Research Article

In Vitro Antioxidant Activity and Hepatoprotective Effect of *Genista ulicina* Spach Extract in Chlorpyrifos-Induced Toxicity

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ABSTRACT

In this study, the *n*-butanol extract of *Genista ulicina* Spach was evaluated for its antioxidant properties and for its hepatoprotective effect against Chlorpyrifos-Ethyl (CPF) toxicity. The effect of *n*-butanol extract of *Genista ulicina* at the dose (100 mg/kg) was studied on Chlorpyrifos-Ethyl induced hepatic damage (20 mg/kg) by gavage for 10 days in *Wistar Albinos* rats. The liver marker enzymes, aspartate transaminase (AST), alanine transaminase (ALT) and lipid peroxidation (MDA) were estimated to access liver damage. Severe alterations in all biomarkers were observed after CPF treatment. CPF induced hepatic dysfunction revealed by a significant elevated in serum enzymes, AST, ALT and alkaline Phosphatase relative to control values. It was found that combined *Genista ulicina* (100mg/kg) extract and CPF treatment decreased significantly TBARS, AST, ALT and alkaline Phosphatase level compared to the CPF group ($p<0.01$). The results of *in vivo* experiments showed that the *n*-butanol extract of *Genista ulicina* inhibited lipid peroxidation and protected the experimental animals from CPF-induced hepatic toxicity.

Keywords: Chlorpyrifos-Ethyl; Antioxidant Activity; Hepatotoxicity; Genista ulicina; Phenolic and Flavonoid Compounds.

INTRODUCTION

Organophosphorus insecticides (OPs) constitute a large family of pesticides and are widely used for controlling pests in the household, agricultural and urban environment[1]. These pesticides, lack specificity, and it has been demonstrated that they are also highly toxic to non-target species, including mammals, birds and aquatic organisms. They can reach natural waters either via transfer of the chemicals from the soil or by directing praying organisms[2]. CPF is an effective organophosphate (OP) pesticide used heavily throughout the world for agriculture and domestic purposes. CPF elicits a number of effects, including hepatic dysfunction, immunological abnormalities, embryotoxicity, genotoxicity, teratogenicity, neurochemical, and neurobehavioral changes[3]. In fact, pesticides are known to increase the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues[4,5]. Oxidative damage primarily occurs through the production of reactive oxygen species (ROS), including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues. Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipids bilayers, and they may damage membranes by inducing lipid peroxidation (LPO)[5].

Antioxidants are a group of substances which, when present at low concentration, in relation to oxidizable substances, significantly inhibit or delay oxidative process[6]. They have also been shown to protect against cytotoxicity caused by oxygen radicals. Flavonoids have been reported to exhibit potent antioxidative and free radical scavenging activities. They may scavenge ROS, chelate metal ions, act as chain-breaking antioxidants by scavenging lipid peroxyl radicals, or integrate into the lipid bilayer to prevent lipid damage[7]. Besides, Flavonoids have been recognized to possess anti-inflammatory, anti-allergic, antiviral and antiproliferative activities[8].

The aim of this study was to examine the hepatoprotective effect of *n*-butanol extract of *Genista ulicina* against oxidative stress induced by Chlorpyrifos in Wistar Albinos rats.

MATERIALS AND METHODS

Plant material

Aerial parts of *Genista ulicina* Spach. (Fabaceae) were collected from the area of El Kala in the North-East of Algeria on May 2008 and authenticated on the basis of Quezel and Santa[9] by Professor Mohamed Kaabache (Ferhat Abbas University, Setif 1, Algeria). A voucher specimen (GUF0508-EK-ALG-70) has been deposited in the Herbarium of the VARENBIOMOL research unit (University Frères Mentouri, Constantine 1).

Extraction and Isolation

Air-dried aerial parts of *Genista ulicina* Spach. (495 g) were macerated at room temperature with EtOH-H2O (70:30 v/v) three times for 72 hours. The filtrates were combined, concentrated under reduced pressure, diluted in H2O (250 mL) under magnetic stirring and extracted with petroleum ether to remove a maximum of chlorophylls. The remaining aqueous solution was extracted successively with chloroform, ethyl acetate and

n-butanol to obtain CHCl3 (2.04 g), EtOAc (1.31 g) and *n*-BuOH (21.76 g) extracts.

