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# Effect of Zinc Oxid Nanoparticles on the Oxidative Stress (Malonaldehyde MDA, Lipid Peroxidation Level LPO) and Antioxidants (GSH glutation)

**Nibras AL-Ibrahemi[1] , Ruqayah Manoon Hasan[1] , Kaiser Alslman[2]**

*[1]Faculty of Education for Girls / Department of Biology / University of Kufa,*
*[2] College of Education for Pure Sciences / Department of Biology / University of Kerbala*

## Abstract

The plant was selected as the source of the nanoscale study because it possesses more chemical compounds than the other plants,ZnO nanoparticles ( ZnONPs) were prepared from the plant *Artemisia herba alba* extract and from the flavonoid compound. The samples were diagnosed by Atomic Force Microsco (AFM) and Fourier transform infrared (FT-IR) spectroscopy , and effect ZnONPs the synthesis from *Artemisia herba alba* extract , from the flavonoid compound , *Artemisia herba alba* and flavonoid on the criteria for oxidative stress (Malonaldehyde MDA, Lipid peroxidation level LPO) and antioxidants (GSH glutation) were determine effective time and effective dose. The results showed that MDA and LPO showed a significant decrease compared with the control group, while the period of 32 days showed a significant decrease compared to 62 days. The analysis of GSH found that the coefficients of the study showed a significant increase compared with the control, while the period of 32 days showed a significant decrease compared with 62 days.

***Key words :*** *ZnO Nanoparticles , Artemisia herba alba , Flavonoid , MDA, LPO, GSH*

## Introduction

Nanoparticles is one active modern science, rapid growing field with technology and science for the purpose manufacturing new materials at nanoscale level [1] , The term nanotechnology was first used by Norio Taniguchi, using the word to describe tools that use nanoparticles , Nanotechnology is defined as the ability to monitor, measure, process and manufacture nanomaterials, and the nanometer-nm is one billionth of a meter, which is very small [2]. Zno nanoparticles was one of the most nanparticles used for used make ointments and cosmetic creams , etc .. which protect skin from cancerogenic and burns of ultraviolet radiation [3] Free radical generation is a natural consquance of living in an oxidising environment, cells generate small a mount of free radical or reactive oxygen speciese ( ROS) , accumulation of ROS may damage biological macromoleculese such is DNA, carbohydrate , protein , lipid [4], External factors such infection , radiation , heat , toxins and exericies can lead to increase free radicals and other ROS [5] , oxidant damage may be minimised by antioxidant defence mechanism that protect the cell against cellular oxidant and repaire system that prevent the accumulation of oxidatively damaged molecules , antioxidant enzyme play a vital role in protecting cellular damage from harmful effect of ROS [6] plants contain phytochemicals are any compound such as phenol, terpenoid , polysaccharide and flavone that contribute in reduction and stabilizing nanoparticles [7] , plants are natural antioxidant sources which are used in traditional medicine for healthy of many disease [8] as *Artemisia herba alba* plant ( Asteraceae family commonly known as annual wormwood) is aplant used many centries in medicine treatment of many of disease [9] , moreover , *Artemisia* leave a high content of cineole , Artemisia ketone , camphene [10] being a rich source of variouse phenolic compounde as asource of natural antioxidant [11] . Such flavonoid have great potention to inhibit the generation of reactive oxygen speciese ( ROS) and once they are formed perform antioxidant function , flavonoid are found in the chloroplast which play role as scavenger of oxygen and stabilizers of chloroplast outer envelope memberane [12] Since our food is rich in antioxidants, including flavonoids, and the current time research

has been manufactured with some caveats and that nanoparticles have taken into account the application of biological, industrial and medical in an attempt to reduce the oxidant stress, so the study aimed at:-1-synthesis ZnO nanoparticles from *Artemisia herba alba* , 2 - preparation best of ZnO nanoparticles,3- studying evalution properties antioxidant – oxidant of *Artemisia herb alba* extract Flavonoid,4- analysis oxidant strese Malonaldehyd (MDA ) and Lipid peroxidase (LPO). 5-analysis antioxidant glutation GSH.1: Materials and method

1-1-plants collection: *Artemisia herba alba* obtain from karbalaa city in 2018 during the flowering stage the plant were air dried and at oven degree 45cº and powder.

