

Original research

## **Protection potency assessment of the Ethanol Extract of *Artemisia herba-alba* against chronic exposure to cadmium toxicity in adult male rats: Biochemical and molecular studies.**

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### **Abstract:**

Cadmium (Cd) is a common environmental heavy metal contaminant, which has severe toxicity to human health. Traditional medicine plants frequently use *Artemisia herba-alba* (ArtHA) to treat a variety of illnesses. Three different groups, each of seven Wistar rats were set up. A normal control group: given saline solutions only. Rats in group two: received CdCl<sub>2</sub> (5 mg/kg/b.w. i.p). In group three: (CdCl<sub>2</sub>+ArtHA) treated orally by ArtHA ethanol extract (100 mg/kg/b.w.) and CdCl<sub>2</sub> (5 mg/kg/b.w. i.p). Oxidative stress parameters and antioxidant enzymes were assessed in the liver, kidney, lung, and testis homogenates, as well as DNA damage. With a rise in malondialdehyde (MDA) levels in the Cd-treated group, the antioxidant enzyme activities were markedly downregulated. The amount of DNA damage was much higher in the Cd-treated group. Supplementing with ArtHA ethanol extract was extremely efficient and effectively restored the Cd-induced metabolic changes, making them close to the control values. The liver indicators (AST and ALT) as well as the renal damage markers (s. creatinine and s. urea) all showed significantly elevated levels.

**Keywords:** Cadmium, Toxicity, *Artemisia herba-alba*, Oxidative stress, DNA damage.

### **1. Introduction**

One of the biggest threats to human existence today is heavy metal pollution. Heavy metals' harmful effects on biological systems are gradually getting worse as a result of the world's ongoing industrialization (**Jaishankar et al., 2014; Hussain et al., 2017**). One of the most dangerous metals, cadmium, is easily accessible in the environment. Cd is naturally present in soil, water, and air. Due to its widespread application in plastics, batteries, metal plating, pigments, fertilizers, and different alloy industry sectors (**Kumar et al., 2019; Gupta et al., 2016; Kumar and Sharma, 2018**). Humans are mostly exposed to Cd through infected food and water. Workers who produce batteries or paint are more likely to get Cd-intoxicated than those who are not exposed to Cd-infested environments.

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The other common ways that Cd may build up in people are through cigarette smoking and drinking tainted drinks (**Wuana and Okieimen, 2014**). The majority of human activities lead to the buildup of Cd in the environment. According to reports, administering Cd to people causes both acute and chronic tissue damage and adversely impacts many crucial organs, including the liver (**Mitra et al., 2012; Jarup and Åkesson, 2009**). Bioindicators are being researched for low levels of Cd as a water pollutant because of the genetic and epigenetic impacts of Cd-induced toxicity (**Khalil et al., 2014**). Chronic exposure to Cd in humans can harm a variety of organs, including the kidneys and liver in particular, as well as the lungs, gastrointestinal tract, nervous system, testicles, immunological system, and endocrine system (**Unsal et al., 2020**). Additionally, Cd has been linked to cancer and causes oxidative damage to blood and other tissues, which impairs the functionality of cellular membranes (**Jemai et al., 2007**). Recent research suggests that Cd can alter the genetic and epigenetic makeup of mammalian and plant cells both in vitro and in vivo (**Genchi et al., 2020**).

The development of a disturbance in the antioxidant state in biological systems is the most common and widely recognized strategy used by Cd. Free radical production is increased, and essential antioxidant enzyme activity is suppressed (**Cuyppers et al., 2010; Lushchak, 2011; Nair et al., 2013; Sharma et al., 2014**). It also interferes with the regular functioning of several biological macromolecules, such as metallothionein and molecules containing sulfhydryl, which are vital to the healing process and protect live cells from free radical damage. Increased production of reactive oxygen species (ROS) and decreased antioxidant enzyme activity cause proteins and DNA, in particular, to degrade quickly (**Le et al., 2016; del Pino et al., 2016; Person et al., 2013**). These biological macromolecules become oxidized and lose their functionality, which leads to the emergence of metabolic diseases. The kidney, liver, brain, and testes are only a few of the vital organs whose activities are impacted by the following injection of Cd. The numerous antioxidant enzymes that are normally expressed by living cells include superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), esterases, and several non-enzymatic antioxidants like GSH and sulfhydryl group-containing molecules (**Renugadevi and Prabu, 2010; Newairy et al., 2007**). The inhibition of these protective enzymes and an increase in lipid peroxidation are both effects of chronic Cd exposure (**Eybl et al., 2006; Valko et al., 2005**).

Over time, natural ingredients have made an essential contribution to the formulation of current medicinal medications. Plants provide a variety of natural sources of beneficial substances that might be used as a starting point for the creation of new medicinal products. Medicinal herbs are generally thought to be less poisonous and cause less harm than synthetic medicines. (**Pari and Saravanan, 2004**). Therefore, it is now essential to do pharmacological studies on phytochemicals to confirm the alleged therapeutic benefits of plants (**Jayashree et al., 2011**). One of the species that develops in Sinai is the well-known medicinal plant *Artemisia herba-alba*, commonly referred to as "Sheh" in Egypt. This plant has been used in Middle Eastern traditional medicine to cure a variety of illnesses. The native populations of multiple nations use it as an antidiabetic (**Kamal et al., 2007; Ashraf et al., 2010**). This species' herbal infusions have been employed as analgesic, antibiotic, and hemostatic substances (**Tilaoui et al., 2011; Mohamed et al., 2010**). This herb's medicinal functions as a disinfectant, anthelmintic, and antispasmodic were discovered to be due to its essential oil (**Mohamed et al., 2010**). This species is also recommended for neurological issues because the ethanol-derived extract of ArtHA has shown activity in the GABAA-benzodiazepine receptor test (**Salah and Jager, 2005**). The plant's strong

antioxidant content, which is higher than that of green and black tea, is assumed to be the cause of its beneficial benefits (Abid et al., 2007).

## 2. Material and Methods

### 2.1. Preparation of plant extract.

The plant's dried aerial portions have been crushed using an electric grinder after being obtained from Egyptian marketplaces. The plant powder was dissolved in 70% ethyl alcohol for approximately three days, filtered through paper, concentrated under a vacuum using a rotary evaporator, and then continuously percolated until it was drained. Out of 100g of dried powder, 25g of ArtHA extract could be used for the study.

### 2.2. Chemicals

CdCl<sub>2</sub>, which has a 99.99% purity level. The following items were acquired from Sigma Chemical Co. (St. Louis, MO, U.S.A.): epinephrine, ethylene diamine tetra-acetic acid (EDTA), dimethyl sulfoxide, sodium dodecyl sulfate (SDS), and thiobarbituric acid (TBA). From MERK, I purchased sodium hydrogen phosphate, sodium dihydrogen phosphate, and NaOH. The highest-quality chemicals and solvents commercially available were employed in the investigation. Using commercially available kits, assess AST, ALT, s. creatinin, and s. urea (Spectrum Diagnostics Egyptian Company for Biotechnology).

