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# Improvement of the Health Status of Sheep by the Use of Medicinal Plants and Their Effects on Oxidative Stress Parameters

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**Original Research Article**

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**Abstract:** Medicinal plants are used for their beneficial properties to the health of animals. Recently, it has been found that the use of medicinal plants eliminates industrial pollutants. The objective of our work is to determine and evaluate the effect of two medicinal plants (*Thymus algeriensis* and *Artemisia campestris*) on oxidative stress parameters in sheep. The results obtained in liver cells show that medicinal plants have produced a globally antioxidant preventive effect, this is revealed by the significant decrease in the GSH level (199.976±1.913), and the enzymatic activity of CAT (0.699±0.02). In addition, an increase in the enzymatic activities of GST (0.435±0.017), GPx (1.881±0.040) and MDA (29.009±0.086) was observed. These results clearly show an antioxidant effect of medicinal plants on the organism of sheep.

**Keywords:** Medicinal plant, *Thymus algeriensis*, *Artemisia campestris*, oxidative stress, sheep.

## INTRODUCTION

In Algeria, the sheep population is the largest animal resource, estimated at more than 26.88 million head of the national livestock population [1]. Sheep farming occupies a very important place in the field of livestock production in Algeria [2]. It has always constituted the sole income of one third of the Algerian population, especially in the region of Tebessa (eastern Algeria). Sheep has always been and continues to be the preferred and primary resource of animal proteins.

In terms of geographical distribution, about 60% of the national sheep population is located in the steppe, which is currently experiencing many difficulties due mainly to the often-irreversible degradation of pastoral resources and drought [3].

The plains, particularly the semi-dry in eastern Algeria, constitute one of the most important pastures of sheep in this country because of the richness of its vegetation. It contains many medicinal plants that help strengthen the body and improve its effectiveness by reducing the possibility of oxidative stress and activating the antioxidant system. A number of researchers have concluded that oxidative stress is directly related to the environment and environmental health. Inadequate environmental factors (such as pollution) result in severe oxidative stress [3]. In addition to environmental factors that increase the ability to improve the performance of the antioxidant system in the body, it facilitates the resistance and elimination of all free radicals, including improved body performance [4, 5]. In this work, we studied the effects of medicinal plants in the Tebessa region and their role in improving the sheep health and production. By calibrating the oxy/antioxidant balance by evaluating some antioxidant enzymes and peptides in the liver of two groups of sheep; one of which is consuming the mixture of medicinal plants and the other is a control, oxidation negatively affects animal production by causing many diseases and critical situations, which were the main source of these free radicals products.

## MATERIALS AND METHODS

### Animals of experimentation

A total of eighty-two sheep weighing between 35 and 40 kg were used for this study, animals were divided into a control group and a medicinal plants treated group. The temperature of the pet store was maintained at 23°C, with a moderate humidity level and a half 24-hour photoperiod.

### Extraction yield

The decoction of 50g and 100g of the leaves powder of *Thymus algeriensis* and *Artemisia campestris* in 0.5 L and 1 L distilled water, respectively, gave the

yield of 14.10 % and 17.39 % of aqueous extract (AQE). The maceration of 50g and 100g of the leaves powder of *Thymus algeriensis* and *Artemisia campestris* in 0.5 L and 1 L hydro-alcoholic mixture (MeE, methanol/distilled water, 85/15 v/v), respectively, gave the yield of 11.84 % and 25.56 % of methanolic extract.

The highest yields were obtained in *Artemisia campestris* extracts, where MeE had the best yield (25.56 %) followed by AQE (17.39%). The highest yield for *Thymus algeriensis* was obtained in AQE (14.10%) followed by MeE (11.84 %).

**Determination of Reduced Glutathione (GSH)**

Brain glutathione level (GSH) was measured using the method described by Habig et *al*., [6]. 0.2 ml of a sulfosalicylic acid (SSA, 0.25 %) was centrifuged for 5 min at 1000 rpm. 1 mL of Tris HcL-EDTA buffer (0.02M), pH=9.6 was added. The mixture was added to 0.025 mL of 5, 5`dithiobis-2-nitrobenzoic acid (DTNB) 0.01M dissolved in absolute methanol and the absorbance was measured at 412 nm.