Determination of total phenolic content

The total phenolic content of *n*-butanol extract of Genista ulicina Spach was determined using *Folin-Ciocalteu* reagent according to the method of Singleton[10]. The reaction mixture was composed by mixing 20µl of sample solution, 1580 µL of distilled water, and 100µL of the *Folin–Ciocalteu* reagent. The mixture was shaken. After 3 to 8 min, 300 µl of 20 % sodium carbonate was added and the mixture was allowed to stand for 30 min at 37 C° in bath water. The absorption at 765 nm was measured against a blank, which contained 20 µl of methanol in place of the sample. The total phenolic contents were expressed as micrograms gallic acid equivalents (µg GAE) per gram of extract. All samples were analyzed in three replicates.

Determination of total flavonoid content

Total flavonoid content was determined using the method of Ordonez[11]. A volume of 0.5 ml of 2% $AlCl_3$ of methanol solution was added to 0.5ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml and calculated as quercetin (µg /mg) using the calibration curve. Results were expressed as µg quercetin equivalents (QE)/ mg extract. Measurements were performed at least in triplicate.

2-4-Antioxidant activity assay

The scavenging activity of the DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical was assayed according to the method of Farhana[12]. Briefly an aliquot (0,1ml) of each sample (With appropriate dilution if necessary) was added to 0.3ml of methanol DPPH solution (0.004%). Discolorations were measured at 517nm after incubation for 30min at 30°C in the dark. Measurements were performed at least in triplicate. The percentage of DPPH which was scavenged was calculated by the following equation:

$$I\ \% = (Acontrol-Asample/Acontrol) \times 100$$

Then % inhibitions were plotted against respective concentration uses and from the graph, IC50 was calculated using Myricetin as standard.

Experimental animals

Male Wistar Albinos rats weighing (166 - 229g) were used in the present study. The animals were kept in 12h light/dark cycles and maintained in an air-conditioned room at 22 to 25 °C, with free access to food and water ad libitum for two weeks. The general guidelines for the use and care of living animals in scientific investigations were designed according to the ethical standards for animals use and approved by the local ethical committee of animal use and followed[13].

The animals were divided into 4 groups, six animals each. All treatments were administered orally by gavages.

Group 1: untreated rat and served as controls.

Group2: CPF Received 20 mg/kg Chlorpyrifos daily for 10 days.

Group3: Ext. Received extract at the dose of 100mg/kg daily for 10 days.

Group4: Ext + CPF (Received 100 mg/kg extract and 20 mg/kg Chlorpyrifos (the extract was administered after 35-40 minutes CPF treatment) for 10 days.

After treatment, rats were sacrificed and dissected; blood was collected and liver was removed for measurement of various biochemical parameters, such as levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Malondialdehyde (MDA).

Lipids peroxidation assay

The extent of LPO was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) using the method of Uchiama and Mihara[14]. The principle of the method is based on spectrophotometric measurement of color produced during the reaction of thiobarbituric acid (TBA) with MDA[15]. For this purpose, 0.5 ml of plasma or 10% homogenate was taken in a 10 ml centrifuge tube to which 3 ml of 1% phosphoric acid and 1 ml of 0.67% aqueous thiobarbituric acid (TBA) were added. The mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml of *n*-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and its absorbance was measured at 532 nm. Malondialdehyde was used as standard.

Biochemical evaluation

The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), was determined by the spectrophotometric method of Reitman-frankel[16] using BioMerieux kits. Also, activity of alkaline phosphatase (ALP) was estimated using commercial kits.

Statistical analysis

Data are expressed as the mean ± SD. The student's test and a one-way analysis of variance (ANOVA) were used for multiple comparisons followed by Tukey-kramer multiple comparisons test. $P < 0.05$ was considered to indicate statistical significance. (SPSS program, version, 13.0).

