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In vivo study of stress oxidative and some organs damage in rats exposed to metribuzin (triazinone herbicide)

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Abstract

The study was conducted to investigate the effects of pesticides Metribuzin (Triazinone herbicide) which is used worldwide as an ecotoxic herbicide and is the most widely used in Algeria and in random ways without respect for the doses approved, on oxidative stress and liver structure in rats. About 30 female rats *Wistar albinos* divided into five groups (n=6), the first group of non-treated rats serve as control, the second group of rats treated with 30 mg kg^{-1} b.wt., of Metribuzin and the third group of rats treated with 30 mg kg–1 b.wt. of Metribuzin followed by aqueu extrcts of *Astragalus gombo* (roots) with dose 80 mg/kg during 15 days, the fourth group treated with 30 mg kg^{-1} b.wt. of Metribuzin followed by aqueu extrcts of *Astragalus gombo* (leaves) with dose 60 mg/kg during 15 days and the fifth group of rats treated with 30 mg kg^{-1} b.wt. of Metribuzin, followed by drug (vitamin E) with dose 200 mg/ml. Metribuzin added in their drinking water for 30 days. This plant administered in combination with the pesticide shows a highly significant decrease ($p \leq 0.01$) in maintaining the equilibrium of the antioxidant/prooxidant balance cells of all tissues studied. The treatment with the two extracts of *Astragalus gombo* decreases the state of oxidative stress induced by metribuzin exposure by limiting free radical phenomena and repairing oxidative damage by decreasing lipid peroxidation in the liver, kidney and liver.

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Introduction

Free radicals are atoms or molecules that have unpaired electrons, usually unstable and highly reactive (Finkel and Holbrook, 2000). Natural antioxidants contained in edible or medicinal plants often possess strong antioxidant and free radical scavenging abilities as well as anti-inflammatory action, which are also supposed to be the basis of other bioactivities and health benefits.Vitamins E/C, N-acetylcysteine, polyenylphosphatidylcholine, silymarin, and antioxidants cocktail have been attempted for the treatment of alcoholic hepatitis or cirrhosis patients (Groenbaek *and coll*, 2006; Esrefoglu, 2012).

The liver is the most frequently targeted organ in terms of drug toxicity. The production of radical species, specifically ROS and RNS, has been proposed as an early event of drugs hepatotoxicity and as an indicator of hepatotoxic potential (Videla, 2009).

The aim of our study is to evaluate the effect of the extracts of *Astragalus gombo* on different biochemical and biological parameters in the rats *wistar albinos* whitch are exposed to the pesticide metribuzin

Material and methods

Animals and handling

In our study we used females rats wistar with inital weight between 180- 250g were obtained from the Animal service of Pasteur institute, Algeria. They were placed in five groups of 6 rats in each and kept in animal's house of department of cellular and molecular biology. University of El Oued, Algeria. The animals werecarried in a laboratory place for adaptation with conditions of temperature (25±2 °C), humidity (65,3 %) and photoperiod (12 hours of light/ 12 hours of black). Access to standard diet and water is free for animals ad libitum during the experiments, according to previous work. The realization of the experimental part is respect to the ethical approval.

Experimental design

After a period of adaptation, the animals, at the age of 6 weeks, were divided into five groups (n=6). The frist group of rats received standard diet and water (controls) and the second group of rats recevied standard diet with 30 mg kg^{-1} b.wt., of Metribuzin and the third group of rats treated with 30 mg kg^{-1} b.wt. of Metribuzin followed by aqueu extrcts of *Astragalus gombo*(roots) with dose 80 mg/kg during 15 days, the fourth group treated with 30 mg kg^{-1} b.wt. of Metribuzin followed byaqueu extrcts of*Astragalus gombo*(leaves) with dose 60 mg/kg during 15 days and the fifth group of rats treated with 30 mg kg^{-1} b.wt. of Metribuzin followed by drug (vitamin E) with dose 200 mg/ml. Metribuzin added in their drinking water for 30 days.

Blood collection and tissue preparation

After of 30 days of Metribuzin exposure, rats were fasted for 24 hours, anaesthetized with chloroform (94%) by inhalation. After the rats were sacrificed, the blood was collected in secs tubes for biochemical analysis. The serum was obtained by centrifuging the blood at 3000 rpm for 5 min and then stored at 4°C, and used for biochemical analysis. The liver was treated by the paraffin technique. Liver of each group are cut 5 µm thick and then rinsed in hematoxylin and eosin for histological analysis.