**Determination of glutathione-S-transferase (GST)**

The activity of GST was measured according to the method of Habig [6]. The P-nitro benzyl chloride was used as substrate. The absorbance was measured at 340nm at 30 second intervals for 3min.

**Determination of Glutathione Peroxidase (GPx)**

Glutathione peroxidase (GPx) activity was measured according to the method of Flohe & Gunzler [7]. Supernatant obtained after centrifuging 5% of liver homogenate at 1500 rpm for 10 min, followed by 10000 rpm for 30 min at 4°C was used for GPx assay. 1 mL of reaction mixture was prepared which contained 0.3 mL of phosphate buffer (0.1M, pH7.4), 0.2mL of GSH (2mM), 0.1 mL of sodium azide (10mM), 0.1mL of $H_2O_2$ (1mM) and 0.3mL of liver supernatant. The reaction was terminated by addition of 0.5mL 5% TCA after 15 min of incubation at 37°C. Tubes were centrifuged at 1500 rpm for 5 min and the supernatant was collected. 0.2 mL of phosphate buffer (0.1M, pH7.4) and 0.7 mL of DTNB (0.4 mg /mL) were added to 0.1 mL of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

**Determination of catalase activity (CAT)**

The catalase (CAT) activity was determined according to the method of Aebi [8]. The $H_2O_2$ decomposition rate was followed by monitoring absorption at 240 nm. One unit of CAT activity is defined as the amount of enzymes required to decompose 1µmol of hydrogen peroxide in 1 min. The enzyme activity was expressed as *µmol of $H_2O_2$ consumed/mn/mg protein.*

**Determination of malondialdehyde acide (MDA)**

The lipid peroxidation level in heart homogenate was measured as malondialdehyde (MDA) which is the product of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red colored complex with a peak absorbance at 532 nm (28). *125µL of supernatant* were homogenized by sonication with 50 µL of PBS, 125 µL of TCA–BHT (trichloroacetic acid–butylhydroxytoluene) in order to precipitate proteins. The mixture was then centrifuged at 1000 rpm for 10 min at 4°C. Afterwards, 200 µL of supernatant were mixed with 40 µL of HcL (0.6M) and 160 mL of TBA dissolved in Tris**.** The mixture was heated at 80 °C for 10 min. The absorbance of the resultant supernatant was obtained at 530 nm.

**Data processing**

The experiments data were analyzed using statistical software SPSS (SPSS 22.0 for Windows. SPSS Ins. USA), and all statistical comparisons were made by means of T-test and values of $P<0.05$ were considered significant. Results were expressed as means ± SEM, All histograms were obtained using the Office Excel 2013.

**RESULTS**

The parameters of oxidative stress (GSH, MDA, GPx and GST) in the livers of control sheep and medicinal plants-treated sheep are illustrated in Figures (01-05) and Table 01.

**Table-1: Oxidative stress parameters in sheep's livers**

| Parameters | CAT | GST | GSH | GPx | MDA |
|---|---|---|---|---|---|
| Control sheep | 0.867 ± 0,03 | 0,387±0,014 | 209,155±1,685 | 2,143±0,022 | 29,773±0,130 |
| Treated sheep | 0.699±0,02 | 0,435±0,017 | 199,976±1,913 | 1,881±0,040 | 29,009±0,086 |

**Reduced Glutathione (GSH)**

The GSH assay in the liver of sheep fed with medicinal plants results in a very highly significant (P> 0.0001) decrease in glutathione cell content compared to the control group (Fgure-1).