### 2.3. Experimental procedure:

Twenty-one male Wister rats that weighed between 130 and 150g were utilized. The animals were taken from the faculty of veterinary medicine at Quena University's animal home. All animals were housed at a constant temperature (22 ± 2 °C), subjected to a 12-hour cycle of light and darkness, and given access to clean water and pellet food as needed. Three groups of seven rats each were created out of the rats: Group I: Normal controls received saline solution (0.9%) by oral gavage; Group II: CdCl<sub>2</sub> (5 mg/kg b.w.) was administered intraperitoneally; and Group III: (CdCl<sub>2</sub>+ ArtHA) was administered ArtHA ethanol extract (100 mg/kg b.w.) via oral gavage and then CdCl<sub>2</sub> (5 mg/kg b.w.) intraperitoneally an hour later. Every therapy was administered once every day for 21 days in a row. The animals were killed by cervical dislocation 24 hours after the previous treatment, and blood was collected into heparinized tubes. The cooling centrifuge was used to extract serum samples, which were then processed to determine various biochemical parameters. The testis, kidney, lung, and liver tissues were removed, homogenized in saline phosphate buffer, and then rinsed with ice-cold saline.

### 2.4. Detection of antioxidant enzymes and oxidative products

The liver, kidney, lung, and testis tissues were immediately removed from the animals after they were killed and homogenized in an ice-cold solution of saline sodium phosphate buffer. The supernatants were collected after the tissue homogenates were centrifuged at 10.000 rpm for 10 min at 4°C. Based on the thiobarbituric (TBA) reaction products, the degree of lipid peroxidation in the tissues of the liver, kidney, lung, and testis was determined spectrophotometrically (Ohkawa et al., 1979). Based on the inhibitory impact of SOD on epinephrine oxidation using the technique of Misra and Fridovich (1972), superoxide dismutase was determined in the

homogenate of the liver, kidney, lung, and testis. Using the **Beers and Sizer (1952)** technique, catalase was measured in the homogenate of the liver, kidney, lung, and testis.

## **2.5. Biochemical analysis:**

According to the **Reitman and Frankel (1957)** approach, serum was obtained and submitted for evaluation of the activities of aspartate aminotransferase (AST) and alanine transaminase (ALT) using corresponding commercial kits. The colorimetric technique developed by **Fawcett and Soctt in 1960** is used to assess urea in addition to the levels of renal functions (s. urea, s. creatinine) in blood samples. Utilizing a colorimetric technique developed by **Bartels et al. in 1972**, creatinine was measured.

## **2.6. DNA fragmentation assay in serum.**

The procedure of **Kurita-Ochiai et al., (1999)** was used to determine DNA fragmentation using a spectrophotometer set to 600 nm and a reagent blank. The following formula was used to calculate the proportion of fragmented DNA:

$$\% \text{ of fragmented DNA} = (\text{fragmented DNA} / \text{fragmented} + \text{intact DNA}) \times 100.$$

## **2.7. Molecular analysis: Detection of DNA fragmentation by Agarose gel electrophoresis.**

Rat liver, kidney, lung, and testis tissues were used to extract the genomic DNA, which was then, precipitated using phenol/chloroform. The extracted DNA concentrations were tested at Nanodrop levels. By using 2.0% agarose gel electrophoresis at 85V in 1.0 M Tris-acetate-EDTA (TAE) buffer, the reaction products (10 µL) were separated. The DNA-loading buffer (MyTaq Red Reaction buffer, Bioline, UK) was combined with the DNA samples. Ethidium bromide dye was used to view the gel, and after that, the gel documentation system (UVP- BioDoc-IT) was used to take pictures. Using the GeneRuler 1.0 kb Plus DNA Ladder (Bioline, UK) to contrast the amplified products, DNA fragments, and DNA profiles were identified. In TAE buffer, 5 µg were loaded onto a 1.5% agarose gel. The gel was stained with ethidium bromide, inspected under UV light, and photographed after one hour of electrophoresis at 85 V (**Jing et al., 2005**).

# **3. Results and Discussion**

## **3.1. Antioxidant activity of enzymes and oxidative stress markers**

Figures 1-3 show the impact of cadmium and *Artemisia herba alba* on lipid peroxidation and antioxidant enzymes activity. From the obtained results, Cadmium high significance ( $p < 0.01$ ) increased MDA in the liver, lung, and testis and very high significance ( $p < 0.001$ ) in the kidney compared to the untreated control group. Conversely, it is noticed that the quantity of lipid peroxidation as assessed by malondialdehyde (MDA) decreased significantly ( $p < 0.05$ ) in the lung homogenate after rats were processed with ArtHA ethanol extract, high significant decreased ( $p < 0.01$ ) in the liver and testis homogenates, and very high significant ( $p < 0.001$ ) in the kidney homogenate in the rats supplemented with ArtHA compared to cadmium groups Figure.1.

Figure. 2. shows A high significant reduction ( $p < 0.01$ ) was observed on superoxide dismutase (SOD) in liver, kidney, and lung homogenates, and a very high significant reduction ( $p < 0.001$ ) in testis homogenate of cadmium rats compared to the control group. The treatment with ArtHA ethanol extract protected against the depletion of antioxidant enzyme activity induced by

cadmium intoxication. SOD activity showed a very high significant increase ( $p < 0.001$ ) in the liver, kidney, and lung homogenates, and a high significant ( $p < 0.01$ ) in the testis homogenate in rats supplemented with ArtHA compared to the cadmium group. Additionally, catalase (CAT) activity showed a very high significant reduction ( $p < 0.001$ ) in the liver and kidney homogenates, high significance in the lung ( $p < 0.01$ ), and a significant reduction in the testis ( $p < 0.05$ ) compared with the control group. Conversely, the activity of CAT was significantly ( $p < 0.05$ ) normalized in the testis rats supplemented with ArtHA ethanol extract compared to the untreated cadmium rats and very high significance ( $p < 0.001$ ) in the liver, kidney, and lung homogenates which reflected the antioxidant properties of the ArtHA ethanol extract. Figure. 3.