Determination of oxidative stress parameters

Preparation of homogenates

One gram of tissue (liver, kidneys, and lung) from each rat of the different groups studied was used. After grinding and homogenizing the tissues in TBS (50mM Tris, 150mM NaCl, pH 7.4), the cell suspension wascentrifuged at 3000 rpm for 15 min. The supernatant obtained was stored at -20°C until use for the oxidative stress marker assay.

Determination of tissue proteins

It's necessary to know the total concentration of all the proteins contained in the tissues. The protein assay is done by the method of BRADFORD (1976) which uses Coomassie blue binding on proteins and in particular on amino groups ($-NH_2$) proteins to form a blue complex. The appearance of the blue color reflects the degree of ionization of the acid

medium and the intensity corresponds to the concentration of the proteins.

Determination of lipid peroxidation (MDA)

Liver lipid peroxidation levels were measured as malondialdehyde (MDA) which measured according to the technique of Sastre *and coll.* The method is based on the reaction between the carbonyl compounds of malondialdehyde with thiobarbituric acid (TBA) to give absorbent pink chromophores at 532 nm. MDA level was expressed asnmol of MDA/mg prot.

Level of Reduced Glutathione (GSH) assay

Liver Reduced Glutathione (GSH) level was determined by a colorimetric method according to the technique described by Ellman, the measurement of optical density results from the formation of thionitrobenzoic acid (TNB) from the reduction of The 5,5'dithiodis-2-nitrobenzoic acid (DTNB), which is called the Ellman reagent with the SH groups exist in GSH, which has an absorbance at 412nm. Total GSH level was expressed as nmol GSH/mg prot.

Enzymatic activity of catalase (CAT)

The enzymatic activity of catalase is determined in cell tissues according to the method of (FLOHE *and coll.,* 1984). The assay for catalase enzymatic activity is based on the decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxide (H_2O_2) by catalase.

Histological study

The histological sections of tissue specimens were performed at a private laboratory. The technique used is that described by (Hougroup, 1984). And histological sections obtained were observed by binocular light microscope (Optica) at the Laboratory University of El Oued Algeria.

Statistical analysis

The results obtained are expressed as mean ± standard deviation.

The analysis of the data was carried out by applying the Student's T-test, which is based on the comparison between two averages, using the MINITAB (Version 17) and EXCEL (Version 2013).

Results

Histopathological studies

The histological observation (figure1) show that the liver of all the groups are taken to make the histological test, the observations of histological sections allow me to confirm the toxic effect of the pesticide (metribuzin) and the detoxification power of the two extracts of the plant *Astragalus gombo* (leaves and roots) on the rats. Generally, the treatment of the rats with the aqueous extracts of the plant studied has a remarkable efficiency against inflammations and necrosis caused by the exposure of the pesticide (metribuzin) at the hepatic tissue.

Table 1. Level of malondialdehyde (MDA) in the liver, kidneys and lungs

Parameters		GROUP1(T) N=5	GROUP2(P) N=5	GROUP3(R) N=5 80 mg/kg	GROUP4(F) N=5 60mg/kg	GROUP5(M) N=5
Malondialdéhyde (MDA)(µM/mg prot)	Liver	0.5175± 0.128	1.837± 0.372***	1.023± 0.069** b	0.634± 0.014 NS c	0.672± 0.046NS c
	Kidney	0.92± 0.069	2.29± 0.035***	0. 7914± 0.025* c	0.8628± 0.066NS c	0.8853± 0.051NS c
	lungs	0.274± 0.031	0.266± 0.0069NS	0.237± 0.0237NS b	0.216± 0.030* c	0.247± 0.0185NS a

Effect on level of malonaldehyde (MDA)

The table 1 show increase in the levels of the oxidative stress marker, the malonaldehyde (MDA) of rats exposed to metribuzin in liver and kidney cells (P≤0.001) and a decrease in significant pulmonary concentration (p> 0.05), and a slight increase in the group treated by the extract root compared to healthy rats.

Intraperitoneal injection by the two aqueous extracts of *Astragalus gombo* (roots and leaves) and treatment with the drug induces a decrease in the level of MDA hepatic and renal with a very highly significant difference (p≤0.001) for each of the groups (F and M), but treatment with the root extract causes a very highly significant decrease in renal concentration (p <0.001) and decrease (P≤0.01) of hepatic concentration compared to metribuzin-treated rats (pesticide control).