1-2-Soxhlet Extraction : 50 gm of fin powder was plased in to an extraction thimble and extracted with 50 ml of (80%) methanol in flask round volum (500 ml ) for 24 hours, ( Iqbal, 2012) then evaporated at ( 45Cº) by a rotary evaporator and using freez drayer ( lyophilizer ) to dried crude extract.

1-3-Exrtraction of total flavonoids from *Artemisia herba alba* : Material has been according to this method [14] .

1-4: preparation ZnO nanoparticles from the extraction *Artemisia herb alba* and flavonoid. Material has been done according to his method [15].

1-5: Diagnosis using FT-IR infrared spectrum: Work has been done according to his method [16]

1-6-Diagnosis using Atomic Force Microscope AFM:

1-7 Design experiment: Atotal adult male rat , weight (200-320) gm, age ( 8-10 ) weak , wer used in this study and were isolated in a relatively controlled environmental at temperature of about 25Cº in animal house , rats were divided in to 5 groups , G1-the control group has given normal saline (0,9%) only, G2- injection 150 mg/kg B.W of *Artemisia herb alba* extracted , G3-injection 150mg/kg B.W of Flavonoid extracted, G4-injection 0. 4 mg/kg B.W ZnO nanoprticles synthesis from *Artemisia herba alba* extract, G5- injection 0.2 mg/kg B.W ZnO NPS synthesis from Flavonoid.

1-2- Oxidant -Antioxidant analysis :

1-2-1 : Malondialdehyde (MDA) U/ L: Work has been done according to his method [17]

1-2-2 Lipid peroxidation : Work has been done according to his method[18, 19].

## Results And Discusion

Identification of nanoparticles :

Atomic Force Microscopie (AFM):

The topography of surface was studied using AFM , Fig (1) showed two dimention image semispherical and spherical of ZnONanoparticles synthesis extract of *Artimesia herb alba* and Flavonoid compounds , nano particles high to the ZnO show 33.33nm , 18.00 for ZnONanoparticles synthesis extract *Artimesia herb alba* and Flavonoid in tabl 1,

comparition with study of [20] the morphological character of ZnO nanoparticles by flavonoid was analysis by SEM which showed spherical shape and cluster.

**Tabl (1 ) show shape , nanoparticles , size particles , diameter average of ZnONPS *Artimesia herb alba* and ZnONPS Flavonoid.**

| Sample | Sample | Nanoparticles | Size particle | Diameter average |
|---|---|---|---|---|
| ZnO Artimesia herb alba | Semispherical | 33-33 nm | 55-100.00nm | 80-55 nm |
| ZnONPS Flavonoid | Spherical | 18.00 nm | 55-85.00 nm | 75-04 nm |