### **3.2. Effect of treatments with ArtHA ethanol extract on liver and kidney function markers.**

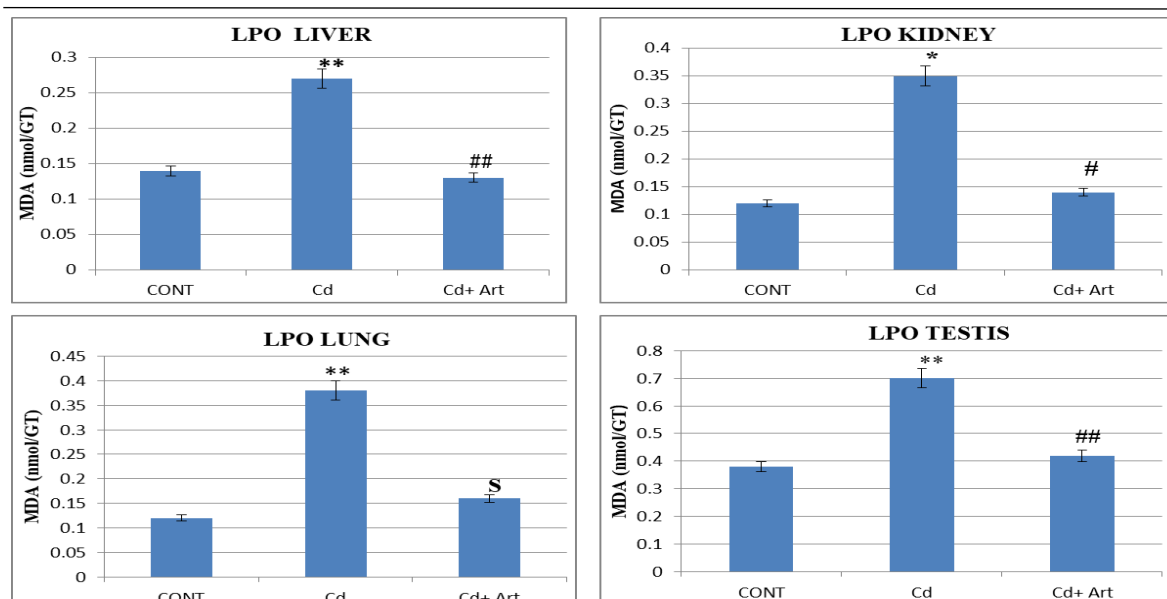
As shown in Table 1. Serum activities of ALT and AST enzymes showed high and very high significant increase in the Cd-treated group, reaching 106.6 (U/L) and 89.5 (U/L) respectively, as compared to the control group. The protective effect of the plant extract on liver enzymes was comparable to that of cadmium groups, treatment with ArtHA ethanol extract decreased ALT and AST activities and recorded a significant and very high significant decreased in ALT and AST serum activity of 68.6 (U/L) and 63.7 (U/L), respectively. Levels of s. creatinine and s. urea increased high significantly in the cadmium groups ( $10.8 \pm 2.1$  mg/dl and  $62.3 \pm 3.8$ mg/dl for s. creatinine and s. urea vs  $1.2 \pm 0.09$  mg/dl and  $32.8 \pm 5.6$  mg/dl in normal rats). The ethanol extract of ArtHA improved the kidney function indices of the cadmium rats by significantly reducing the concentrations of s.creatinine and s.urea. It showed a decreased of  $4.7 \pm 1.1$  mg/dl in s.creatinine level and  $35.02 \pm 2.6$  mg/dl in s.urea level compared to the cadmium treated group.

### **3.3. DNA fragmentation in the serum.**

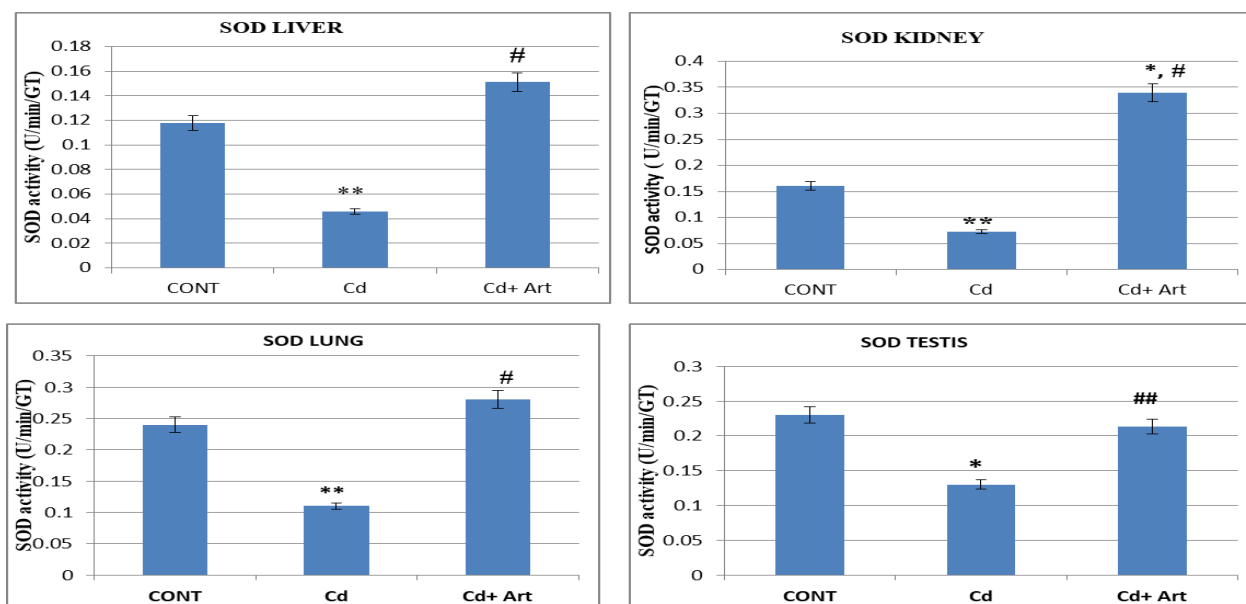
The changes recorded in DNA fragmentation in the rat serum indicate that Cd administration induced a marked and highly significant increase ( $p < 0.01$ ) in the level of DNA fragmentation. This is evident from the data obtained, where the value of 62.6% was calculated as a percent versus the control. On the other hand, pre-treatment of ArtHA with Cd-treated rats exhibited a significant decrease ( $p < 0.05$ ) after 21 days, where a value of 50.1% percent versus the cadmium-treated rats was observed (Figure 4).

### **3.4. DNA fragmentation by Agarose gel electrophoresis**

As shown in Figures 5 and 6, the results of DNA fragmentation analysis by agarose gel electrophoresis and visualization by ethidium bromide fluorescence observed on the agarose gel in liver, kidney, lung, and testis tissues were determined by considering the bands that appeared, which indicated that samples treated with cadmium had higher fragmentation indices than those treated with Artemisia herba alba ethanol extract.