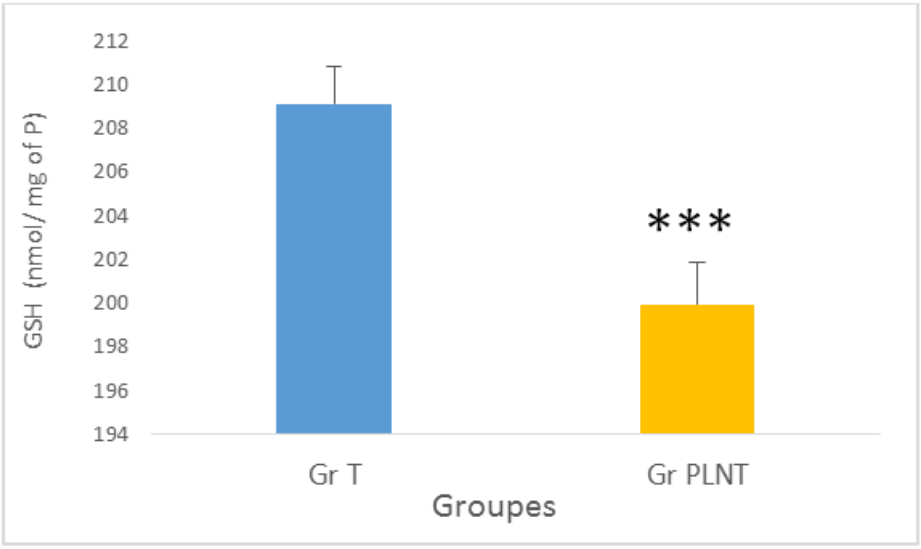


**Fig-1: Variation of glutathione (nmol / mg of prot) in sheep livers**.
***: Highly significant difference compared to control (P ≤0.01)*
***:Very highly significant difference compared to control (P ≤0.001)*
*P: Significance threshold.*

**Peroxidase Glutathione (GPx)**

In contrast, GPx activity in the same groups (Gr PLNT) showed a highly significant (P> 0.01) increase in GPx in livers compared to the control group (Figure-2).

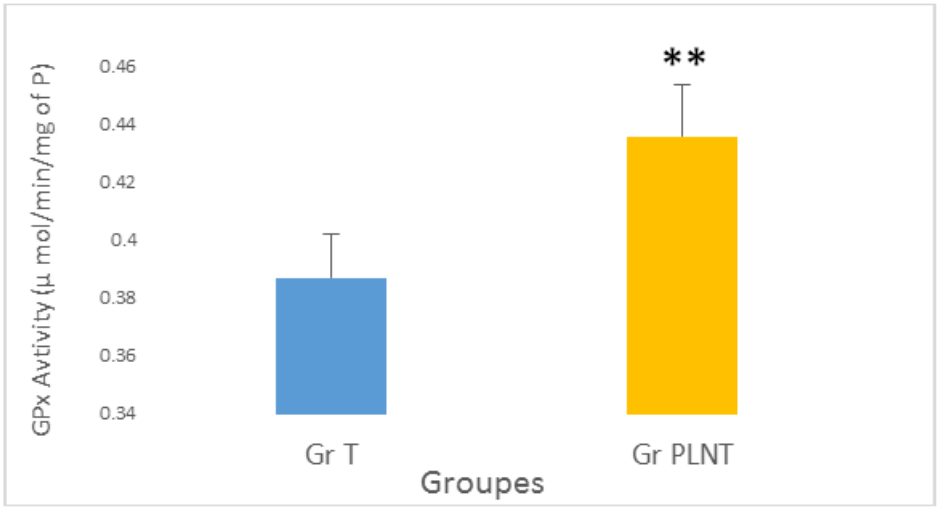


**Fig-2: Variation of the enzymatic activity of glutathione peroxidase (µmol/mg prot) in sheep livers**.
*** :Highly significant difference compared to control (P ≤0.01)*
****: Very highly significant difference compared to control (P ≤0.001).*
*P: Threshold of significance.*

**Malondialdehyde (MDA)**

From the results obtained (Table-1 & 3), a very highly significant increase (P≤0,001) in the level of MDA in livers in sheep fed with medicinal plants compared to control sheep.

