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# Bio-Synthesis of Zinc Nanoparticle Using *Helianthemum Lippii* L. Extract and Improving in Rats Their Hepatoprotective Effects against Carbon Tetrachloride Induced Liver Damage

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**ABSTRACT:**

The aim of this study is the green biosynthesis and spectroscopic characterization of Zinc Oxide Nanoparticle (ZnO-NPs) from aqueous extract of plant species: *Helianthemum lippii* L. and the evaluation of its variation of biochemical parameters against oxidative damage induced by carbon tetrachloride in female rats of the Wistar Albino type. Our result compared with a medication of a plant base; Silymarine.

Our *In vivo* experiment was carried out on 30 rats; divided into 6 batches of five rats each. The phytochemical study results obtained show that our aqueous extract has a yield of around 7.08%. According to the Fourier Transforms Infra-Red (FT-IR) results of the green biosynthesis of ZnO-NPs, we notice: functional group involved in the synthesis of ZnO-NPs illustrated in parts 900 and 700 cm -1, likewise the observations of the Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX) analysis we keep Nanoparticles cluster of different shapes with a size of the order of nanometer. For evaluation of variation of biochemical parameters in rats; there is a considerable decrease in body weight of individuals poisoned by $CCl_4$ and an increase in relative liver weights in the group contaminated with $CCl_4$ compared to the control. In addition, for the products tested: Aqueous extract and ZnO-NPs impressed us with their regulatory capacity with respect to the levels of serum enzymes (TGO, TGP, PAL and GGT) that they are disturbed by $CCl_4$ with a

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very significant decrease ($p < 0.001$), even for biochemical parameters such as (Bilirubin, Cholesterol, Triglyceride, Urea and Creatinine).

In conclusion, the results of this study show that: Aqueous extract and ZnO-NPs and especially the synergy between ZnO-NPs and aqueous extract of the plant *Helianthemum lippii* L. can be proposed to protect the liver against oxidative damage induced by tetrachloride of carbon.

**Keywords:** Green biosynthesis, Zinc Oxide Nanoparticle, *Helianthemum lippii* L, Antioxidant power, $CCl_4$**.**

## 1. Introduction

Over the last few years, many studies and research have been carried out on nanomaterials. They are no longer confined to research laboratories, but are now integrated into many industrial processes and contribute to the composition of a wide variety of products or systems used in everyday life (Sunscreens, Textiles, Food, Transport, etc.) [1]. Nanotechnologies have developed energetically as an essential area of modern research with effects in electronics, biochemistry and biotechnology. [2].

A major aspect of nanotechnologies is the bio-synthesis (green synthesis) of nanoparticles. With the advent, new techniques and improved scientific knowledge have paved the way for the use of biological entities in the synthesis of nanoparticles instead of chemicals such as chemical solvents for-example : Carbon tetrachloride, Benzene, Chloroform, Dichloropropane, Dibromoethane, Dichloroethane, Chlorobenzene, Tetrachlorobenzene… etc., (This gives us an overview of the public health problem posed by pathologies at the global level) among which the use of plants in particular plant extracts rich in bioactive substances for the synthesis of nanoparticles may be advantageous compared to other biological entities [3]. The biological synthesis of nanoparticles by the plant extract is currently being exploited on the grounds that developing a synthesis of benign nanoparticles in the environment and to avoid adverse effects for humans. [4,5]. In addition, recent reports on plants towards the production of nanoparticles would have such advantages as readily available [6]., safe to handle and a wide range of bioactive compounds especiallythe antioxidants such as flavonoids tannins, terpenoids, phenols, ... etc [7].

The green synthesis of nanoparticles needs to be undertaken within plant biodiversity using ethno-pharmacological data. This approach is used to select potentially active plants and significantly increase the number of discoveries of new assets. So far, out of the 300000 plant

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species identified, it is estimated that only 15% of them have been studied phytochemical, of which 6% of their biological activities against xenobiotic intoxication, which makes plants a reservoir of bioactive molecules yet little explored [8].

The objectif of this study is the green synthesis and spectroscopic characterization of Zinc Oxide Nanoparticle (ZnO-NPs) from plant specie: *Helianthemum lippii* L. and the evaluation of its variation of biochemical parameters against oxidative damage induced by carbon tetrachloride in rats.

