefully completed by interviewing each targeted participant.

It lists all the information useful to our investigation, in particular the mentions relating to the use, the instructions for use, the method of preparation as well as the method of administration of the remedy.

The ginseng sample is obtained from an expert herbalist in Sidi Bel Abbes North-West of Algeria in January 2022. A maceration is carried out using 20g of root ‘s dried powder of red panax ginseng and every 10g homogenized in the mixture (ethanol/distilled water) at 80% for 72 h, at room temperature, with replacement of the solvent every 24 h. The extract is filtered with Whatman No. 1 paper, then evaporated separately to remove the solvent in a rotary evaporator at 40°C.

The ginseng sample is obtained from an expert herbalist in Sidi Bel Abbes North-West of Algeria in January 2022. A Decoction is carried out using 15 g of root ‘s dried of white panax ginseng and homogenized in distilled water (450 ml) for 15min, in 100 Celsius. The extract is filtered with Whatman No. 1 paper, then evaporated separately to remove the solvent in an oven at 38°C.

During this study, the peripheral analgesic activity of the extracts obtained (red ginseng extract & white ginseng extract) was highlighted, the biological material chosen for this experiment are Wistar rats.

**Koster's test (abdominal constriction)**

**Experimental protocol**

The analgesic effect of the natural remedy is evaluated according to the number of abdominal constrictions induced by the intraperitoneal (IP) injection of acetic acid (0.6%) v/v according to the method described by **(Seungyeop Lee, 2017)** and **(Sawadogo et al., 2006).**

One hour after administration of the extract by gavage, the animals receive 0.6% acetic acid intraperitoneally at a dose of 10 ml/kg. The principle consists in counting, five minutes after the injection of acetic acid, the number of abdominal constrictions in each mouse for 10 minutes.

The rats were fasted 18 hours before the experiment to avoid any digestive food interaction. Batches of Wistar rats were formed.

**Group1:** The control group (untreated)

The rats (n=03) receive distilled water (10ml/kg) by gavage, one hour before the injection of 0.6% acetic acid at a dose of 10ml/kg intraperitoneally.

**Group 02**: The reference group (paracetamol 100 mg/kg)

The rats (n=03) receive, by gavage, paracetamol at a dose of (100mg/kg), one hour before the injection of 0.6% acetic acid.

**Group 03**: the treated group (Hydroalcoholic Red Extract)

The rats (n=04) receive the hydroalcoholic extract by gavage at doses of (500mg/kg), one hour before the injection of 0.6% acetic acid at a dose of 10ml/kg intraperitoneally.

**Group 04:** the treated group (Aqueous white extract)

The rats (n=04) receive the aqueous extract at a dose of (500mg/kg) by gavage, one hour before the injection of 0.6% acetic acid at a dose of 10ml/kg intraperitoneally.


**Figure 1**. abdominal constriction induced by injection of 0.6% acetic acid

**Analgesic effect measurement**

The analgesic effect was evaluated according to the following formula:

% inhibition = {1-Wt/Wb}x 100

Wb: represents the mean number of constrictions of the rats of the control group (untreated).

Wt.: represents the mean number of constrictions of the rats of the treated group.

***Results***

**1. Ethnobotanical study**

Our study focused on an ethnobotanical survey on the use of the panax ginseng plant over a period of 2 months. During which we approached people in public places, universities, herbalists, traditional healers, family and our relatives are also among the subjects questioned, these people are a sample of 66 individual cases from different slices of ages.

The age group most concerned is 26-36 years old, 30% among them use the plant. This population is divided between the two sexes, of which 39 are female (i.e. 59%), and 27 are male (i.e. 41%).

Regarding the level of education, 56% of the population was graduated, and the rest of the respondents (41%) were divided between an average level of education (6%) and secondary level (27%), and only 9% for informants had levels of higher education (post-graduation). The data collected from the survey according to the level of education, revealed that the people they have at a higher level (graduation, post-graduation) use panax ginseng much more, as a treatment against certain diseases compared to the les people who have other levels (medium, secondary) who have not used the plant. Moreover, 62.12% of the graduates questioned use ginseng daily for its therapeutic virtues, against 19.69% of the population at another level. So, there is a relationship between the level of education and the use of this plant (p= 0.0006); (**table 1**).

