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# Neuroprotective Effects of Grape Seed Extract against Cadmium Toxicity in Broilers

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## ABSTRACT

Ration contamination with cadmium chloride ($CdCl_2$) leads to serious economic loss. The current study aimed to explain the ameliorative effect of grape seed extract (GSE) either alone or in combination with $CdCl_2$. One hundred and fifty chicks were used in the current study. They were equally alienated into 6 groups; group I was kept as control. Group II: given grape seed extract by the first dose (GSE1) of 250 mg/kg; group III: given grape seed extract by the second dose (GSE2) of 500 mg/kg; group IV: given cadmium chloride ($CdCl_2$) to evaluate the undesirable effects of the dose (100 mg/kg diet). Group V: given combination of GSE1+ $CdCl_2$, group VI: given combination of GSE2+ $CdCl_2$. At the end of the 3rd and 5th weeks, the following parameters were measured: Serum oxidants and antioxidants (GSH, SOD, CAT and MDA), brain oxidants and antioxidants (SOD, CAT, MDA, and NO), semi-quantitative RT-PCR detection of brain and liver GST and GPx, as well as serum cytokines (IL-1β, IL-10, and TNF-α), were determined. The results put on display show that GSE extract considerably ameliorated the levels of serum and tissue oxidants and antioxidants, as well as cytokines that ramshackle $CdCl_2$. Histopathological assessment of brain tissue and BAX brain sections was in concurrence with the immunological, oxidant, antioxidant, and RT-PCR results. It is important to take into consideration that the immunostimulant, antioxidative properties of GSE are mechanistically achieved. So, GSE could be used as a protective agent against ration contamination.

***Keywords:*** Antioxidant, Brain, Broilers, Cadmium chloride, Cytokines, Grape seed extract.

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## INTRODUCTION

Over the past few decades, environmental pollution has increased, posing a risk to all biological systems, including the chicken industry **(Alagawany *et al.*, 2016)**. It is widely acknowledged that cadmium is one of the most hazardous industrial and environmental pollutants that contaminate food, water, and air **(Tchounwou *et al.*, 2012)**. Cadmium's extraordinarily long biological half-life effectively renders it a cumulative poison, meaning that leftover metal exposure from a long time ago may have direct harmful effects **(Berglund *et al.*, 2015)**.

Recently, one of the main components of complementary and alternative medicine has been herbs and herbal extracts **(Dhama *et al.*, 2018)**, which have exhibited numerous properties against many toxic materials **(Waman *et al.*, 2018)**. Consequently, the use of herbs and their extracts has been the primary concern of treatment and prevention approaches for cadmium toxicity **(Abedi *et al.*, 2016)**. According to **Asmaa *et al.* (2019)**, herbs have the ability to bind to heavy metals and lessen their absorption in the intestine. They can also help to release the body's excretion mechanisms in areas of heavy poisoning and assist the metals' evacuation from the body.

Flavonoids, in particular, which play a significant role as antioxidants, have drawn a lot of attention lately. Thus, grape polyphenols (GP) could be used as an efficient feed supplement to boost birds' immune and antioxidant status because of their potent antioxidant qualities and low cost **(Iqbal *et al.*, 2015)**.

In Egypt, grapes are widely grown and regarded as the second most significant fruit crop, behind citrus. Egypt's grape-growing region stretches from Aswan in the south to Alexandria in the north **(Abu Hafsa and Ibrahim 2018)**. According to **Viveros *et al.* (2011)**, grapes are one fruit crop that has a high concentration of phenolic compounds. Important plant flavonoids and proanthocyanidin oligomers are found in a natural extract called grape seed extract (GSE) that is made from grape seeds **(Asl and Hsseinzadeh 2009)**. Due to their wide diversity of effects, like antimicrobial, hepatoprotective, antioxidant, anti-inflammatory, cardioprotective, and neuroprotective, grape seed extract is used as an antioxidant nutritive additive **(El-Ashmawy *et al*., 2007)**.