## 2. Material and Methods

### 2.1 Chemicals and reagents

All chemicals were of analytical grade and purchased from Sigma-Aldrich, Mo, USA.

### 2.2. Plant Material:

Vegetal material *Helainthemum Lppii* L. aerial parts were collected in 2019 in Wilaya of El-Oued, south-eastern Algeria; and were identified by the botanist Prof. Atef Chouikh (Faculty of Natural and Life Sciences, University of El Oued, Algeria). The aerial parts of plant material was washed, dried and grounded into powder then stored at room temperature until use.

### 2.3. Preparation of aqueous extract:

100 g of vegetable material *Helainthemum Lppii* L. is macerated in 1000 ml of distilled water for 24 hours in the shade and at room temperature. The extract recovered by filtration, the solvent was removed using a rotary evaporator and incubated at 40°C to dry completely. The extract was weighed and stored in a refrigerator at 4°C for future analysis. [9].

### 2.4. Green synthesis of Zinc Oxide nanoparticle:

50 ml of dehydrated zinc acetate (0.02M) was prepared with distilled water. After 10 minutes of agitation in a magnetic shaker, 1 ml of aqueous extract of *Helianthemum lippy* L. (0.5 mg/ml) was added and followed by sodium hydroxide (2.0 M). The mix was left agitated for 2 hours until a white precipitate formed. Centrifugation was performed at 4000 rpm for 10 minutes; the pellet was continually removed and washed with distillate water and ethanol to remove impurities from the final product. The mixtures were heated at 60°C and incubated in a dark for 24 h. The nanoparticle was carefully collected and dried to get a final mass of ZnO-NPs for further future experimentations and characterizations [10,11].

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### 2.5. Characterization of Zinc Oxide nanoparticle:

The most effective aspect for identifying and characterization of nanoparticles is the Fourier Transforms Infra-Red (FT-IR) spectrum confirms the surface chemistry and organic functional group responsible for the stabilization of nanoparticles in the spectral region of 4000-600 cm-1 using a resolution of 4 cm-1 and 64 coadded scans.

Used the analysis Scanning Electron Microscope (SEM) for determining the surface morphology of ZnO-NPs. The elemental content of the reaction mixture was determined using Energy Dispersive X-ray (EDX). The (SEM) (JEOLJSM 5800) machine was used to perform the (EDX) analysis on the ZnO-NPs sample. To validate the conversion of silver ions into elemental zinc (Zn).

### 2.6. Determination of biochemical parameters variation:

*Animals and Handling*

*In-vivo* study Animals and Handling, Thirty six female rats Wistar albino rats, weighing 208.64 ± 5 g, were obtained from Pasteur Institute, Algeria. They were placed in animal's house of the molecularand cellular biology department, the university of El-Oued, Algeria, in controlled (24 ± 1 °C) temperature, photoperiod (12 h light/12 hdark) cycle, standardrat food and water were supplied ad libitum for the duration of the experiment. The rats were adapted for two weeks before tests under the same laboratory conditions. The experimental procedures were performed according to the National Institute of Health Guidelines for Animal Care and approvedby the Ethics Committee of our Institution.

*Experimental design*

The female rats were randomly divided into six groups, each containing 5 rats as follow: Group 1 (Control group): Animals served as normal control, Group 2 ($CCl_4$): Animals received I.P carbon tetrachloride (25 µl/kg/2 day) for15 days, Group 3 ($CCl_4$+*H.lippii*): Animals received I.P Carbon Tetrachloride (25 µl/kg/2 day) and administered 200 mg/kg of *Helianthemum lippii* daily in drinking water for 15 day, Group 4 ($CCl_4$+ ZnO-NPs): Animals received I.P Carbon Tetrachloride (25 µl/kg/2 day) and administered 100 mg/kg of ZnO-NPs daily in drinking water for 15 day, Group 5 ($CCl_4$+ZnO-NPs+*H.lippi*): Animals received I.P Carbon Tetrachloride (25 µl/kg/2 day) and administered 200 mg/kg of *Helianthemum lippii* and 100 mg/kg of ZnO-NPs daily in drinking water for 15 day, Group 6 ($CCl_4$+ Sly): Animals received I.P Carbon Tetrachloride (25 µl/kg/2 day) and administered 200 mg/kg of

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Silymarin daily in drinking water for 15 day. During treatment, body weight was recorded periodically during the experiment weeks.