Fourier Transform infrared ( FT-IR) analysis: The FT-IR spectra of the four samples are shown in figure (1 ) , The results of FTIR spectrum of ZnONPs A.herba alba presence of alcohols and phenols with a peak at 3411.19 , 3344.68, 3300.31, 3219.30 , 3173.01, 3117.07 , 3057.27 cm-1 , The peak at 2960.83 , 2883.68 and 2883.68 cm-1 to the C-H stretching presence some, faty acid , alkene compounds, wax, carotenoid and phytosteroil are present. The peak 17570.21 cm-1 presence to the C-C stretching which means that some glycoside, The peak value at 1614.47 cm-1 assigned to the C=O stretching which means the presence of some Flavonoid, polyphenol and catechins. The peak value at 1508.38 cm-1 assigned to the C=C stretching which means that some aromatics , flavonoid . The peak values at 1396.51, 1332.86 cm-1 assigned to the C=N stretching confirms aromatic amines. The peak value at 1274.99 cm-1 assigned to theC-N stretching confirms aliphatic amines, The peak value at 1168.90 , 1111.03 cm-1 assigned to the C-O stretching which means that Alcohols , ester, carboxylic acids, and the peak value at 852.56, 78120 , 61724 cm-1 assigned to the C-N stretching which means that Aliphatic amins and secondary alcohols , the absorption at in peak 478.36 cm-1 identifies the presence of ZnO nanoparticles , compared with result of [21] which indicate the presence of ZnO nanoparticles at peak 450-540cm-1 that synthezed from agueous leaf extract of corriandrum sativum using zinc acetate sodium hydroxide as in Figure 1 and Table 3 . the studies [22, 23] ) at peak between 1543-1028 cm-1 the affinity of the confirmed material of the nanoparticles of ethylene glycol and ethyl alcohol .