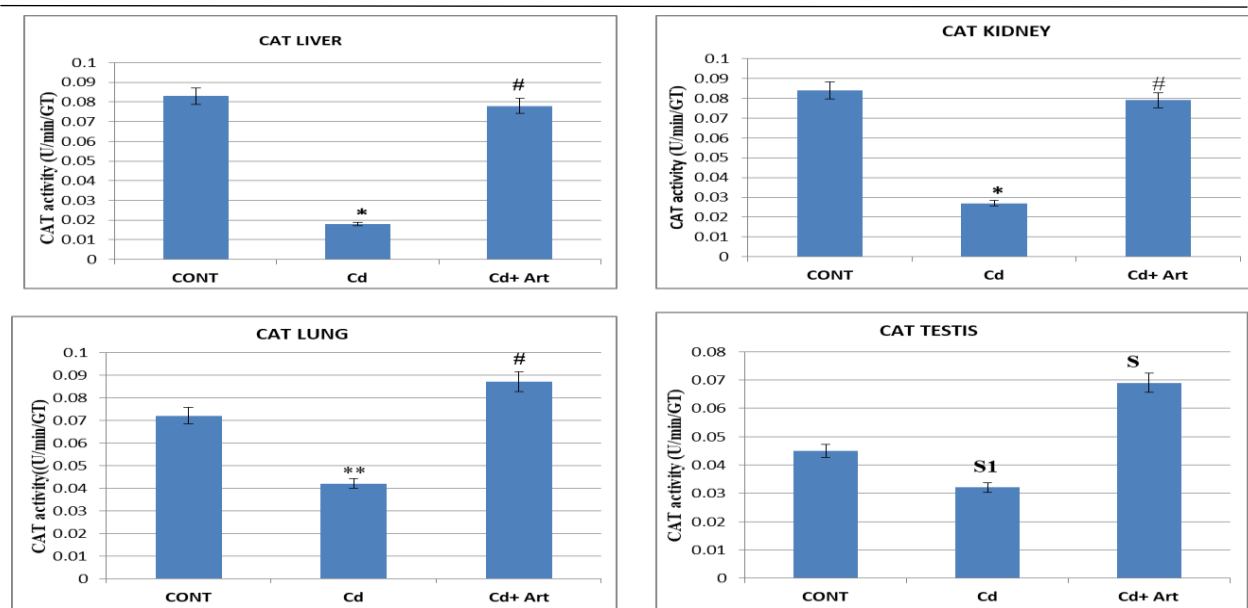


**Fig.1.** Effect of supplemented with *Artemisia herba-alba* on Lipid peroxidation (MDA) induced by cadmium (Cd) in the liver, kidney, lung, and testis of control and treated animal groups. Values are expressed as Mean ( $\pm$ SEM). No of rats = 7 for all groups. P\*\*high significant with Cont, P<sup>\$</sup> significant with Cd, P<sup>##</sup> high significant with Cd, P\* very high significant with Cont, and P<sup>#</sup> very high significant with Cd.



**Fig. 2.** Effect of supplemented with *Artemisia herba-alba* on superoxide dismutase (SOD) induced by cadmium (Cd) in the liver, kidney, lung, and testis of control and treated animal groups. Values are expressed as Mean ( $\pm$ SEM). No of rats = 7 for all groups. P\*\*high significant with Cont, P<sup>\$</sup> significant with Cd, P<sup>##</sup> high significant with Cd, P\* very high significant with Cont, and P<sup>#</sup> very high significant with Cd.





**Fig. 3.** Effect of supplemented with *Artemisia herba-alba* on catalase (CAT) induced by cadmium (Cd) in the liver, kidney, lung, and testis of control and treated animal groups. Values are expressed as Mean ( $\pm$ SEM). No of rats = 7 for all groups. P\*\*high significant with Cont, P<sup>S1</sup> significant with Cont, P<sup>S</sup> significant with Cd, P<sup>##</sup> high significant with Cd, P\* very high significant with Cont, P<sup>#</sup> very high significant with Cd.

**Table. 1.** Effect of 70% ethanol extract *Artemisia herba-alba* treatment on selected biochemical parameters in treated rats exposed to cadmium (Cd) toxicity.

Groups	Liver function		Kidney function	
	SGPT, ALT (U/L)	SGOT, AST (U/L)	S. Creatinin (mg/dl)	S.Urea (mg/dl)
Control	49 $\pm$ 6.2	38.7 $\pm$ 2.3	1.2 $\pm$ 0.09	32.8 $\pm$ 5.6
Cd	106.6 $\pm$ 11.9**	89.5 $\pm$ 3.9*	10.8 $\pm$ 2.1**	62.3 $\pm$ 3.8**
Cd+Artemia	68.6 $\pm$ 13.5 <sup>S</sup>	63.7 $\pm$ 3.2 <sup>#</sup>	4.7 $\pm$ 1.1 <sup>S</sup>	35.02 $\pm$ 2.6 <sup>##</sup>

Values are expressed as Mean ( $\pm$ SEM). No of rats = 7 for all groups. Serum creatinine, Serum urea, Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), \*\* high significant with Cont, <sup>S</sup> significant with Cd, <sup>##</sup> high significant with Cd, \* very high significant with Cont, <sup>#</sup> very high significant with Cd.

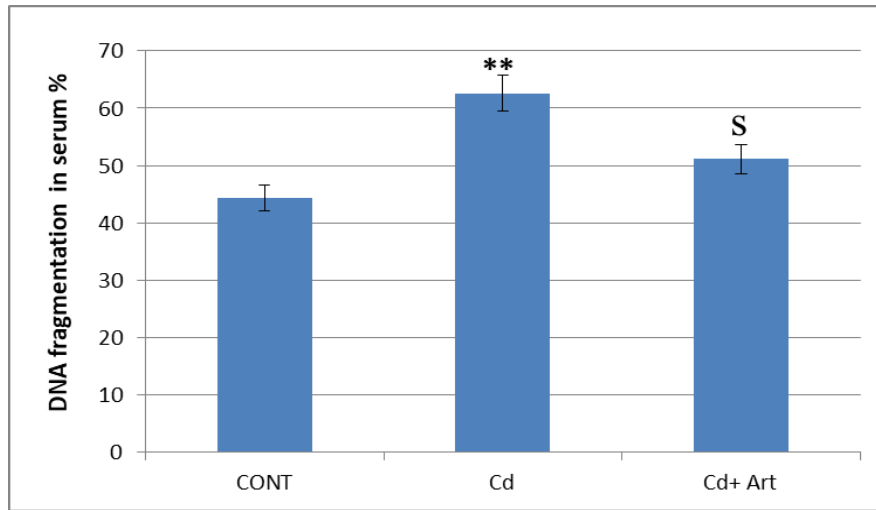


Fig. 4. Effects of *Artemisia herba-alba* treatment on DNA fragmentation % level in Serum of rats exposed to cadmium (Cd) toxicity. Values are expressed as Mean ( $\pm$ SEM). No of rats = 7 for all groups. P\*\*high significant with Cont, P<sup>S</sup> significant with Cd.