*Blood collection and Samples preparation*

At the end of the experiment, rats fasted for 16 h, sacrificed after anesthetized with chloroform by inhalation and then decapitated, the blood samples were transferred into serum separating tubes and prepared by centrifugation for 10 min at 3000 revolutions/min and it was utilized for biochemical parameters analysis.

*Measurement of biochemical parameters*

(AST) : Serum Aspartate Transaminase, (ALT) : Alanine Transaminase, (ALP) : Alkaline Phosphatase, (GGT) : Gama-Glutamil Transferase., Bilirubin, Total protein, Cholesterol, Triglycerides, Creatinine and Urea.(parameters were measured by kinetic colorimetric method using technical cards and commercial kits obtained from Spinreact and BioSystems (Barcelona, Spain)).

### 2.7 Statistical analysis

Data was expressed as mean ± standard deviation (M ± SD) of six animals. Statistical analysis was carried out by using Student T-test to compare means between two groups. Results were evaluated using the Minitab and EXCEL software. Differences were considered significant at $p \leq 0.05$.

## 3. Results

### 3.1. Characterization of Zinc Oxide nanoparticle:

#### 3.1.1. Fourier Transforms Infra-Red (FT-IR) analysis

The Fourier Transforms Infra-Red (FT-IR) spectral analysis result was illustrated in Figure: 01. The spectrum was recorded in the wavelength region between 650 cm-1 to 4000 cm-1. The FT-IR spectrum showed a peak at 3400 cm-1 is attributed to the presence of O-H stretching of alcohols and phenols in the precursor. Stretch, 1650 cm−1 assigned to C=C Amino acid stretch, 1384 cm−1 assigned to C–H alkenes stretch, 1090 cm−1 assigned to C–N amines stretch and 700-900 Zn-O stretch. All this indicates that there are many fundamental groups involved in the transformation of zinc ions into zinc nanoparticles and their stabilization.

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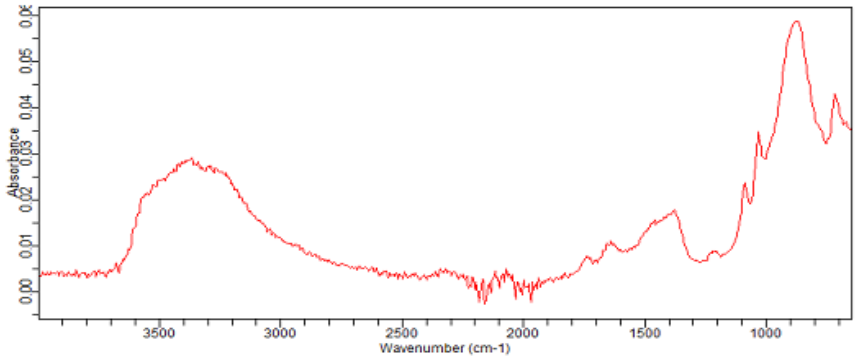


Figure 01: Fourier Transforms Infra-Red (FT-IR) spectra of ZnO-NPs.

### 3.1.2. Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX) analysis

The presence of very small and evenly nanoparticles is confirmed by Scanning Electron Microscope SEM images of samples obtained from colloidal Zn solutions produced (Figure : 02 A). The Energy Dispersive X-ray EDX profile revealed a high signal for Zinc (Figure : 02 B), as well as certain peaks that could have come from biomolecules coupled to the surface of ZnO-NPs, indicating that zinc ions were reduced to elemental zinc. While lesser signals from the O atom were detected. And therefore, ZnO-NPs were clearly successfully reduced by *Helianthemum Lippii* L. based on the EDX findings.