According to recent studies, GSE improved the antioxidant status and reduced the frequency of lipid peroxidation caused by free radicals in older rats **(Balu *et al*., 2005)**. It increases antioxidant capacity in chicken and turkey meat **(Mielnick *et al*., 2006)**. Grape seed extract contains 92 to 95% proanthocyanidin oligomers **(Murray, 1995)**.

Due to elevated levels of polyphenols called proanthocyanidins and oligomers of flavan-3-ol units, particularly epicatechin and catechin, grape seed has a twenty-fold and fifty-fold higher antioxidant potential than vitamins E and C, respectively **(Shi *et al*., 2003)**. It has been demonstrated that grape seed proanthocyanidins extract has anticancer, cholesterol-lowering, antibacterial, antiviral, and antifungal properties **(Cos *et al*., 2003)**. Even though almost every organ has evidence of cadmium toxicity at this point, the central nervous system is still acutely aware of even minute amounts of exposure. A growing body of research suggests that cadmium increases the death of neurons (**Patra *et al*., 2011**).

The current study aims to assess the impact of cadmium chloride on broilers and investigate if GSE can mitigate the toxic changes caused by cadmium. This will be achieved by measuring several immunological parameters, including IL-1β, IL-10, and TNF-α; evaluating oxidative status by measurement of some antioxidants and oxidative stress markers (GSH, SOD, CAT, and MDA); detecting some neurotoxicity markers (SOD, CAT, MDA, and NO) together with RT-PCR detection of brain and liver GST and GPx; and recording the associated pathological changes in the brain together with brain BAX sections.

## MATERIALS AND METHODS

### Experimental animals

One hundred fifty (150) one-day-old Ross chicks were used in the study. They were split into six equal groups, each with 25 chicks per pen, and the design was entirely random. We purchased the chicks from the Ismailia/Misr Poultry Company. As stated by **Ayoub *et al*., (2019)**, the following table describes the vaccination schedule of all chicks.

Table 1: Vaccination program

| Age of vaccination | Applied vaccine |
|---|---|
| Day 1 | HB+ Inactivated GVII |
| Day 7 | Inactivated GVII |
| Day 10 | ND Elite |
| Day 18 | LaSota |

Chickens were raised for a total of 42 days, during which time they were fed starter (0–10 d), grower (11–24 d), and finisher (25–35 d) diets. In order to satisfy the recommended nutritional needs as stated by the NRC (1994). During the experimental period (days 0–35), the chickens were raised in floor pens measuring 100 × 120 cm, with unrestricted access to feed and water. On the 28$^{th}$ day, the room temperature steadily dropped from 33 to 22 °C and then stayed at that level. There were 20 hours of light and 4 hours of darkness during the lighting program.

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### Ethical approval

The birds used in experiment were cared for and treated well in accordance with the regulations set forth by Suez Canal University's Animal Use Research Ethics Committee in Egypt (approval No. 2020052).

### Drugs and Chemicals

### Grape seed extract (GSE)

Was supplied as grape seeds proanthocyanidins, United States Pharmacopeia (USP) from Sigma-Aldrich. CAT-Package 1298208.

### Cadmium (Cd)

Was provided as 99.99% trace metal-free cadmium chloride ($CdCl_2$) by Sigma-Aldrich. Package 202908, CAT.

### Experimental design

A total of 150 chicks were equally alienated into 6 groups: group I: kept as control; group II: given

grape seed extract by the first dose (GSE1) 250 mg/kg diet (**Shahid *et al*., 2017**); group III: given grape seed extract by the second dose (GSE2) 500 mg/kg diet (**Shahid *et al*., 2017**); group IV: given cadmium chloride ($CdCl_2$) to evaluate the undesirable effects by the dose (100 mg/kg diet) (**Hashem *et al*., 2019**); group V: given combination of GSE1+ CdCl2; and group VI: given combination of GSE2+ $CdCl_2$. All experimental animals were fed a basal diet of water *ad libitum* throughout the entire experimental duration and received GSE and $CdCl_2$ orally as a diet mixture.