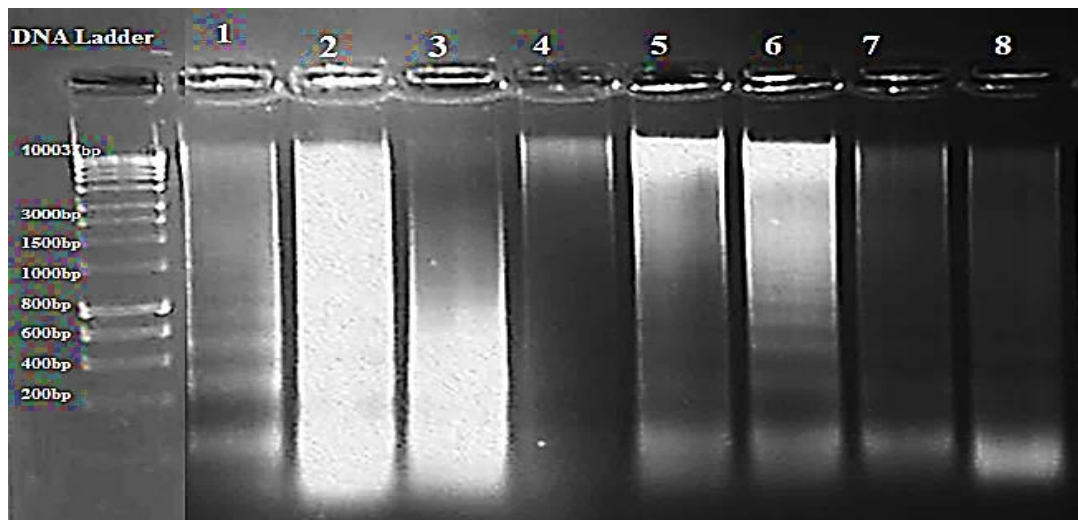
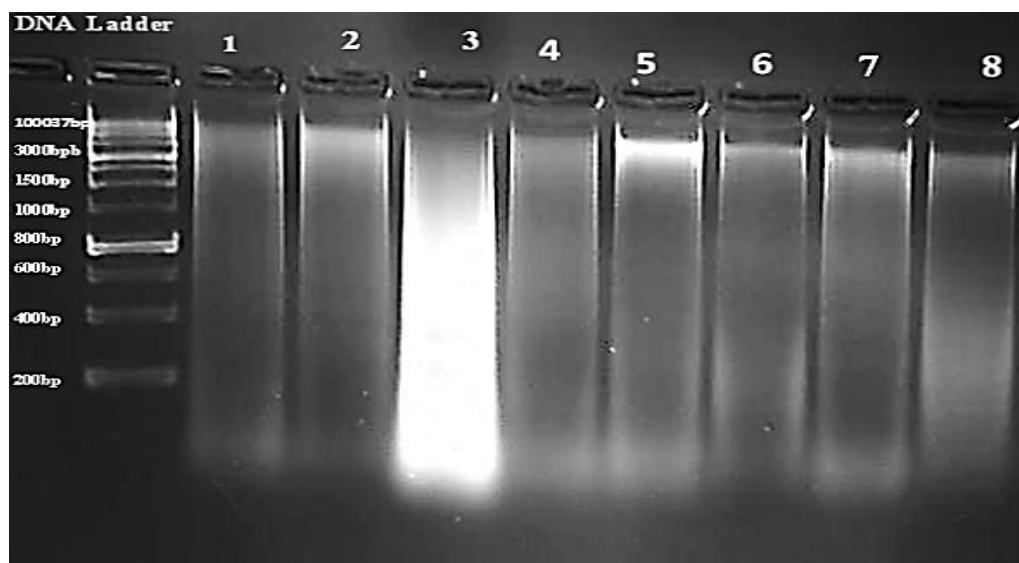


Fig .5 Effect of *Artemisia herba-alba* and Cd on DNA extracted from liver and kidney tissues of normal and treated groups of rats. Agarose gel electrophoresis photograph of DNA ladder-marker, lane 1: normal liver control group showed no DNA laddering, lane 2, 3: Liver Cd -treated group showed DNA laddering band, lane 4: Kidney control group showed no DNA laddering, lane 5, 6: Kidney Cd -treated group showed DNA laddering band. Lane 7,8: Liver Cd +ArTHA and kidney Cd+ ArTHA treated groups respectively showed restoration similar to control.





**Fig .6 Effect of Artemisia herba-alba and Cd on DNA extracted from lung and testis tissues of normal and treated groups of rats. Agarose gel electrophoresis photograph of DNA ladder-Marker, lane 1,2 normal lung, testis control group respectively showed no DNA laddering, lane 3,4: Lung Cd -treated group showed DNA laddering band, lane 5, 6: Testis Cd -treated group showed DNA laddering band, lane 7,8: lung Cd +ArtHA and testis Cd+ ArtHA treated groups respectively showed restored similar to control.**

The preventive effects of an Artemisia herba alba ethanol extract supplement against cadmium exposure were investigated in the current study. Cd, one of the most poisonous heavy metals ever documented, disturbs all creatures, including humans, by producing free radicals and causing cellular oxidative stress (Tchounwou et al., 2012; Rahimzadeh et al., 2017). It nearly exclusively affects all vital organs, making it a problem for public health. The natural or pathological stimulation of cells in response to environmental changes or minor injury is caused by oxidative stress and apoptosis (Denault and Boatright, 2004).

The production of free radicals, which causes lipid peroxidation and the rupturing of cellular membranes, is what causes oxidative stress (Nemliche, 2017; Semenza, 2011). ROS have been demonstrated to play a significant role in Cd-induced toxicity, which can cause cell damage and apoptosis. The disturbance of intracellular redox equilibrium can be caused by excessive ROS generation directly or indirectly activating signal transduction pathways (Szuster-Ciesielska, et al., 2011), which may hasten the pathological alterations of liver damage brought on by Cd poisoning. According to reports, oxidative stress is a significant factor in the pathological alterations of liver damage brought on by Cd exposure (Turner, 2019; Zhang et al., 2019). In this study, it was discovered that Cd treatment raises the amount of lipid peroxidation (increased MDA content) in the liver, kidney, lung, and testis homogenates, which was decreased by the administration of ArtHA ethanol extract. These findings were supported by the discovery that one of the key processes behind Cd-induced toxicity is the production of free radicals and the oxidative stress it causes (Thevenod, 2009). Previous research has demonstrated that exposure to Cd causes hepatocellular damage, which is manifested by an increase in the activity of liver enzymes and many histological changes in liver tissue (Abu-El-Zahab et al., 2019; Refaie et al., 2018; Shen et al., 2017).

Antioxidant treatment may therefore be a useful strategy for treating liver damage brought on by Cd poisoning. According to reports, exposure to Cd reduces the activity of the cellular antioxidant enzymes SOD and CAT, which leads to a rise in intracellular oxidative stress and the formation of ROS (**Abu-El-Zahab et al., 2019; Salama et al., 2019**). According to the data, the defense system's SOD and CAT enzymes were much less active in the cadmium group than they were in the controls. Reactive oxygen species, which are highly produced under oxidative stress situations and lead to protein oxidation, may be the cause of abnormal enzyme activity (**Bouasla et al., 2016; Sekiou et al., 2018**). The antioxidant qualities of the ethanol extract from ArtHA leaves may be responsible for the increased enzyme activity. By scavenging free radicals, the natural antioxidant molecules keep the ratio of antioxidants to free radicals close to normal. The liver, kidney, lung, and testis of Cd-treated rats demonstrated that administering an ethanol extract of ArtHA leaves reduced oxidative stress and increased the activity of cellular antioxidant enzymes.