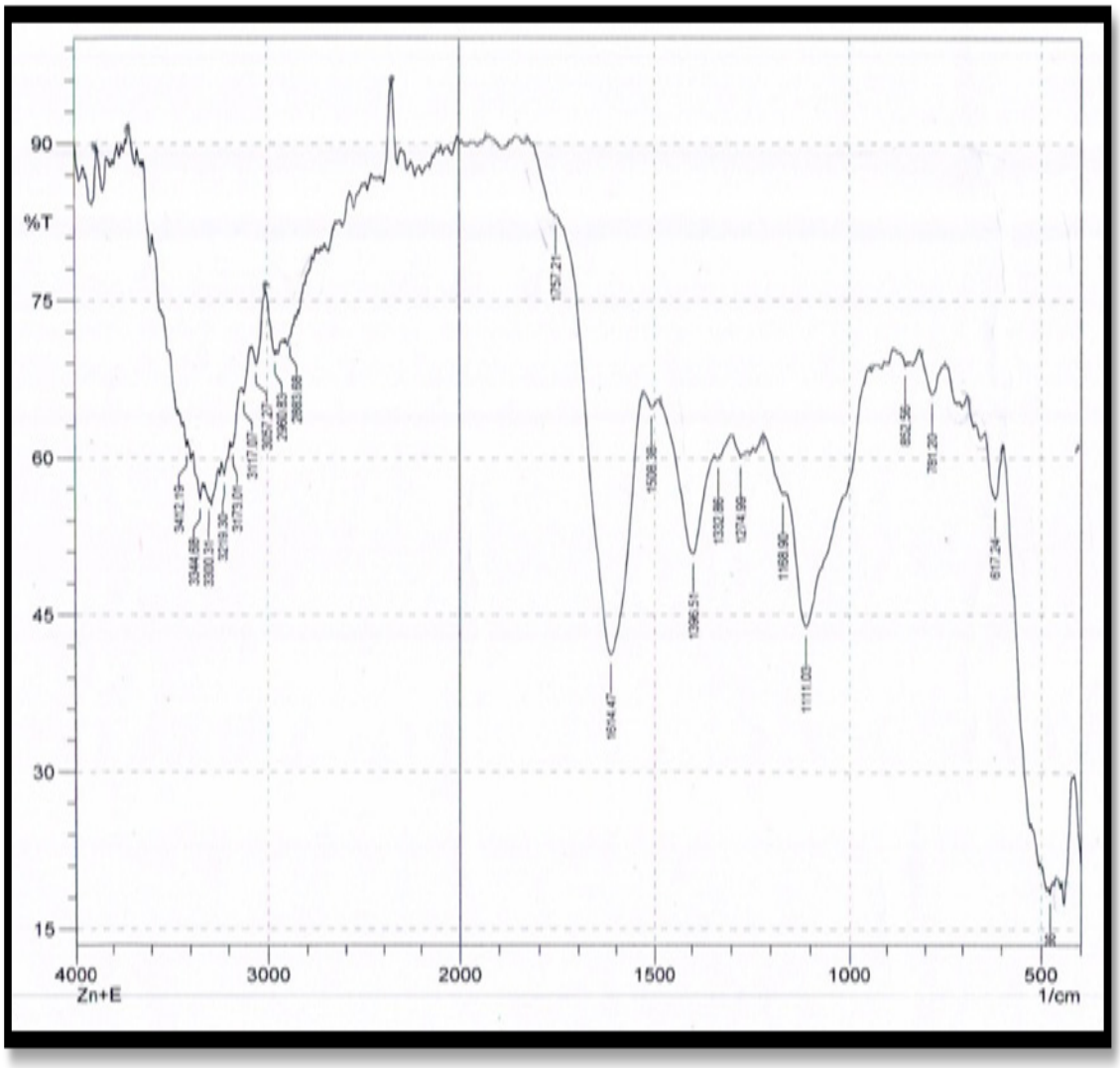


**Fig (1) FTIR ZnO synthesis from *A. herba alba*.**

The results of FTIR spectrum of Flavonoid at peak 3205.80 cm-1 the presence of alcohols and phenols The peak at 2993.62 $cm^{-1}$ and 2071.62 $cm^{-1}$ presence to the C-H stretching alkene compounds , faty acid , carotenoid and phytosteroil are present. The peak value at 1633.76 $cm^{-1}$ assigned to the C=O stretching which means that Flavonoid , polyphenol and catechins , The peak value at 1396.51, 1332.86 $cm^{-1}$ assigned to the C=N stretching which means aromatic amines. The peak value at 1274.99, 1238.34 and1047.38 assigned to the C-O stretching which means alcohol,ester, carboxylic acid , the peak values at 985.66 and 729.12 $cm^{-1}$ assigned to the C-N stretching which means Aliphatic amines as in Figure (6) **( 24, 25,26, 27)**