## Sampling

### Blood Sampling

At the end of the 3rd and the 5th weeks, blood samples were taken from the wing veins of ten randomly chosen birds (n = 10) in each group. Samples of blood were taken in a dry, clean plan centrifuge tube without anticoagulant, allowed to clot at room temperature, and then centrifuged for five minutes at 1198×g relative centrifugal force (RCF) to separate the serum and test the serum immunological, antioxidant and oxidative stress parameters.

### Tissue sampling

After scarification, part of brain and liver tissues were collected for semiquantative RT-PCR. Another part of brain tissue was collected for both histopathological and immunohistochemical studies.

### Serum cytokines

Tumor necrosis factor alpha (TNF-α), interleukin-10 (IL-10), and interleukin-1 beta (IL-1β were assessed by the specific ELISA kits using a commercially available kits (Cusabio Technology LLC, USA) following the instructions provided by the manufacturer.

### Serum and brain antioxidants and oxidative stress parameters

Reduced glutathione (GSH), super oxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and nitric oxide (NO) kits obtained from (Cell Biolabs, Inc, USA) according to the manufacturer's instructions.

### RT-PCR assessment

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of glutathione s transferase (GST) and glutathione peroxidase (GPx) genes was performed according to **El-Tarras *et al*., (2016) and Liu *et al*., (2020).** Liver and brain were collected and preserved in RNA Later (Sigma-Aldrich Chemie GmbH). Kits were obtained from Thermo Fisher Scientific Inc.

Table 2: Primer sequences (5'→3') used in RT-PCR.

| Gene | organ | Forward primer | Reverse primer |
|---|---|---|---|
| GST | Liver | GCCTGATGCACTTGCAAAA | AAAATTGCCATCAGTCTTGGT |
| GPx | | GACGGCTGAGAGTTGATCCT | CTGCGGGTATTTGATGTCC |
| GST | Brain | GCTGGAGTGGAGTTTGAAGAA | GTCCTGACCACGTCAACATAG |
| GPx | | AAGGTGCTGCTCATTGAGAATG | CGTCTGGACCTACCAGGAACTT |
| GAPDH | | TCCTAGGATACACAGAGGACCA | CGGTTGCTATATCCAAACTCA |

Gene expression: RT-PCR analysis of the following genes was performed and the expression levels semi-quantified: GST and GPx.

### Histopathological examination

Formalin-fixed brains for each experimental animal were subjected to dehydration using ascending ethyl alcohol concentrations (70, 80, 90, and 100%) for a duration of one hour each. Clearing was done to the specimens using two changes of xylene (1 hour each), then they were embedded in paraffin blocks. A tissue section of 5µm µm was obtained. The microscopic slides were stained using the eosin and hematoxylin stains (H&E), according to **Bancroft and Cook (1994)**.

For BAX staining, the tissue was preserved for 24 hours in 4% paraformaldehyde buffered at pH 7.4 with 0.1 M phosphate buffer in preparation for immunohistochemistry. Following fixation, the specimens were sectioned at a thickness of 4 µm and embedded in paraffin. The sections were boiled in 10 mM citrate buffer for 40 minutes at pH 9.8 for Bcl-2 or pH 6.0 for Bax (ChemMate Target Retrieval Solution; Dakocytomation, Kyoto, Japan) after being submerged in 0.3% hydrogen peroxide to inhibit the

endogenous peroxidase. The sections were then successively incubated for an additional night at 4 °C with the biotinylated anti-rabbit swine polyclonal antibody (Dakocytomation), streptavidin-biotin-horseradish peroxidase complex (Dakocytomation), and anti-Bcl-2 rabbit polyclonal antibody (N-19; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-Bax rabbit polyclonal antibody (N-20; Santa Cruz Biotechnology). After developing the immunoreaction using 3,3-diaminobenzidine, the sections underwent a light counterstaining with hematoxylin. In the absence of primary antibodies, control sections were incubated with normal rabbit serum (**Narjes *et al.*, 2018**).

### Statistical Analysis

The current study's data were presented as mean ± standard error (SE), and One-Way Analysis of Variance (ANOVA) was used to analyze the data for each of the tested groups in accordance with **Snedecor and Cochran (1989)**. According to **Duncan (1955)**, means separations were performed using Duncan's Multiple Range test. The current data were examined with Windows' (SPSS, 20). When $P \leq 0.05$, the results are deemed significant at the probability level.