Table 2. Level of Reduced Glutathione in the liver, kidneys and lungs.

Parameters		GROUP1(T) N=5	GROUP2(P) N=5	GROUP3(R) N=5 80 mg/kg	GROUP4(F) N=5 60mg/kg	GROUP5(M) N=5
Glutathion reduced (GSH) (nM/mg prot)	Liver	0.180± 0.015	0.0487± 0.008***	0.092± 0.020* b	0.210± 0.031NS c	0.179± 0.037NS c
	Kidney	0.335± 0.013	0.237± 0.023*	0.3602± 0.036NS b	0.2915± 0.033NS a	0.3519± 0.035NS b
	lungs	0.590± 0.086	0.1492± 0.074***	0.324± 0.105** c	0.2312± 0.008*** b	0.279± 0.026** b

Effect on level of Reduced Glutathione (GSH)

With regard to GSH. (in the liver, kidneys and lungs), we notice in Table 2 a decrease in its concentration (P≤0.001) in liver and pulmonary cells and a highly significant (p≤0.01) in renal cells in the group exposed to pesticide compared to control rats (table 2). On the other hand, in order to study the effect of aqueous extract of *Astragalus gombo* on the level concentration of GSH, an increase in hepatic glutathione content was observed with a very highly significant difference (p≤0.001) in batches (F and M) and highly significant (p≤0.01) hepatic and renal for group (R) and slightly reduced in lung content compared to metribuzin-exposed rats.

Table 3. Level of Catalase activity in the liver, kidneys and lungs

Parameters		GROUP1(T) N=5	GROUP2(P) N=5	GROUP3(R) N=5 80 mg/kg	GROUP4(F) N=5 60mg/kg	GROUP5(M) N=5
Catalase(CAT) (UI/g prot)	Liver	24.82± 0.235	31.63± 0.479**	26.01± 1.87NS b	25.20± 1.30NS b	22.091± 0.312NS c
	Kidney	39.79± 0.812	51.932± 0.58***	39.85± 0.842NS c	42.93± 0.735* b	38.14± 0.593NS c
	lungs	49.93± 1.33	68.85± 0.896**	63.12± 0.862** a	60.62± 0.932* b	67.30± 0.730**

Effect on the level of Catalase (CAT)

The results shown in table 3 that the exposure of rats to metribuzin caused an increase in the enzymatic activity of the Hepatic and pulmonary catalase (p≤0.01) and increased renal activity with a very highly significant difference (p≤0.001) compared to healthy rats. We also note, the effect treatment of rats by the root extracts of the plant induced a significant decrease in the level of the enzyme in the kidneys (p≤0.001) and the liver (p≤0.01), the lungs (P ≤ 0.05) compared to the group exposed to the pesticide alone. The treatment of pesticide-exposed rats with plant leaf extracts and drugs (groups F and M) lead to a significant decrease in catalase activity in all the tissues studied mainly in the liver (p≤0.01) compared with to the pesticide control.

Histopathological studies

GSH level (in the liver, kidneys and lungs), there were a decrease in its concentration (P ≤0.001) in liver and

pulmonary cells and a highly significant (p≤0.01) in renal cells in the group exposed to pesticide compared to control rats.On the other hand, in order to study the effect of the aqueous extracts of d'*Astragalus gombo* the concentration of GSH, it was observed an increase in hepatic glutathione content with a very highly significant difference (p≤0.001) in the batches (F and M) and highly significant (p≤0.01) hepatic and renal for group (R) and slightly reduced in lungs content compared to metribuzin-exposed rats.

Discussion

The effect of metribuzin on oxidative stress parameters is increased concentration of tissue MDA in all the organs studied at the exception of Lungs. These results are comparable with the study of Chialif (2014), which shows an increase in tissue MDA concentration in metribuzin-exposed rats. This indicates a decrease in antioxidants that play a very important role in the inhibition of lipid peroxidation (Shalanand Coll, 2005). Metribuzin is able to induce intracellular oxidative stress (Medjdoub and Coll, 2011). It's important to know that lipid oxidation makes membranes more rigid resulting in the development of many pathological processes (Levin and Coll., 1990; Das and *Coll,* 2008). These results can also be explained by the accumulation of free radicals, generated by metribuzin all of which results in lipid peroxidation in liver and kidney tissues.