The vital liver enzymes ALT and AST are rising, which is a sign of liver damage because they weaken the normal level in cases of viral illnesses and other liver disorders, as well as while taking various drugs. The measurement of these enzymes serves as the primary signal for evaluating liver function (**Thapa and Walia, 2007; Lala et al., 2021**). Since the liver is largely responsible for metabolizing these medications because the drug accumulates in Kupffer cells in the liver, the reason for the increase in enzymes may be related to the buildup of drug dosages in the liver. As a result, the liver's lysosomes become overburdened with indigestible compounds, growing in size and number, which in turn causes the plasma membrane to stop functioning (**Kurz et al., 2008**). Free radicals may also be to blame for the increase, as oxidative stress increases liver enzyme levels and is associated with cellular damage, loss of plasma membrane function, and the release of enzymes into the interfluid and blood (**Janani et al., 2009; Cheraghi et al., 2019**). According to the study's findings, as compared to the control group, Cd therapy significantly increased serum AST and ALT activity. The serum activities of these enzymes were significantly decreased in groups treated with ArtHA extract. It is most likely that supplementation with this extract lowered the amount of enzyme leakage from tissues to serum. According to these data, the considerable drop in AST and ALT levels following administration of the ArtHA ethanol extract is evidence of the hepatic cells. According to research by **Irshaid et al. (2012)**, the oil extract of Artemisia has a substantial protective effect on rats with diabetes-induced damage to their hearts, livers, and kidneys. This is because it contains potent antioxidants. An oral dose of 500 mg/kg body weight of the extract twice daily protected the liver from the effects of these compounds and decreased the levels of AST and ALT. **Ahamad, (2019)** studied the effect of the alcoholic and aqueous extracts of Artemisia absinthium against hepatotoxicity caused by CCl<sub>4</sub> and acetaminophen. According to **Jayasimha et al. (2011)**, Artemisia absinthium extract has an antidiabetic effect that lowers excessive levels of urea and creatinine in diabetic rats. In the current investigation, increased levels of renal function indicators showed that rats receiving Cd injections were experiencing decreased kidney function, and these serum activities of renal functions returned to normal in the groups treated with ArtHA extract. These results were consistent with those of **Karadeniz et al. (2011)**.

In the current study, rat liver, kidney, lung, testis and in serum DNA damage from cadmium exposure was reduced in groups treated with ArtHA ethanol extract compared to cadmium ones. These results suggest that ArtHA was more effective in protecting against the effects of Cd-intoxication in rats. According to **Araki and Nishikawa (2010)** and **Mckillop and Schrum**

(2005), one of the primary causes of diabetic problems is oxidative stress, which results in the production of highly reactive OH and causes significant oxidative damage to cell components, including DNA. As a result, the study demonstrates that, in addition to lowering oxidative stress in diabetic rats through free radical scavenging. This is in line with the findings of **Iriadam et al. (2006)**, who discovered that ArtHA had antidiabetic benefits. As shown by the significantly decreased cell viability of human colon cancer cells, increased DNA fragmentation, and induction of apoptosis via activation of caspase-3 and an increase in Bax and p53 proteins, the ethanol extract was reported to trigger cell death (**Giulio et al., 2011**).

#### 4. Conclusion

According to the study's findings, CdCl<sub>2</sub> builds up in and affects the tissues of the liver, kidney, lung, and testes. This heavy metal's toxicity can be assessed in future clinical trials on humans with the addition of supplements containing an extract of ArtHA in the daily drinks and foods of workers in many industries, such as battery and paint manufacturing. In the event of similar results in humans, the administration of ArtHA ethanol extract is effective in the treatment of Cd toxicity. Additionally, it was shown that the ethanol extract of ArtHA has significant antioxidant qualities, enabling it to lessen the negative effects of free radicals that are produced in large quantities. The outcomes showed that *Artemisia herba Alba* has highly effective anti-inflammatory and antioxidant properties.

#### Ethical recommendation

The experimental techniques and processes used in this work have been approved by the Aswan University Ethical Committee for the Care and Use of Laboratory Animals.

#### References

- Abid, Z.B.; Feki, M.; Hedhili, A. and Hamdaoui, M.H. (2007)** : *Artemisia herba-alba* Asso (Asteraceae) has equivalent effects to green and black tea decoctions on antioxidant processes and some metabolic parameters in rats. *Ann Nutr Meta.* 51:216-22.
- Abu-El-Zahab, H.; Hamza, R.Z.; Montaser, M.M.; El-Mahdi, M.M. and Al-Harathi, W.A. (2019)**: Antioxidant, antiapoptotic, antigenotoxic, and hepatic ameliorative effects of Lcarnitine and selenium on cadmium-induced hepatotoxicity and alterations in liver cell structure in male mice. *Ecotoxicol. Environ. Saf.* 173:419–428.
- Ahamad, J. (2019)** : A pharmacognostic review on *Artemisia absinthium*, *International Research Journal of Pharmacy.* 10: (1) 25-31.
- Araki, E. and Nishikawa, T. (2010)** : Oxidative stress: A cause and therapeutic target of diabetic complications. *J Diabetes Investig.* 1: 90-96.
- Ashraf, M.; Hayat, M.Q; Jabeen, S.; Shaheen, N.; Khan, M.A. and Yasmin, G. (2010)** : *Artemisia L.* species recognized by the local community of northern areas of Pakistan as folk therapeutic plants. *J Med Plant Res.* 4:(2) 112-9.
- Bartels, H.; Bohmer, M. and Heirli, C. (1972)**: Serum creatinine determination without protein precipitation. *Clin.Chem. Acta.* 37 - 193.