## RESULTS

### Serum cytokines

As shown in **Table (3)** at the end of the 3rd week of age, $CdCl_2$ treated group showed a noteworthy increment in both IL-1β and TNF-α compared to control group. For GSE1+ $CdCl_2$ and GSE2+ $CdCl_2$ groups, both showed a significant decrease than $CdCl_2$ group. Moreover, GSE2+ $CdCl_2$ revealed a significant decrease than GSE1+ $CdCl_2$. The results at the end of the fifth week of life was the same as it was in the third week. Regarding to IL-10, at the end of the 3rd week of age, $CdCl_2$ treated group manifested a significant fall in IL-10 when compared to control group. GSE1+ $CdCl_2$ and GSE2+ $CdCl_2$ exhibited a weighty rise compared to $CdCl_2$ group. GSE2+ $CdCl_2$ revealed a notable rise when compared to GSE1+ $CdCl_2$ group. Same results were obtained in the 5th week of age.

Table 3: The effect of cadmium chloride ($CdCl_2$), grape seed extract by the first dose (GSE1) and grape seed extract by the second dose (GSE2) on serum cytokines at 3rd and 5th weeks of chickens.

| Parameters / Treatments | IL-1β (pg/ml) | IL-10 (pg/ml) | TNF-α (pg/ml) |
|---|---|---|---|
| The third week of age | | | |
| Control | 3.71 ± 0.03 $^{d}$ | 35.22 ± 0.54 $^{a}$ | 7.54 ± 0.07 $^{d}$ |
| GSE1 | 3.73 ± 0.04 $^{d}$ | 35.25 ± 0.53 $^{a}$ | 7.48 ± 0.04 $^{d}$ |
| GSE2 | 3.65 ± 0.02 $^{d}$ | 35.19 ± 0.44 $^{a}$ | 7.45 ± 0.03 $^{d}$ |
| $CdCl_2$ | 6.38 ± 0.04 $^{a}$ | 23.68 ± 0.91 $^{d}$ | 18.91 ± 0.27 $^{a}$ |
| GSE1+ $CdCl_2$ | 6.07 ± 0.04 $^{b}$ | 26.52 ± 0.09 $^{c}$ | 16.09 ± 0.18 $^{b}$ |
| GSE2+ $CdCl_2$ | 5.79 ± 0.07 $^{c}$ | 28.14 ± 0.22 $^{b}$ | 14.93 ± 0.10 $^{c}$ |
| The fifth week of age | | | |
| Control | 3.63 ± 0.02 $^{d}$ | 35.16 ± 0.39 $^{a}$ | 7.61 ± 0.08 $^{d}$ |
| GSE1 | 3.66 ± 0.05 $^{d}$ | 35.20 ± 0.26 $^{a}$ | 7.53 ± 0.08 $^{d}$ |
| GSE2 | 3.66 ± 0.01 $^{d}$ | 35.47 ± 0.21 $^{a}$ | 7.46 ± 0.06 $^{d}$ |
| $CdCl_2$ | 6.02 ± 0.07 $^{a}$ | 28.29 ± 0.08 $^{d}$ | 17.65 ± 0.21 $^{a}$ |
| GSE1+ $CdCl_2$ | 5.23 ± 0.20 $^{b}$ | 30.04 ± 0.12 $^{c}$ | 15.42 ± 0.22 $^{b}$ |
| GSE2+ $CdCl_2$ | 4.37 ± 0.08 $^{c}$ | 32.06 ± 0.40 $^{b}$ | 11.97 ± 0.41 $^{c}$ |

Values are expressed as mean (Mean ± SE).
Means with the same letter in the same column are non-significant at P<0.05.
GSE1= grape seed extract 1st dose GSE2= grape seed extract 2nd dose. $CdCl_2$ = cadmium chloride.