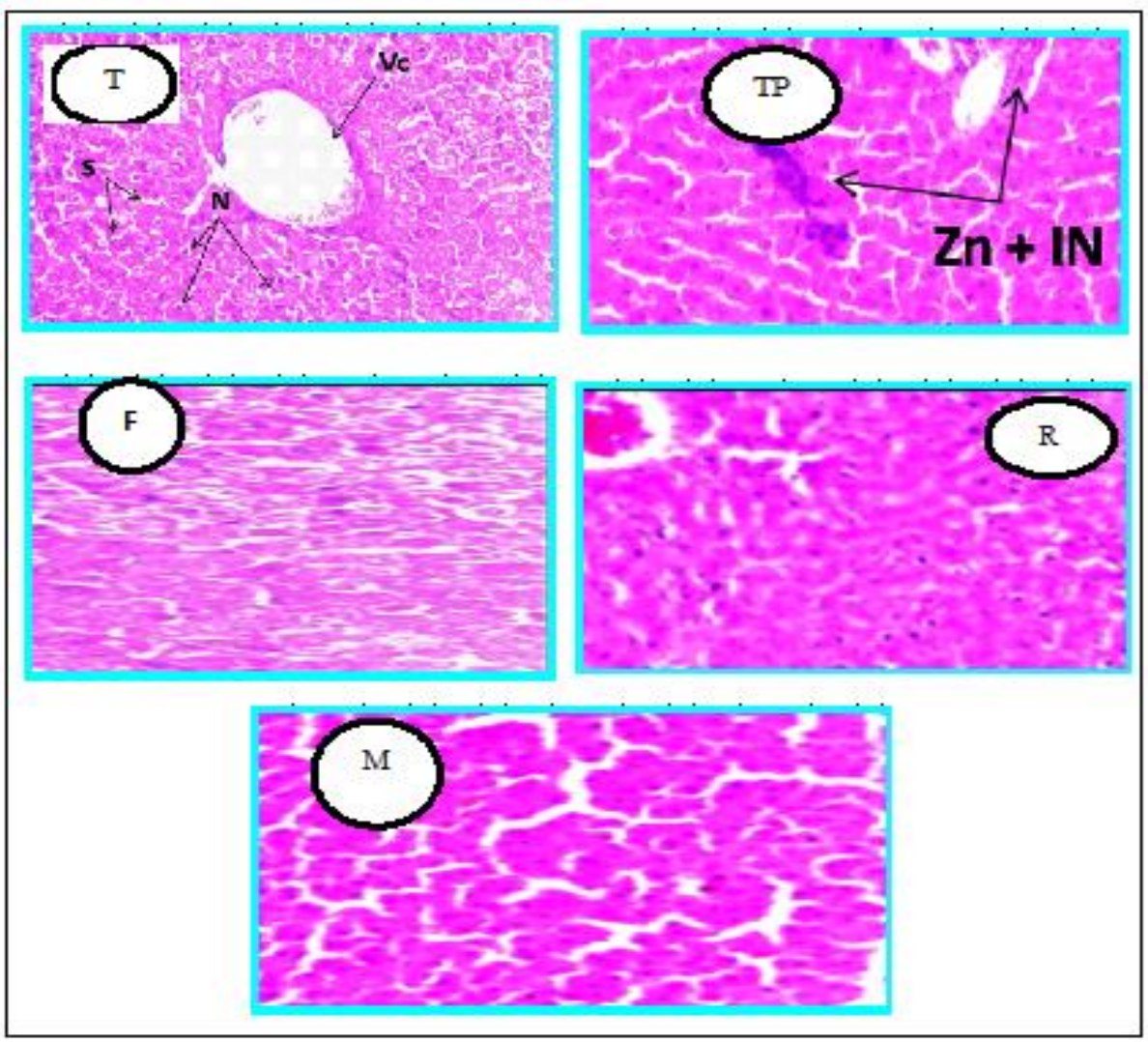

Fig. 1. Microscopic observation of a histological section of the liver (x40) (Original photos, 2018).
T: Control, TP: Control Pesticide, R: Root, F: Leaf, M: Drug
Zn + IN: Zone of necrosis with inflammation.

On the other side, the rats treated with the drug and the aqueous extract of the studied plant which contains cellular chemoprotective compounds provided with an antiperoxidant power (Selami and Boukhezza, 2014). These compounds could prevent the metribuzin-induced alteration and play an important role in the prevention of complications due to lipid peroxidation, also used against the deleterious effect of free radicals. In an in vivo model (mouse), administration of a diet supplemented with *Astragalus gombo* significantly decreased malondialdehyde (MDA) content in the liver of treated animals (Yin and *Coll,* 2010). All this explains the maintenance of MDA at its normal cytosolic levels in the organs studied. This repair is clearly observed in the rats treated with the leaves and the drug.