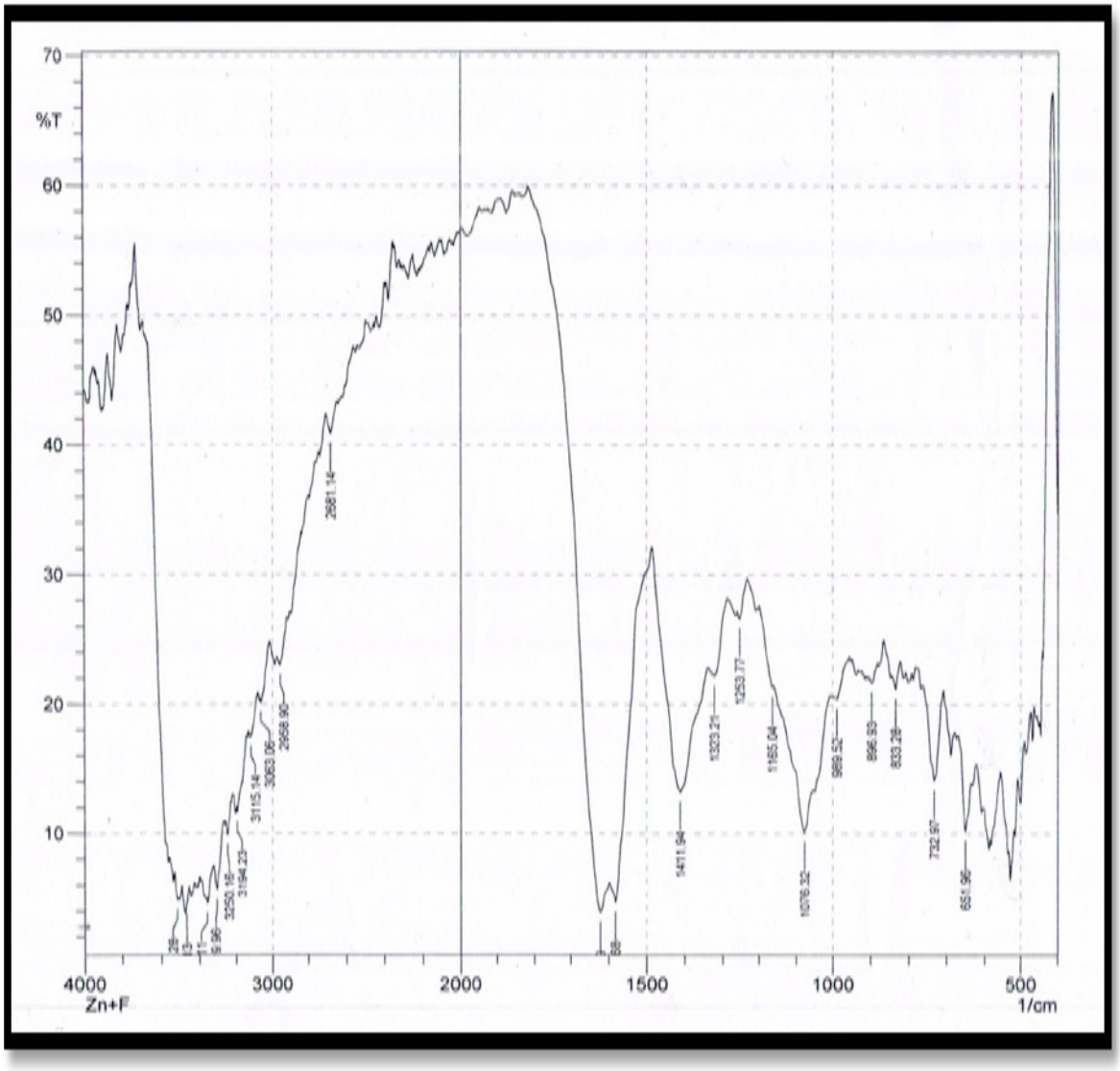


**Fig (2) FTIR of the ZnO nanoparticles from Flavonoid.**

**oxidant – Antioxidant assay :**

Determination of Malonaldehyde ( MDA) level U/L:

MDA level of group G2, G3,G4,G5 of rat showed significant decrease (1.95±0.26, 2.26±0.17, 1.38±0.26, 1.21±0.19) respectively compared with contro 2.26±0.17at 30 day , while at 60 day G2,G3,G4,G5, show significant decrease (2.06±0.24 , , 2.01±0.17 , 1.49±0.24, 1.29±0.22) for respectively compared with control 2.31±0.17 Table (4).

While groups at 32 day of MDA show significant decrease (2.26±0.17, 1.95±0.26, 2.26±0.17, 1.38±0.26,

1.21±0.19) of control , G2,G3,G4,G5, respectively compared with at 62 day (2.31±0.17, 2.06±0.24 , 2.01±0.17 , 1.49±0.24, 1.29±0.22 ) of control , G2,G3,G4,G5, respectively.

**Table (2) MDA percentage for rat groups treated with study coefficients.**

| At 32 day | | |
|---|---|---|
| **Group** | **mean± SD** | **Sig** |
| Control | 2.26±0.17 | 0.23 |
| G2 | 1.95±0.26 | |
| G 3 | 2.26±0.17 | 1.00 |
| G4 | 1.38±0.26 | 0.00* |
| G5 | 1.21±0.19 | 0.00* |
| Sig between group | 0.000 | |
| At 62 days | | |
| Control | 2.31±0.17 | |
| G2 | 2.06±0.24 | 0.41 |
| G 3 | 2.01±0.17 | 1.00 |
| G4 | 1.49±0.24* | 0.00* |
| G5 | 1.29±0.22* | 0.00* |
| Sig between group | 0.000 | |
| n | 5 at $p \leq 0.05$ | |

Determination of Lipid peroxidation ( LPO ) assay :