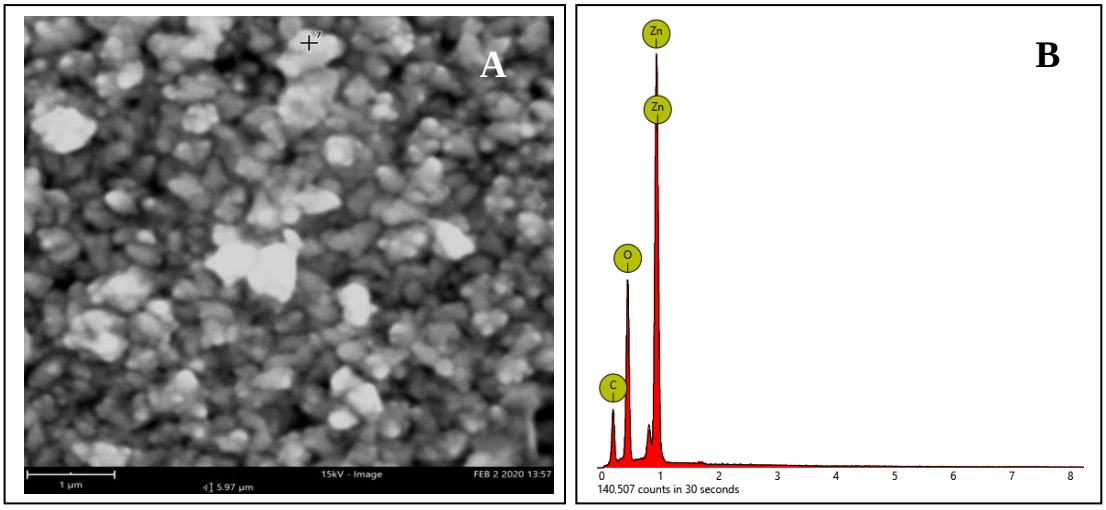


Figure 02: Scanning Electron Microscope and Energy Dispersive X-ray (SEM/EDX) spectra of ZnO-NPs.

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*A: Scanning Electron Microscope (SEM) images of* ZnO-NPs *samples, B: Energy Dispersive X-ray EDX profile of* ZnO-NPs *samples.*

### 3.2.1. Body weight and relative liver weight

Table: 01 shows that body weights rats within days of the experimental; a significant elevation of relative liver weight was seen in Carbon Tetrachloride treated group, indicating that Carbon Tetrachloride induced hypertrophy of these tissues. By contrast, areal part of *Helianthemum lippii* and Zinc Oxide Nanoparticle in combination with Carbon Tetrachloride significantly reduced the elevated weight of liver, suggesting the possibility of *Helianthemum lippii* and Zinc Oxide Nanoparticle to give protection against liver injury upon Carbon Tetrachloride induction.

Table: 01 Initial body weight, Liver weight, and relative liver weight in control and experimental groups.

| **Group** | **Weight** | | |
|---|---|---|---|
| | Initial body weight (g) | Liver weight (g) | Relative liver weight (%) |
| **Control** | 214.80±11.92 | 4.965±0.699 | 2.265± 0.429 |
| **$CCl_4$** | 200.2 ±8.93$^{*}$ | 6.096±0.333$^{***}$ | 3.051±0.257$^{**}$ |
| **$CCl_4$+ *H.lippii*** | 209.20± 4.21$^{b}$ | 5.296±4.66$^{a}$ | 2.555±0.267$^{b}$ |
| **$CCl_4$+ ZnO-NPs** | 213.2± 24.6$^{Ns}$ | 5.400±0.307$^{b}$ | 2.558±0.317$^{a}$ |
| **$CCl_4$+ *H.lippii* + ZnO-NPs** | 208.80 ±5.81$^{a}$ | 5.198±0.584$^{a}$ | 2.528±0.361$^{a}$ |
| **$CCl_4$+Sly** | 223.4±22.6$^{Ns}$ | 5.451±0.680$^{Ns}$ | 2.4416±0.2133$^{b}$ |

$CCl_4$: Carbon Tetrachloride. *H.lippii* : *Helianthemum lippii* L. ZnO-NPs: Zinc Oxide Nanoparticle. Sly : Silymarin**.** Results are presented as the mean ± S.E. (Standard Error) (n = 5). $^{(*/a)}p\leq0.05$, $^{(**/b)}p\leq0.01$ and $^{(***/c)}p\leq0.001$. $^{(NS)}p > 0.05$ as compared with Carbon Tetrachloride model group. bp < 0.05 as compared with normal control group.

### 3.2.2. Liver and serum enzymes activities

The protective effect of *Helianthemum lippii* extract, ZnO-NPs at dose level (100 mg/kg) and *Helianthemum lippii* + ZnO-NPs on the Carbon Tetrachloride induced modification in serum ALT, AST, ALP and GGT levels was shown in Table 01. A single dose of Carbon Tetrachloride caused hepatotoxicity in rats as indicated by an increase ($p\leq0.001$) in all serum

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enzymes activities after Carbon Tetrachloride administration. Whereas, Animals pretreated with plant extract and nanoparticle showed a significantly decreased ($p \leq 0.001$) activity of serum marker enzymes better than silymarin.