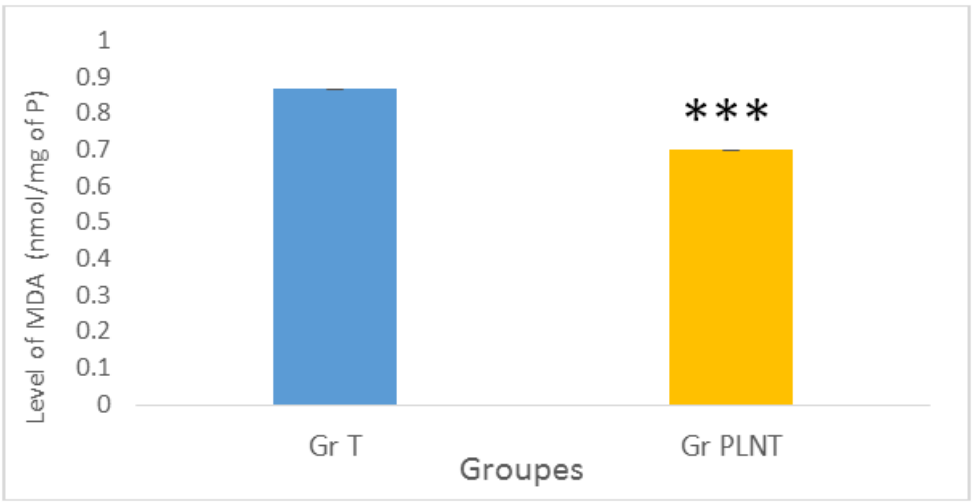


**Fig-3: Malondialdehyde variation (nmol/mg protein) in sheep livers.**
*** :Highly significant difference compared to control (P ≤0.01).*
**** : Very highly significant difference compared to control (P ≤0.001).*
*P: Significance threshold*

**Glutathione-S-transferase (GST)**

A very highly significant decrease (P≤0.001) in the enzymatic activity of glutathione S-transferase (GST) in sheep treated with medicinal plants compared to the control sheep (Figure-4).

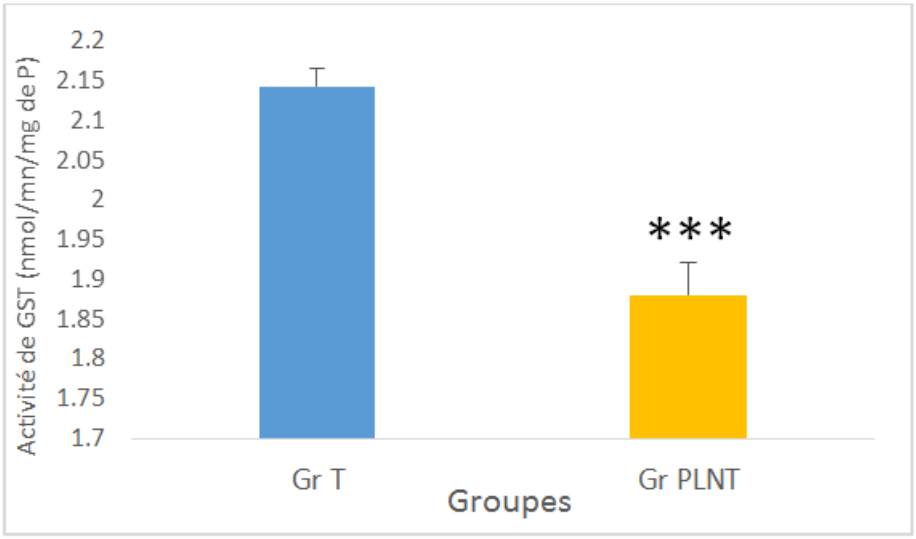


**Fig-4: Variation of the enzymatic activity of glutathione-S-transferase (mmol / min / mg of prot) in sheep livers**.
***: Highly significant difference compared to control (P ≤0.01).*
****: Very highly significant difference compared to control (P ≤0.001).*
*P: Threshold of significance.*