LPO level of group G2, G3,G4,G5of rat showed significant decrease (19.71±3.7, 16.19±3.25, 12.57±7.37, 12±4.24) respectively compared with control 24.28±7.37at 32 day , while at 62 day G2,G3,G4,G5 show significant decrease (20.28±7.24, 16.86±5.84, 15.99±2.12 , 14.57±5.84*) respectively compared with control 24.28±5.72. table (4)

While groups at 32 day of LPO show significant decrease (24.28±7.37, 19.71±3.7, 16.19±3.25, 12.57±7.37, 12±4.24) of control , G2,G3,G4,G5, respectively compared with at 62day (24.28±5.72, 20.28±7.24, 16.86±5.84, 15.99±2.12 , 14.57±5.84*) of control , G2,G3,G4,G5, for respectively.

**Table (3) LPO percentage for rat groups treated with Study coefficients.**

| **LPO AT 32 days** | | |
|---|---|---|
| **Group** | **mean± SD** | **Sig** |
| Control | 24.28±7.37 | |
| G2 | 19.71±3.7 | 0.83 |
| G 3 | 16.19±3.25 | 0.31 |
| G4 | 12.57±7.37* | 0.05 |
| G5 | 12±4.24* | 0.37 |
| Sig between group | 0.31 | |
| At 62 days | | |
| Control | 24.28±5.72 | |
| G2 | 20.28±7.24 | 0.87 |
| G 3 | 16.86±5.84 | 0.34 |
| G4 | 15.99±2.12 | 0.23 |
| G5 | 14.57±5.84 | 0.11 |
| Sig between group | 0.98 | |

Determent Glutathione (GSH ) level:

GSH level of group G2, G3,G4,G5, of rat showed significant increase (2.79±0.81, 2.94±1.38, 3.24±1.11, 3.97±0.66,) respectively compared with control 2.502±1.12 at 32 day , while at 62 day G2,G3,G4,G5 show significant increase (2.354±0.96, 2.80±0.96, 3.09±0.62, 3.82±0.61) respectively compared with control 1.47±0.90.

While groups at 32 day of GSH show significant increase (2.502±1.12, 2.79±0.81, 2.94±1.38, 3.24±1.11, 3.97±0.66) of control , G2,G3,G4,G5 respectively compared with at 62 day (1.47±0.90, 2.354±0.96, 2.80±0.96, 3.09±0.62, 3.82±0.61) of control , G2,G3,G4,G5, respectively table (4)

**Table (4) GSH percentage for rats groups treated with study coefficients.**

| **GSH at 32 days** | | |
|---|---|---|
| **Group** | **mean± SD** | |
| Control | 2.502±1.12 | |
| G2 | 2.79±0.81 | 0.99 |
| G 3 | 2.94±1.38 | 0.98 |
| G4 | 3.24±1.11* | 0.88 |
| G5 | 3.97±0.66* | 0.27 |
| Sig between group | 0.04 | |
| At 62days | | |
| Control | 1.47±0.90 | |
| G2 | 2.354±0.96 | 0.51 |
| G 3 | 2.80±0.96 | 0.13 |
| G4 | 3.09±0.62 | 0.38 |
| G5 | 3.82±0.61 | 0.01 |
| Sig between group | 0.001 | |

## Discussion

The experiment result show that ( *A. herb alba* , flavonoid ,ZnONPs ) improved antioxidant enzyme activity and effectively free radicals scavenger that can enhance the antioxidant capacity ,because *Artemisia* contained bioactive substance such polysaccharide and flavonoid which has very good radicals scavenging capacity and antioxidant effect. Because flavonoid have antioxidant effect at extracellular or intracellular antioxidant substance levels and act inhibition xanthine oxide activity is free radicals defense mechanism .that has conversion of xanthine oxide product to xanthine dehydrogenase so that decrease free radicals substance produce. ZnO nanoparticles is able to protect cell membrane against radical ferr damage , increase antioxidant enzymes and decrease MDA level and decrease level ROS.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Biology/ University of Kufa and all experiments were carried out in accordance with approved guidelines.

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