Table 2: Liver and serum enzymes activities in the control and experimental groups.

| Group | Parameters | | | |
|---|---|---|---|---|
| | ALT (U/l) | AST (U/l) | ALP (U/l) | GGT (U/l) |
| **Control** | 54.6 ±7.98749 | 20.4 ±1.67332 | 60 ±9.380832 | 0.84 ±0.114018 |
| **$CCl_4$** | 131.2 ±24.92388$^{**}$ | 53.2 ±4.91935$^{***}$ | 181.2 ±18.5661$^{***}$ | 2.04 ±0.114018$^{***}$ |
| **$CCl_4$+ *H.lippii*** | 82 ± 14.61164$^{b}$ | 25.2 ±1.095445$^{c}$ | 80.4 ±6.107373$^{c}$ | 1.132 ±0.202781$^{c}$ |
| **$CCl_4$+ ZnO-NPs** | 80.6 ± 6.268971$^{c}$ | 28 ±1$^{c}$ | 90 ±22.83637$^{c}$ | 0.52 ±0.192354$^{c}$ |
| **$CCl_4$+ *H.lippii* + ZnO-NPs** | 83 ±5.385165$^{c}$ | 31.2 ±1.30384$^{c}$ | 92.8 ±6.610598$^{c}$ | 1.1 ±0.2$^{c}$ |
| **$CCl_4$+Sly** | 77.6 ±1.949359$^{c}$ | 30.4 ±3.209361$^{c}$ | 65.6 ±18.11905$^{c}$ | 0.98 ±0.148324$^{c}$ |

$CCl_4$: Carbon Tetrachloride. *H.lippii* : *Helianthemum lippii* L. ZnO-NPs: Zinc Oxide Nanoparticle. Sly : Silymarin**.** Results are presented as the mean ± S.E. (Standard Error) (n = 5). $^{(*/a)}p \leq 0.05$, $^{(**/b)}p \leq 0.01$ and $^{(***/c)}p \leq 0.001$. $^{(NS)}p > 0.05$ as compared with Carbon Tetrachloride model group. bp < 0.05 as compared with normal control group.

### 3.2.3. Measurement of other biochemical parameters

As shown in Table 03, Carbon Tetrachloride ($CCl_4$) supplement caused a significant increase in serum of Cholesterol, Triglyceride, Urea and total Bilirubin ($p \leq 0.001$). Also, a significant increase for Creatinine ($p \leq 0.01$) and serum total protein levels ($p \leq 0.05$). During this time, *Helianthemum lippii* extract and Zinc Oxide Nanoparticle have recovered to the above biochemical parameters for curative or preventative treatment. In addition, treatment with the

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*Helianthemum lippii* extract and Zinc Oxide Nanoparticle does affect most of these parameters.

Table 03: Biochemical parameters in the control and experimental groups.

| Parameters | Control | $CCl_4$ | $CCl_4$ +*H.lippii* | $CCl_4$ +ZnO-NPs | $CCl_4$ + *H.lippii*+ ZnO-NPs | $CCl_4$ +Sly |
|---|---|---|---|---|---|---|
| **Cholesterol (g/L)** | 0.87 ±0.064 | 1.20 ±0.225$^{***}$ | 0.86 ± 0.054$^{c}$ | 0.90 ± 0.065$^{c}$ | 0.82 ±0.032$^{c}$ | 0.82 ±0.010$^{c}$ |
| **Triglyceride (mg/L)** | 0.13 ±0.01 | 0.18 ±0.008$^{***}$ | 0.17 ±0.019$^{b}$ | 0.09 ±0.015$^{b}$ | 0.17 ±0.023$^{a}$ | 0.09 ±0.01$^{c}$ |
| **Creatinine (mg/L)** | 5.01 ±0.707 | 7.03 ±0.707$^{**}$ | 6.20 ±0.447$^{b}$ | 6.21 ±0.447$^{b}$ | 6.80 ±0.448$^{Ns}$ | 6.81 ±0.836$^{Ns}$ |
| **Urea (g/L)** | 0.30 ±0.046 | 0.62 ±0.064$^{***}$ | 0.53 ±0.047$^{Ns}$ | 0.47 ±0.061$^{a}$ | 0.54 ±0.028$^{Ns}$ | 0.56 ±0.032$^{a}$ |
| **Bilirubin (mg/L)** | 1.41 ±0.098 | 5.02 ±0.342$^{***}$ | 4.16 ±0.545$^{a}$ | 4.16 ±0.545$^{a}$ | 3.12 ±0.521$^{c}$ | 2.92 ±0.286$^{c}$ |
| **Protein (g/L)** | 69.6 ±13.126 | 98.2 ±22.196$^{*}$ | 79.8 ±17.655$^{Ns}$ | 86.4 ±5.319$^{c}$ | 87.2 ±2.167$^{b}$ | 89.2 ±3.420$^{b}$ |