59% of this population know the plant and its importance, besides 49% they didn’t hear about it, this is due to the fact that the source of the plant is Asian. (**Figure 2).**

There are three interests which is the therapeutic one, 67% of the informants use for that purpose, on the other hand, there are 19% and 14% use it for cosmetic and food purposes respectively (**Figure 3).**

**Table 1:** Distribution of use of the plant according to different situations

|  |  |  |
| --- | --- | --- |
|  | using  |  |
| No | Yes |  |
| Effective | % | Effective | % | P |
| Knowing the importance | Yes | 7 | 58.3% | 33 | 61.1% | 1.000 |
| No | 5 | 41.7% | 21 | 38.9% |
| Gender | Male | 5 | 41.7% | 22 | 40.7% | 1.000 |
| Female | 7 | 58.3% | 32 | 59.3% |
| Age | 15-25 y. o | 4 | 33.3% | 8 | 15.1% | 0.323 |
| 26-36 y. o | 3 | 25.0% | 16 | 30.2% |
| 37-47 y. o | 2 | 16.7% | 15 | 28.3% |
| 48-59 y. o | 1 | 8.3% | 11 | 20.8% |
| 59-69 y. o | 2 | 16.7% | 3 | 5.7% |
| interrogates | Scientific | 9 | 75.0% | 28 | 51.9% | 0.638 |
| Household | 2 | 16.7% | 14 | 25.9% |
| Herbalist | 1 | 8.3% | 8 | 14.8% |
| Traditional therapist | 0 | 0.0% | 4 | 7.4% |
| Educational level | Medium | 1 | 8.3% | 3 | 5.6% | 0.006 |
| Secondary | 8 | 66.7% | 10 | 18.5% |
| Graduation | 3 | 25.0% | 35 | 64.8% |
| Post graduation | 0 | 0.0% | 6 | 11.1% |

**Figure 2**. The percentage of people who know the panax ginseng.

**Figure 3**. The interest of use the plant by the informants

**2- Study of peripheral analgesic activity**

▪ Koster's test (abdominal constriction)

We recall that this activity is evaluated from the abdominal constructions of the rats. The results of this test which result in the number of constructions are mentioned in (Table 2) below:

**Table 2.** Test results of koster analgesic effect of paracetamol and the extracts studied

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | Doses | Number of constrictions | % Inhibition |
| **Control**  | 10mg/Kg | 46.7±10.0 | --- |
| **Paracetamol** | 100mg/Kg | 18.3±1.5 | 60.81% |
| **White Extract** | 500mg/Kg | 15.5±6.6 | 66.80% |
| **Red Extract** | 500mg/kg | 12.3±5.0 | 73.66% |

**Values expressed as an average ± standard deviation. SEM**

 Abdominal contractions induced by injection of acetic acid were used to evaluate the peripheral analgesic effect of panax ginseng extracts.

We report that the mechanism of pain onset results from a tissue lesion responsible for an increase in the release of many chemical mediators such as: histamine, prostaglandin and serotonin, in the intraperitoneal fluid, which stimulate nociceptive receptors located at the peritoneal level. In rats, the pain is manifested in the form of abdominal cramps (writhing).

According to the results obtained, it can be seen that acetic acid induces an average of 46.7 ± 10.0 cramps recorded after 10 minutes in the control group. These cramps occurring under the effect of acetic acid, involve the action of peritoneal receptors

local and are accompanied by pain due to the release of serotonin, histamine, bradykinin, substance P, prostaglandins (PGE2α, PGF2α) and cytokines (TNF-α, IL-1β, IL- 8) (Bentley, 1983; Negus, 2006). These chemical mediators stimulate peripheral nociceptive neurons and induce an increase in vascular permeability, causing pain in our treated rats (**Figure 4).**

**Figure 4. Analgesic effect of panax ginseng extracts by injection of acetic acid.**

**Bars with different letters are significantly different (p<0.05).**

Our results show that the administration of the aqueous extract at a dose of 500mg / kg BW inhibits abdominal contractions with a satisfactory rate but, less important compared to the hydroalcoholic extract inhibits in a highly significant p<0.001 abdominal contractions compared to the batch witness on the one hand and even compared to paracetamol.

Panax ginseng is a species rich in natural compounds including flavonoids and Saponosides these compounds are therefore very likely and with others, responsible for inhibiting the release of chemical mediators responsible for the appearance of the painful symptom

The results obtained also indicate that the administration of the white and red extracts at a dose of 500mg/kg reduced abdominal contractions in a dose-dependent and significant manner p < 0.05, at a rate of (15.5±6.6) and (12.3± 5.00) respectively compared to the control group, with an inhibition rate of 66.8% and 73.66% after 10 minutes of the injection of acetic acid. The extracts also reveal a significant effect, compared to the reference batch, treated with paracetamol, at a dose of 100mg/Kg. CP which is (18.30±1.5).