RESULTS AND DISCUSSION

Determination of total phenolic and flavonoid content

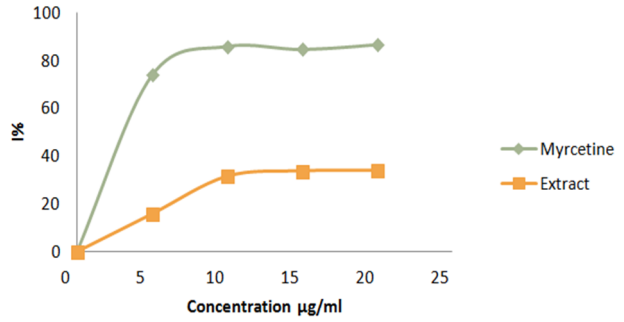
Total phenol content is expressed in microgram of gallic acid per milligram of freeze dried sample. The total phenolic content of *Genista ulicina* Spach of *n*-butanol extract was estimated to be (260 ± 0.002 µg gallic acid equivalent/mg) extract. However, the flavonoid content was also high (318±0.01 µg quercertin equivalent/mg

extract). The antioxidant properties of flavonoids are due to their ability to directly scavenge some radical species. Flavonoids may also act as chain-breaking antioxidants and/or may recycle other chain-breaking antioxidants, such as α-tocopherol, by donating a hydrogen atom to the tocopheryl radical[7]. The high phenolic and flavonoid content of the *n*-BuOH extract correlate very well with the strong scavenging activity observed of this extract[17].

Antioxidant activity

DPPH is the most used tests to evaluate antioxidative activity. *n*-Butanol extract of *Genista ulicina* Spach presented concentration dependent hydrogen donation ability as shown in (Fig 1).

The antioxidant test (DPPH) indicates that *Genista ulicina* extract showed a good scavenging activity with IC50 value 3.64 µg/ml compared to Myrcetine used as standard (IC50 = 2.11 µg/ml).



Values are mean± SD (n=3)

Figure 1: DPPH scavenging activity of *n*-butanol extract of *Genista ulicina* Spach.

Evaluation of Malondialdehyde (MDA) levels plasma MDA level

MDA is a major oxidation product of peroxidized polyunsaturated fatty acids, and increased MDA content is an important indicator of lipid peroxidation[7]. The obtained results indicated a significant increase (P<0. 01) in plasma MDA level in CPF treated group and a significant decrease (P <0.05) in extract treated groups compared to control group respectively. Where the extract showed a lower decrease in plasma MDA level in animals treated with both CPF and extract compared to CPF group (fig2).

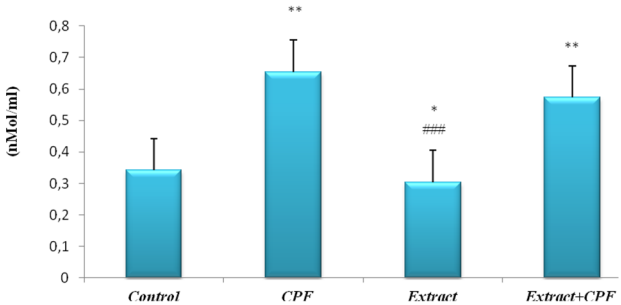


Figure 2: MDA plasma levels in control and experimental groups.

*P <0.05 control vs. other groups; #P <0.05 CPF vs. other groups.

Values are mean± SD of Six rats in each group

b-Liver MDA level

Compared to control group we observed a significant increase (P<0. 01) in liver tissues MDA level in the CPF treated group, when we observed a significant decrease in the MDA tissues levels in the *Genista ulicina* extract treated group and extract+CPF treated group compared to CPF group (fig3).

The increase of MDA formation may be due to the OPI's themselves, inducing LPO or possibly due to an increase in ROS induced by OPI's[18]. LPO may be considered as the first step of cellar membrane damage by OP[19], however pretreatment of *Genista ulicina* extract lead to significant decrease in MDA level indicated that it may have a beneficial role in lowering CPF –induced toxicity.

Due to their antioxidant property, polyphenols in plant extracts may afford protection. The data showed a significant increase in plasma and tissue LPO levels in animals treated with Chlorpyrifos pesticide. The decrease in LPO levels after treatment with the plant extract revealed its antioxidant property[20].