T0: Control. $CCl_4$: Carbon Tetrachloride. *H.lippii* : *Helianthemum lippii* L. ZnO-NPs: Zinc Oxide Nanoparticle. Sly : Silymarin**,** AST : Serum Aspartate Transaminase, ALT: Alanine Transaminase, ALP : Alkaline Phosphatase, GGT : Gama-Glutamil Transferase. Results are presented as the mean ± S.E. (Standard Error) (n = 5). $^{(*/a)}p\leq0.05$, $^{(**/b)}p\leq0.01$ and $^{(***/c)}p\leq0.001$. $^{(NS)}p > 0.05$ as compared with Carbon Tetrachloride model group. bp < 0.05 as compared with normal control group.

## 4. Discussion

The phytochemical analysis carried out on the aqueous extract plant *H.lippii*. has shown the presence of several active principles of the secondary metabolism in the aerial parts such as: terpenoids, saponins, alkaloids and tannins. [12]. Additionally. Important tenures of natural products existence, it can be said that its different compounds of secondary metabolism are

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probably responsible for the different biological activities of the plant *H.lippii*, the natural compounds have an interesting biological activities and that justify their abundant and varied uses in traditional medicine [13]. These results suggest also that some plants such as plant *H.lippii* can be useful as a source of chemicals compounds with strong antioxidant activity [14].

The spectroscopic analysis of Zinc Oxide Nanoparticle ZnO-NPs synthesized from plant *H.lippii*. extract by Fourier Transforms Infra-Red; shows different absorption bands of these molecules. The large peak appearing at 980 and 700 cm-1 in ZnO-NPs Fourier Transforms Infra-Red spectrum, is the characteristic peaks of ZnO molecules. The small peak at 1650 cm -1 could be attributed to the stretch C [15]. The net band at 1090 cm 1 indicates the C–O–C group [16]. We also observed transmission peaks around 1384 concerning the stretching mode C-N [17]. and a wide peak (O-H) has also been observed stretching 3000 cm-1 to 3600 cm-1 is due to the stretching of O-H group vibrations in water, alcohol and polyphénols [6].

The characterization of ZnO-NPs with Scanning Electron Microscope (SEM) showed that the molecules combine in clusters of different forms, with a size of the order of nanometer, the results obtained confirm the ability of the plant extract to synthesize ZnO-NPs. The mechanism through which these extracts from its responsible for the synthesis of nanoparticles, may be due to the coordination of ZnO-NPs with the polyphenol -OH and C-O groups. [6]. The synthesis is due to the presence of polyphenolic compounds like flavonoids, that are involved in the synthesis of ZnO-NPs from plant *H.lippii* extract.

Our results show that intraperitoneally injection of $CCl_4$ (25 µl/kg/2) causes disturbances in the physiological growth of animals. In fact, there was a decrease in the growth of rats intoxicated with xenobiotics compared to controls. [18]. This finding suggests that $CCl_4$ has adverse effects on the body growth of experimental animals. The decrease in weight is accompanied by an increase in relative liver weight in female rats treated with $CCl_4$. It can be inferred that the liver represents the tissues targeted for $CCl_4$ poisoning, which increases their size and indicates hepatomegaly caused by the chemical xenobiotic $CCl_4$. This result is consistent with studies [19]. On the other hand, rats group 2 ($CCl_4$+*H.lippii)* have recorded a significant increase in body weight could be due to polyphenol constituent that can improve nutritional and healthy states leading to an improved body weight gain poids. [20]. Moreover, hepatoprotective properties confirmed the role of medicinal plants phenolic compounds as