Discussion

Panax ginseng is one of the most well-known herbal health tonics worldwide. The drug is traditionally believed to strengthen general health and boost vital energy. Several animal and clinical studies have provide scientific evidence for various health benefits of P. ginseng, including immunomodulation, increased cognitive function, reductions in fatigue and biological stress, improved glucose metabolismand sexual function and cancer prevention (**Yun *et al*, 2010**). This study was designed to obtain clinical evidence explaining the mechanism underlying the pharmaceutical benefits of P. ginseng.

The survey on the use of ginseng made it possible to know that the population obtains ginseng from species contained in packed boxes, with a slightly expensive price and then generally stored away from light, it is also used as a remedy by decoction, in order to treat several pathologies and more frequently cardiovascular, infectious pathologies and pathologies such as Diabetes and Infertility, it is also used for well-being, These results are in agreement with several studywho revealed the therapeutic importance of panax ginseng on certain infectious, cardiovascular and endocrine-metabolic diseases.

Based on scientific research, ginseng has multiple biological bioactivities, such as vasorelaxation (angio-modulation), but it also has antioxidant, anti-inflammatory, anticancer, and antidiabetic-related (for obesity), properties. All these biological effects are closely connected to inflammation and the immune system (**Sung *et al*, 2016**).

In reality, it is useless to compare our yields with those of the literature, because this parameter is linked to the climatic conditions of cultivation, namely temperature, rate of exposure to the sun, drought, salinity, cultural practices, maturity at harvest. and storage conditions.

Another study also of ginseng on rats shows that ginseng reduces oxidative stress in certain tissues by altering specific enzymatic activities that are necessary to scavenge free radicals, also protecting against oxidative stress (**Kitts & Hu, 2000**)

Combinations of different ginsenosides have been shown to have synergistic effects. Although ginsenoside Rb1 or Rg1 alone only slightly induces the expression of NrF2, which is a key transcription factor that binds to the antioxidant response element (ARE) and is essential for regulating the expression of numerous antioxidants and detoxifying enzymes (**Wong et al, 2014**). Similar in vitro studies with ginsenosides have indicated some degree of protection against free radical induced endothelial cell damage (**Kitts & Hu, 2000**)

The analgesic mechanism of action in inﬂammatory pain models is thought to be the down regulation of pro-inﬂammatory cytokine expression (TNF-αIL-1β, and IL-6).
Several studies have also demonstrated that ginsenosides regulate neuropathic pain through the modulation of oestrogen receptors. Recently, an increasing number of pathways have emerged in relation to the antinociceptive effect of ginseng and ginsenosides. Therefore, this review presents our current understanding of the effectiveness of ginseng in pain and how its active constituents regulate analgesic responses and their mechanisms of action, In the current study, it was observed that both phases of the acetic acid-induced writhing were significantly reduced by pre-treatment of rats with the WG and RG. The result of this study suggests that aqueous extract WG of has analgesic effect comparable to those of the standard drugs such as PARACETAMOL. Moreover, the ethanolic extract RG was more effective as an analgesic as it reduced the number of writhes (in acetic acid induced writhing)

This observation is in line with a similar study carried out **Haoming luo et al. (2018)** which the Ginseng showed long aging analgesic effect, the experimental results indicated that Ginseng may possess the effect of peripheral analgesic. At present, ginsenosides are considered to be the active substance of ginseng. The research about its anti-inflammatory and analgesic activities mainly focused on ginsenosides, Rb1 and Rg1. The glycopeptides which are active macromolecules, is a new exploration for the anti-inflammatory and analgesic substances of ginseng.

**Conclusion**

Ginseng and its bioactive constituents the ginsenosides include the modulation of neurotransmitter function in both peripheral and central systems, inhibition of inﬂammatory cytokine expression, modulation of ion channel activity in DRG and spinal cord neurons, regulation of the TLR4/NF-κB signal transduction axis, and anti-inﬂammatory effects. This work has provided atheoretical basis and practical application for the analgesia effect based on Panax ginseng. Hence, ginsenosides have great potential for future pain treatment or as an adjuvant for pain therapy in multiple pain type.

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