Furthermore, there is a significant decrease in the activity of tissue GSH in the liver, kidneys and lungs in rats exposed to metribuzin. Reduced glutathione (GSH) is the main non-enzymatic antioxidant intracellular agent (Jacob, 2007), which has the detoxification capacity where it appears to play a role in the cellular protection of oxidative stress (Schwab, 2011). GSH shows the majority of glutathione in cells, an increase in oxidized form (GSSG) of reduced glutathione (GSH) indicates the presence of a state of oxidative stress (Pelletier and *coll.,* 2004). The transformation of oxidized glutathione into reduced glutathione is done by glutathione reductase (GR). Aouacheri, (2009) reported, the decrease in GSH levels in lactating rats and their offspring may be a consequence of its increased use by cells to trap radicals.

Consequently, the treatment of rats with the aqueous extract of *Astragalus gombo* and the drug prevented the decrease of GSH in the groups exposed to the pesticide and treated with the extract of the plant and the drug, that is to say instead, the free radicals produced by Metribuzine neutralize by GSH they will rather be captured by the phenolic compounds thus maintaining the normal rate of cellular glutathione which in agreement with the study of (Yan and *Coll*, 2010). Antioxidant enzymes (such as CAT) limit the effects of oxidative molecules in tissues and play a role in the defense against cellular oxidative damage as scavengers of free radicals (Gutteridge, 1995). They constitute a ROS defense support team (Ahmed and Coll, 2013, Soudani and *Coll,* 2011). The results obtained show the increase of enzymatic activity of catalase in all tissues in rats exposed to Metribuzine compared to control rats. These results disagree with several authors who have tested the effect of certain types of pesticides resulting in increased activity of catalase in animals, due to the existence of these pesticides in the body and subsequently stimulating antioxidant defense (Sarkar and Coll, 2014; Simeonova and *Coll,* 2013; Adhikari and Coll, 2001; Zaheer and Coll, 2013; Datta and *Coll,* 2011). Also the high activity of catalase is an adaptive response against the increased generation of free radicals produced by Metribuzine (Koner and *Coll,* 1998).

Generally, it can be said that the *Astragalus gombo*(either the aerial or sub-terrestrial part) and also the drug administered in combination with the pesticide shows a highly significant decrease ($p \leq 0.01$) in maintaining the equilibrium of the antioxidant / prooxidant balance cells of all tissues studied. This balance is maintained under the effect of the antioxidant action of the plant against the RL. This indicates the interference of the phenolic compounds of this plant in the capture of free radicals.

There is a gradual return of catalase to its normal range what's confirmed by the study of (Aouacheri, 2009; Ahmed and *Coll,* 2013).

In order to confirm the pesticidal toxicity and the therapeutic effect of the plant at the tissue level, histological sections on the liver were made, in the control rats and in the rats, the histological examination was designed to complete the previous results where it's noted that Metribuzine induced remarkable structural changes (the appearance of necrosis and an inflammatory cell infiltrate).

These damages could be probably due to the generation of reactive radicals and lipid peroxidation.

However, treatment with the two extracts (leaves and roots) and vitamin E (positive control) in the metribuzin-exposed group resulted in a remarkable improvement of the liver tissue, especially the group treated with the leaves of the plant by repair. lesions derived from pesticide, therefore the aqueous extract of *Astragalus gombo* has a hepatoprotective property due to the presence of antioxidants provides significant protective effects can be used as hepatoprotective agent and antioxidant for the treatment of liver disorders (Chouanna, 2017).

Conclusion

The treatment with the two extracts of *Astragalus gombo* decreases the state of oxidative stress induced by metribuzin exposure by limiting free radical phenomena and repairing oxidative damage by decreasing lipid peroxidation in the liver, kidney and liver. Finally, the histopathological analyzes confirm the results obtained, it is good that the tissue analysis performed at the level of livers of rats treated and not treated by the plant allowed to show that the two plant extracts and especially the leaves have an activity antioxidant and provides protection against necrosis and hepatic inflammation. Short term supplementation and lower dose might be the reason, so It's necessary to work with higher dose of roots extract for longer duration.

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