- Beers, R. F. Jr. and Sizer, I. W. (1952) :** A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase. *J. Biol. Chem.* 195 :133-140.
- Bouasla, A.; Bouasla, I.; Boumendjel, A. et al. (2016):** Prophylactic effects of pomegranate (*Punica granatum*) juice on sodium fluoride-induced oxidative damage in liver and erythrocytes of rats. *Can J Physiol Pharmacol.* 94: 1e10.
- Cheraghi, M.; Ahmadvand, H.; Maleki, A.; Babaeenezhad, E. ; Shakiba, S. and Hassanzadeh, F. (2019) :** Oxidative stress status and liver markers in coronary heart disease, *Reports of biochemistry & molecular biology.* 8:(1) 49.
- Cuypers, A.; Plusquin, M.; Remans, T.; Jozefczak, M.; Keunen, E.; Gielen, H.; Opdenakker, K.; Nair, A.R.; Munters, E.; Artois, T.J.; Nawrot, T.; Vangronsveld, J. and Smeets, K. (2010) :** Cadmium stress: an oxidative challenge, *BioMetals* 23 :927–940.
- Denault, J.B. and Boatright, K. (2004) :** Apoptosis in Biochemistry and Structural Biology. 3–8 February 2004, Keystone, CO, USA. *IDrugs.* 7:315–317.
- del Pino, J.; Zeballos, G.; Anadon, M.J.; Moyano, P.; Díaz, M.J.; García, J.M. and Frejo, M. T. (2016) :** Cadmium-induced cell death of basal forebrain cholinergic neurons mediated by muscarinic M1 receptor blockade, increase in GSK-3 $\beta$  enzyme,  $\beta$ -amyloid and tau protein levels, *Arch. Toxicol.* 90 :1081–1092.
- Eybl, V.; Kotyzova, D. and Koutensky, J. (2006) :** Comparative study of natural antioxidants - curcumin, resveratrol and melatonin - in cadmium-induced oxidative damage in mice, *Toxicology.* 225 :150–156.
- Fawcett, J.K. and Soctt, J.E. (1960) :** A rapid and precise method for the determination of urea. *J. Clin. Pathol,* 13 :156-159.
- Genchi, G.; Sinicropi, M.S.; Lauria, G.; Carocci, A. and Catalano, A. (2020) :** The Effects of Cadmium Toxicity. *Int J Environ Res Public Health.* 17(11):3782.
- Giulio, L.; Massimo, B.; Luana, Q.; Enzo, F.; Luca, A.; Hala, K. and Hala, G. M. (2011):** Antiproliferative activities of *Artemisia herba-alba* ethanolic extract in human colon cancer cell line (HCT116). *Alternative Medicine Studies.* 1:e1.
- Gupta, V.K.; Kumar, A.; Siddiqi, N.J. and Sharma, B. (2016) :** Rat brain acetyl cholinesterase as a biomarker of cadmium induced neurotoxicity, *Open Access J. Toxicol.* 1: 1–7.
- Hussain, M.M.; Hina, A.; Saeed, A.; Sabahat, S.; Jannat, F.T. and Aslam, M. (2017):** Impact of heavy metals on plants and animals in relation to sewage water, *Sci. Technol. Dev.*
- Iriadam, M.; Musa, D.; Gökhan, H. and Baba, F. (2006):** Effects of two Turkish medicinal plants *Artemisia herba-alba* and *Teucrium polium* on blood glucose levels and other biochemical parameters in rabbits. *Journal of Cell and Molecular Biology* 5: 19-24.
- Irshaid, F.; Mansi, K.; Bani-Khaled, A. and Aburjia, T. (2012) :** Hepatoprotective, cardioprotective and nephroprotective actions of essential oil extract of *Artemisia sieberi*

in alloxan induced diabetic rats, Iranian journal of pharmaceutical research: IJPR 11(4) :1227.

**Jaishankar, M.; Tseten, T.; Anbalagan, N.; Mathew, B.B. and Beeregowda, K.N. (2014)** :Toxicity, mechanism and health effects of some heavy metals, Interdiscip. Toxicol. 7 : 60–72.

**Janani, P.; Sivakumari, K. and Parthasarathy, C. (2009)** : Hepatoprotective activity of bacoside A against N-nitrosodiethylamine-induced liver toxicity in adult rats, Cell biology and toxicology 25(5) : 425-434.

**Jarup, L. and Åkesson, A. (2009)** : Current status of cadmium as an environmental health problem, Toxicol. Appl. Pharmacol. 238 :201–208.

**Jayashree, T.; Kishore, K.K.; Vinay, M.; Vasavi, P.; Chandrashekhar, N. and Manohar, V. et al. (2011)**: Evaluation of the diuretic effect of the chloroform extract of the *Benincasa hispida* Rind (pericarp) extract in Guinea-pigs. J Clin Diagn Res 5(3): 578-82.

**Jayasimha Goud, B.; Danamma, B.; Nizamuddin Basha, S.; Dayananda, K. and Chikka Swamy, B. (2011)** : 307-312. Hypo glycemc activity of a methanol extract of *Artemisia absinthium* leaves in experimental rats, Int J Appl Pat Recog 2.

**Jemai, H.; Messaoudi, I.; Chaouch, A. and Kerkeni A. (2007)** : Protective effect of zinc supplementation on blood antioxidant defense system in rats exposed to cadmium. J Trace Elem Med Biol. 21(4) :269-73.

**Jing, W.; Wei, K.; Ming-tai, G.; Yong-ning, Z.; Ai-qin, W.; Qun-ji, X. and Liang, Q. (2005)** : Effects of {2-[(3-carboxy-1-oxopropyl) amino]-2-deoxy-D-glucose} on human hepatocellular carcinoma cell line. Acta Pharmacologica Sinica. 5 :635–640.

**Kamal, M.; Masalmeh, A. and Hamzah, N. (2007)** : The hypolipidemic effects of *Artemisia sieberi* (A. herba-alba) in alloxan induced diabetic rats. Int J Pharm; 3(6) :487-91.

**Karadeniz, A. et al. (2011)** : Royal jelly modulates oxidative stress and apoptosis in liver and kidneys of rats treated with cisplatin. Oxid Med Cell Longev. 981793.

**Khalil, M.; Furness, D.N.; Zholobenko, V. and Hoole, D. (2014)**; Effect of tapeworm parasitisation on cadmium toxicity in the bioindicator copepod, *Cyclops strenuus*. Ecol Indic. 37:21-6.

**Kumar, A. and Sharma, B. (2018)**: Consequences of heavy metals pollution in environment and bioremediation practices, in: R.N. Bharagava (Ed.), Recent Adv. Environ. Manag., CRC Press, Taylor & Francis Group, USA. pp. 247–273.

**Kumar, R.; Pandey, N.; Siddiqi, J. and Sharma, B. (2019)** : Oxidative stress biomarkers of cadmium toxicity in mammalian systems and their distinct ameliorative strategy, J. Appl. Biotechnol. Bioeng. 6 :126–135.

**Kurita-Ochiai, T.; Fukushima, K. and Ochiai, K. (1999)** : Lipopolysaccharide stimulates butyric acid-induced apoptosis in human peripheral blood mononuclear cells. Infection and Immunity. 67- 1: 22-29.