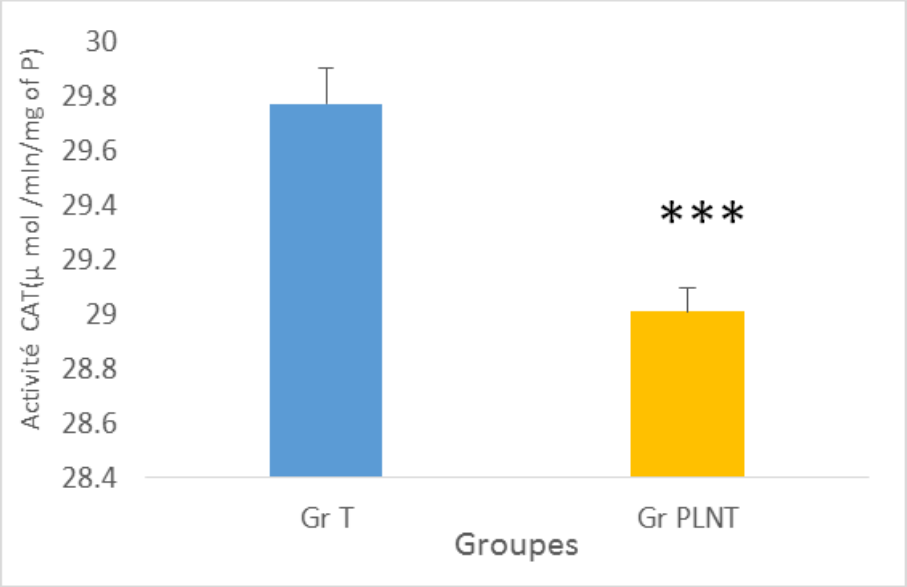


**Fig-5:** Variation of catalase activity (µmol / min / mg prot) in sheep livers.
***:Highly significant difference compared to control (P ≤0.01).*
****: Very highly significant difference compared to control (P ≤0.001).*
*P: Threshold of significance.*

**Catalase (CAT)**

The administration of medicinal plants in sheep induces a very highly significant decrease (P> 0.001) compared to the control groups (Figure-5).

**DISCUSSION**

Medicinal plants are an accessible, affordable and culturally relevant source of health and care for the majority of the world's population [9]. In this study we evaluated the antihepatotoxicity of two medicinal plants (*Thymus algeriensis* and *Artemisia campestris*) in two herds of sheep; one grazed in pastoral areas containing medicinal plants and the other fed inside warehouses (synthetic dry food). Both herds growing in the Cheria region of Tebessa, 600 Km East of Algiers, Algeria.

This is the first study in this field, the purpose of which is to evaluate the effects of natural medicinal plants on the antioxidant system of sheep (antihepatotoxicity) and at the same time on the animals quality. The activity of the antioxidant system may be increased or inhibited by a toxicant or a pollutant, both of which depend on the duration of exposure and the sensitivity of the species [10]. The antioxidant activity can be reinforced by different factors; the nutrition is one of these [11]. Phenolic compounds are considered as major contributors to the antioxidant capacity of plants [12, 4]**.** These compounds also possess various biological activities such as anti-inflammatory, antibacterial, antiviral, antiallergic, antithrombotic and vasodilating activities. These capacities can be related to their antioxidant activity due to the presence of numerous hydroxyls capable of reacting with free radicals [13-16]**.** In the present study, stress parameters were valuated such as GSH, MDA and hepatic detoxification enzymes (GST, CAT and GPx).

**GSH**

Glutathione is a water-soluble non-protein tripeptide consisting of three amino acids; glutamate, cysteine and glycine (L-γ-glutamyl-L cysteinyl glycine), produced naturally in the body [17]**,** found in fairly high concentrations (1-10 mM) in almost all cells of living organisms (animals, plants and humans) [18, 19]**.** Glutathione is a non-enzymatic biomarker which plays a central role in the intracellular defence process. It is the main system involved in the detoxification of peroxide ions and prevention of against oxidative stress [20]. Glutathione exists in two forms, oxidized GSSG and reduced GSH, these enzymes include glutathione peroxidase (GPx) and glutathione S-transferase (GST) that are involved in detoxification processes [21, 22, 19]**.** A deficiency in GSH exposes the cells to a risk of oxidative damage. Thanks to the thiol (-SH) function of cysteine, glutathione in its reduced form is an important compound for maintaining the redox balance of the cell. This thiol function can also fix electrophilic functions, thus serves to detoxify many pesticides that contain such a function [6]. Reduced glutathione oxidation is achieved by glutathione peroxidase and reduction of oxidized glutathione by glutathione reductase [20]**.** Some insecticides act on a very limited number of species, increasing the activity of the various enzymes involved in detoxification [23]. Results of GSH determination in our samples, showed a significant decrease in the GSH level in the group that consumes the medicinal plants compared to the other group. These results are in agreement with several precedent studies, help to better explain the relationship between the

decrease in GSH levels and the administration of polyphenolic compounds in experimented animals [4, 5, 24, 25]**.** The decrease in GSH could be explained by an increased consumption of this cofactor by the GSTs in order to detoxify the organism. In addition, this decrease in GSH also reflects a reduction in the non-enzymatic antioxidant system. The decrease of this small molecule is a widespread consequence of the accelerated formation of reactive oxygen species during sustained cellular activities [26]**.**