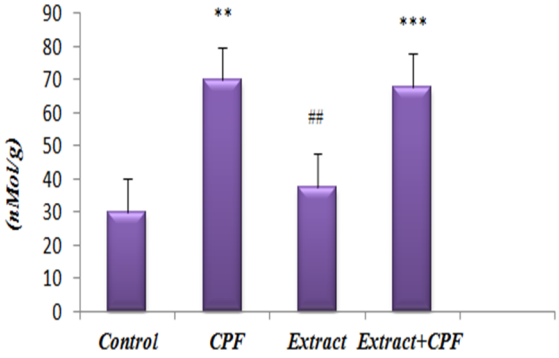


Figure 3: MDA liver levels in control and experimental groups.

*P <0.05 control vs. other groups; #P <0.05 CPF vs. other groups.

Values are mean± SD of six rats in each group.

Change in hepatic function

Liver is the organ where activation and detoxification of chlorpyrifos takes place[21]. When the liver cell membrane is damaged, several enzymes located in the hepatocyte cytosol, including ALT, AST and ALP are secreted into the blood. Consequently, these serum enzymes are markers of liver function[22].

The result of levels of hepatic enzymes ALT, AST and ALP were shown in fig 4 and fig 5. Compared to control group, CPF treated group had significantly increased ALT, AST (P <0.01) and ALP levels (P <0.001). The increase in these enzymes may be due to liver dysfunction and disturbance in the bio synthesis of these enzymes with alteration in the permeability of liver cell membrane[23].

When the extract plant treated group was shown a significant decrease (P <0.01) in ALT, AST and ALP compared to control group. CPF plus plant extract had

significantly lower ALT, AST and ALP level compared to CPF group.

This fact may explain the role of *n*-butanol extract to decreased oxidative stress induced by CPF, and normalized the biological concentration.

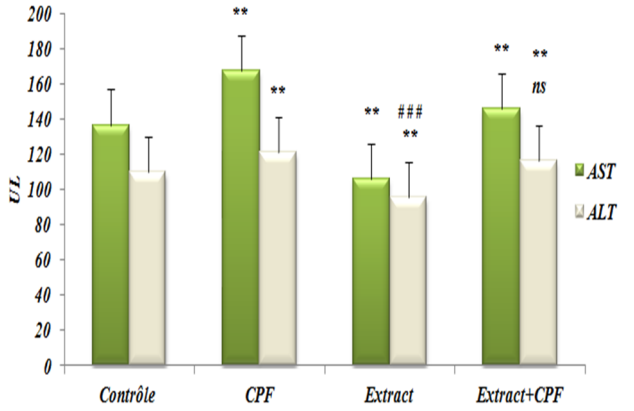


Figure 4: AST and ALT levels in control and experimental groups.

*P <0.05 control vs. other groups; #P <0.05 CPF vs. other groups.

Values are mean± SD of six rats in each group.

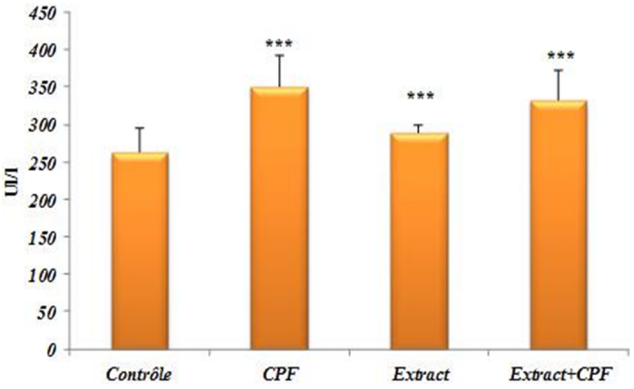


Figure 5: ALP levels in control and experimental groups.

*P <0.05 control vs. other groups; #P <0.05 CPF vs. other groups.
Values are mean± SD of six rats in each group.

CONCLUSION

In conclusion, the present study finding indicate that oral exposure of chlorpyrifos induced cellular oxidative as evidenced by increasing Plasma and liver lipid peroxidation levels. Also, serum enzymes including ALT, AST and ALP are increased. Pretreatment of *n*-butanol extract of *Genista ulicina* may attenuated oxidative stress induced by CPF then ameliorated biological status. We also found that this extract exhibited remarkable antioxidant activity on the DPPH radical scavenging activity. The high levels of antioxidant activity were due to the presence of phenolic compounds.

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