- Kurz, T.; Terman, A.; Gustafsson, B. and Brunk, U.T. (2008) :** Lysosomes and oxidative stress in aging and apoptosis, *Biochimica et Biophysica Acta (BBA)-General Subjects.* 1780(11) 1291- 1303.
- Lala, V.; Goyal, A. and Minter, D.A. (2021):** Liver function tests, StatPearls [Internet], StatPearls Publishing.
- Le, Q.G.; Ishiwata-Kimata, Y.; Kohno, K. and Kimata, Y. (2016) :** Cadmium impairs protein folding in the endoplasmic reticulum and induces the unfolded protein response. *FEMS Yeast Res.* 16: fow049.
- Lushchak, V.I. (2011) :** Environmentally induced oxidative stress in aquatic animals, *Aquat. Toxicol.* 101 :13–30.
- McKillop, I.H. and Schrum, L.W. (2005) :** Alcohol and liver cancer. *Alcohol.* 35:195-203.
- Misra, H. P. and Fridovich, I. (1972) :** The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem,* 247 :3170-3175.
- Mitra, E.; Ghosh, A.K.; Ghosh, D.; Mukherjee, D.; Chattopadhyay, A.; Dutta, S.; Pattari, S. K. and Bandyopadhyay, D. (2012) :** Protective effect of aqueous Curry leaf (*Murraya koenigii*) extract against cadmium-induced oxidative stress in rat heart, *Food Chem. Toxicol.* 50 :1340–1353.
- Mohamed, A.E.H.H.; El-Sayed, M.A.; Hegazy, M.E.; Helaly, S.E.; Esmail, A.M. and Mohamed, N.S. (2010) :** Chemical constituents and biological activities of *Artemisia herba alba*. *Rec Nat Prod;* 4 :1-25.
- Nair, A. R.; DeGheselle, O.; Smeets, K.; Van Kerkhove, E. and Cuypers, A. (2013) :** Cadmium induced pathologies: where is the oxidative balance lost (or not)? *Int. J. Mol. Sci.* 14 :6116–6143.
- Nemniche, S. (2017) :** Oxidative signaling response to cadmium exposure. *Toxicol. Sci.* 156:4–10.
- Newairy, A.A.; El-Sharaky, A.S.; Badreldeen, M.M.; Eweda, S.M.; and Sheweita, S.A. (2007) :** The hepatoprotective effects of selenium against cadmium toxicity in rats, *Toxicology* 242 :23–30.
- Ohkawa, H.; Ohishi, N. and Nagi, K. (1979) :** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction *Anal. Biochem,* 95 351–358.
- Pari, L. and Saravanan, R. (2004) :** Antidiabetic effect of diasulin, a herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. *Diabetes Obes Metab.* 6 :286-92.
- Person, R.J.; Tokar, E.J.; Xu, Y. Orihuela, R.; Ngalame, N.N.O. and Waalkes, M.P. (2013) :** Chronic cadmium exposure in vitro induces cancer cell characteristics in human lung cells, *Toxicol. Appl. Pharmacol.* 273 :281–288.



- Rahimzadeh, M.R. ; Rahimzadeh, M.R.; Kazemi, S. and Moghadamnia, A.A. (2017).** Cadmium toxicity and treatment: an update, *Casp. J. Intern. Med.*
- Refaie, M.; El-Hussieny, M. and Zenhom, N.M. (2018):** Protective role of nebivolol in cadmium-induced hepatotoxicity via downregulation of oxidative stress, apoptosis and inflammatory pathways. *Environ. Toxicol. Pharmacol.* 58:212–219.
- Reitman, S. and Frankel, S.A. (1957) :** Colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. *Am J ClinPathol*, 28 :56–63.
- Renugadevi, J. and Prabu, S.M. (2010) :** Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin, *Exp. Toxicol. Pathol.* 62 :171–181.
- Salah, S.M. and Jager, A.K. (2005):** Two flavonoids from *Artemisia herba-alba* Asso with in vitro GABAA-benzodiazepine receptor activity. *J Ethnopharmacol.* 99:145-6.
- Salama, S. A.; Arab, H. H.; Hassan, M.H.; Al Robaian, M.M. and Maghrabi, I.A. (2019) :** Cadmium-induced hepatocellular injury: modulatory effects of gamma-glutamyl cysteine on the biomarkers of inflammation, DNA damage, and apoptotic cell death. *J. Trace Elem. Med. Biol.* 52:74–82.
- Sekiou, O.; Boumendjel, M.; Taibi, F.; Boumendjel, A. and Messarah, M. (2018):** Mitigating effects of antioxidant properties of *Artemisia herba alba* aqueous extract on hyperlipidemia and oxidative damage in alloxan-induced diabetic rats. *Arch Physiol Biochem.* 1e11.
- Semenza, G.L. (2011):** Oxygen sensing, homeostasis, and disease. *N. Engl. J. Med.* 365:537–547.
- Sharma, B.; Singh, S. and Siddiqi, N.J. (2014) :** Biomedical implications of heavy metals induced imbalances in redox systems, *BioMed. Res. Int.* 1–26.
- Shen, R.; Liu, D.; Hou, C.; Liu, D.; Zhao, L.; Cheng, J.; Wang, D. and Bai, D. (2017):** Protective effect of *Potentilla anserina* polysaccharide on cadmium-induced nephrotoxicity in vitro and in vivo. *Food Funct.* 8:3636–3646.
- Szuster-Ciesielska, A.; Plewka, K. ; Daniluk, J. and Kandefor-Szerszen, ´ M. (2011):** Betulin and betulinic acid attenuate ethanol-induced liver stellate cell activation by inhibiting reactive oxygen species (ROS), cytokine (TNF-alpha, TGF-beta) production and by influencing intracellular signaling. *Toxicology.* 280:152–163.
- Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K. and Sutton, D.J. (2012):** Heavy metal toxicity and the environment, *EXS.*
- Thapa, B. and Walia, A. (2007) :** Liver function tests and their interpretation, *The Indian Journal of Pediatrics* 74(7) 663-671.
- Thevenod, F. (2009):** Cadmium and cellular signaling cascades: to be or not to be? *Toxicol. Appl. Pharm.* 238:221–239.

- Tilaoui, M.; Mouse, H.A.; Jaafari, A.; Aboufatima, R.; Chait, A. and Ziad, A. (2011) :** Chemical composition and antiproliferative activity of essential oil from aerial parts of a medicinal herb *Artemisia herba-alba*. *Rev Bras Farmacogn*; 21(4): 781-5.
- Turner, A. (2019):** Cadmium pigments in consumer products and their health risks. *Sci. Total Environ.* 657:1409–1418.
- Unsal, V.; Dalkıran, T.; Çiçek, M. and Kölükcü, E. (2020) :**The Role of Natural Antioxidants Against Reactive Oxygen Species Produced by Cadmium Toxicity: A Review. *Adv Pharm Bull.* 10(2):184-202.
- Valko, M.; Morris, H. and Cronin, M. (2005) :** Metals, toxicity and oxidative stress, *Curr. Med. Chem.* 12 :1161–1208.
- Wuana, R.A. and Okieimen, F.E. (2014).** Heavy metals in contaminated soils: A review of sources, chemistry, risks, and best available strategies for remediation. *Heavy Met. Contam. Water Soil Anal. Assessment, Remediat. Strateg.*
- Zhang, J.; Wang, Y.; Fu, L.; Wang, B.; Ji, Y. L.; Wang, H. and Xu, D. X. (2019):** Chronic cadmium exposure induced hepatic cellular stress and inflammation in aged female mice. *J. Appl. Toxicol.* 39:498–509.