**MDA**

The increase in MDA is a consequence of tissue damage by excessive free radical formation [80], and as our results show, a very significant decrease in malondialdehyde (MDA) which is a biomarker of lipid peroxidationhas been observed in hepatic tissue [27, 28. The effect of medicinal plants on GSH and the activities of antioxidant enzymes is accompanied by a decrease in the amount of free radicals, such as the hydroxyl radical, which in turn can decrease lipid peroxidation [29, 30]**.**

Results of present work confirmed those of [31, 24] who found an improvement in antioxidant status in mice and rats treated with medicinal plant extracts. This improvement is accompanied by a decrease in lipid peroxidation and cellular GSH in the liver. This is due to the antioxidant effect of plant polyphenolic compounds, which is a cofactor of many antioxidant enzymes such as glutathione peroxidase GPx, glutathione-s-transferase, where the activity of these enzymes is highly dependent on the enzyme [32-34].

Enzymes which can prevent oxidative stress, are considered the first line of an organism defence. Moreover, these enzymes can be enhanced by the polyphenols and flavonoids of medicinal plants, causing the increase of antioxidant enzyme activities such as CAT, GPx, GST [35, 4].

**GPx**

GPx is a key antioxidant enzyme that regulates the level of ROS, it is able to reduce hydrogen peroxide to water, but also hydro peroxides resulting from the oxidation of unsaturated fatty acids, thus protecting cells against the damage caused by xenobiotics [36, 37**]**. Our results show a highly significant increase in hepatic GPx activity in sheep fed with medicinal plants compared to controls. This is mainly due to decrease of ROS production in the cells of the organism [38, 39]

**GST**

GST is a multifunctional enzyme involved in the stage of "reduced glutathione" conjugation to a large number of xenobiotics [40, 41]**.** They are mainly localized in the cytoplasm of cells, fat bodies and wing muscles [42]. They play an important role in the detoxification of xenobiotic substances and intervene by catalysing the conjugation of these substances with the thiol group of endogenous glutathione [43]**.**

This results in the synthesis of a mercapturic acid which would be then readily removable. Thus, the major role of glutathione is to convert lipophilic compounds into readily excretable hydrophilic molecules [6]. GSTs allow the development of resistance to chemotherapeutic agents, insecticides, herbicides and microbial antibiotics. They play an important role in the physiology of stress, intracellular transport and in the various pathways of biosynthesis [44]. Our results show a highly significant decrease in GST activity of the medicinal plant as animal feed compared to controls. The decrease in GST activity reflects the establishment of the process of detoxification which is a form of defences of the body against xenobiotics. A similar increase in the specific activity of GST was also observed in Donax trunculus exposed to environmental pollutants [10]. In addition, different reports showed that low levels of ROS is associated with decreased GST and cytochrome P450 activity in animals [40, 45, 46].

**CAT**

Catalase (CAT) is the second step in the enzymatic defence system. It supports hydrogen peroxide previously produced by SODs and metabolizes it to water [47].

In the liver tissues, the medicinal plants decrease the activity of catalase (CAT), this result suggest that these phenolic compounds indirectly induce an increase in $H_2O_2$, so it can cause by the withdrawal of other detoxification enzymes. The increase in GPx activities results in a decrease in $H_2O_2$ [29].These can be enzymatic and / or non-enzymatic and the cooperation between these antioxidants plays an important role in the elimination of ROS and maintenance of the redox status of the plant [48]

Our results confirm those of Gasmi et *al*., [11] which showed that quercetin supplementation improved the detoxification system of mice.

**CONCLUSION**

In conclusion, the results of this study indicate the effective and clear role of the medicinal plants studied. Which are among the available foods for sheep in the Tebessa region. Where the effect of these plants was very clear by improving the antioxidant system at the level of the liver, this led to the improvement of animals health and production.

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