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antioxidant active ingredients. This results in the detoxification of liver tissue, decreasing the relative weight of the liver. [21]. We also observed an increase in body weight after the administration of ZnO-NPs and following the toxicity of $CCl_4$ (Group 3 and 4), due to the ability of ZnO-NPs to penetrate more easily through their small size and involve in cell proliferation, differentiation and growth [22]. A low dose of ZnO-NPs could improve antioxidant activity and lower levels of free radicals. So ZnO-NPs could protect liver cells from the harmful effect of xenobiotics [23].

Results of $CCl_4$ effect on serum enzyme levels showed a highly significant increase in the activity of ALT, AST, ALP and GGT for the intoxicated ratts compared to the control. Instead, serum ALT, AST, ALP and GGT activities were significantly inhibited after treated with plant *H.lippii* extract, ZnO-NPs and the synergy between ZnO-NPs and *H.lippii* extract indicating predictive of effectiveness of these compounds in liver regeneration after damaged. $CCl_4$ is highly hepatotoxic xenobiotics and causes damage involving biotransformation of $CCl_4$ by cytochrome P-450 system in a free radical trichloromethyl ($CCl_3$.), then by transforming this free radical into a more reactive radical trichloromethylperoxyl ($CCl_3O_2$), which causes lipid peroxidation. In general, this increase reflects the severity of liver injury. [24]. Furthermore. Serum enzymes TGO, TGP, are synthesized in the cell cytoplasm and released into the bloodstream in case of damaged cells and considered the best indicators of hepatic parenchyma cytolysis, which source from the cell membrane and mitochondrial damages in liver cells by different toxic drugs such as Lambda-cyhalothrin insecticide [25,26]. PAL is present in the plasma membrane of hepatocytes and the level of PAL in the blood is directly related to damage in the membrane of liver cells [27]. The results of Rousseau (1978) [28] confirm the notable increase in rats poisoned with carbon tetrachloride and the untreated serum GGT concentration due to the involvement of bile duct epithelial cells.

Elevated bilirubin in the blood could be attributed to high synthesis, increased haemolysis, decreased conjugation or decreased transport [29], and therefore. Bilirubin is used as an indicator to assess the secretory function of hepatocytes [30]. Under our experimental conditions, the treatment of female rats by ZnO-NP, *Helianthemum lippia* extract has caused a decrease ($p \leq 0.01$) of bilirubin compared to female rats poisoned by carbon tetrachloride, this may be due to the important role of ZnO-NP, which has a hepatoprotective effect by trapping free radicals or by increasing antioxidant activity then detoxifies free radicals by

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anti-hepatotoxic effect [31]. In another hand, ZnO-NP increases the level of zinc, which is an important element in all aspects of immunity. These factors protect the liver cells and maintain the regulation of enzymes and protein synthesis essential to the integrity of cell membranes [31]. A significant increase in plasma triglyceride and cholesterol levels was observed in female rats treated with $CCl_4$. It could be explained by the presence of disturbances for Triglyceride association mechanisms with the appropriate apoprotein to form the carrier molecule (lipoprotein) [32]. Our results have also shown the treatment with extract of *Helianthemum lippia* and ZnO-NPS causes significant decrease in hyperlipidemia by minimizing the level of triglyceride and cholesterol. This indicates that the extract of medicinal plants protects against lipid peroxidation, due to their secondary metabolite richness [33]. Concerning the protective effects of *Helianthemum lippia*. We notice an improvement of renal parameter by natural compounds like polyphenols, its different constituents exert their anti-inflammatory actions. in addition to their antioxidant actions by inhibiting the production of superoxide anion. The decrease in renal parameter levels after administration of zinc oxide nanoparticle explained by their protective effect [34].

## 5. Conclusion

The present study demonstrates that *Helianthemum lippia* plant can use in green chemistry like source of secondary metabolites for synthetics a several nanoparticles such as Zinc Oxide nanoparticle. Thus, the data of the present study suggest that *Helianthemum lippia* and Zinc Oxide nanoparticle have a hepatoprotective effect against Carbon Tetrachloride -induced hepatic damage in rats.

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## Conflicts of Interest Statement

The authors report no conflict of interest.

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