## Antioxidants and oxidative stress parameters

### Serum

As shown in **Table 4**, at the end of the 3rd week of age, $CdCl_2$ treated group showed a remarkable decline in the levels of GSH, SOD and CAT compared to control group, GSE1+ $CdCl_2$ and GSE2+ $CdCl_2$ showed a significant increase compared to $CdCl_2$ group. GSE2+ $CdCl_2$ group showed a weighty proliferation when compared to GSE1+ $CdCl_2$ group. Meanwhile, MDA level indicated a significant upturn in $CdCl_2$ fed group in comparison to control group, both GSE1+ $CdCl_2$ and GSE2+ $CdCl_2$ revealed a significant decrease in contrast to the group fed $CdCl_2$. At the end of the 5th week of age, the same results were recorded as the 3rd week in addition to that GSE2+ $CdCl_2$ group showed a significant decrease when compared to GSE1+ $CdCl_2$ group.

Table 4: The effect of cadmium chloride ($CdCl_2$), grape seed extract by the first dose (GSE1) and grape seed extract by the second dose (GSE2) on some serum oxidative stress markers at the 3rd and the 5th weeks age of chickens.

| Parameters / Treatments | GSH (ng/ml) | SOD (U/ μL) | CAT (U/ ml) | MDA (μM ) |
|---|---|---|---|---|
| The third week of age | | | | |
| Control | 56.00 ± 0.03 [a] | 5.12 ± 0.09 [a] | 12.07 ± 0.29 [a] | 1.80 ± 0.02 [c] |
| GSE1 | 55.08 ± 0.40 [a] | 5.18 ± 0.01 [a] | 12.21 ± 0.32 [a] | 1.75 ± 0.01 [c] |
| GSE2 | 54.93 ± 0.31 [a] | 5.18 ± 0.06 [a] | 12.26 ± 0.35 [a] | 1.78 ± 0.01 [c] |
| $CdCl_2$ | 36.09 ± 0.27 [d] | 3.16 ± 0.03 [d] | 7.01 ± 0.13 [d] | 3.34 ± 0.07 [a] |
| GSE1+ $CdCl_2$ | 40.05 ± 0.27 [c] | 3.42 ± 0.06 [c] | 7.65 ± 0.06 [c] | 2.87 ± 0.06 [b] |
| GSE2+ $CdCl_2$ | 43.79 ± 0.23 [b] | 3.89 ± 0.03 [b] | 8.39 ± 0.07 [b] | 2.70 ± 0.08 [b] |
| The fifth week of age | | | | |
| Control | 54.06 ± 0.26 [a] | 5.16 ± 0.04 [a] | 12.26 ± 0.22 [a] | 1.77 ± 0.03 [d] |
| GSE1 | 55.27 ± 0.43 [a] | 5.19 ± 0.02 [a] | 12.34 ± 0.19 [a] | 1.76 ± 0.04 [d] |
| GSE2 | 55.13 ± 0.22 [a] | 5.19 ± 0.05 [a] | 12.53 ± 0.03 [a] | 1.74 ± 0.03 [d] |
| $CdCl_2$ | 40.77 ± 0.76 [d] | 3.63 ± 0.06 [d] | 8.19 ± 0.04 [d] | 2.80 ± 0.06 [a] |
| GSE1+ $CdCl_2$ | 47.43 ± 0.70 [c] | 3.98 ± 0.07 [c] | 9.26 ± 0.08 [c] | 2.41 ± 0.02 [b] |
| GSE2+ $CdCl_2$ | 50.70 ± 0.67 [b] | 4.44 ± 0.09 [b] | 10.11 ± 0.14 [b] | 2.13 ± 0.06 [c] |

### Brain

As shown in **Table 5**, at the end of the 3rd week of age, the CdCl2-treated group displayed a notable decline in the levels of SOD and CAT when compared to the control group. GSE1+ $CdCl_2$ and GSE2+ $CdCl_2$ demonstrated a noteworthy rise in comparison to the $CdCl_2$ group. Additionally, the GSE2+ $CdCl_2$ group revealed a significant increase compared to the GSE1+ $CdCl_2$ group. On the contrary, the CdCl2treated group exhibited significantly higher MDA and NO levels than the control group. GSE1+ $CdCl_2$ and GSE2+ $CdCl_2$ showed a significant decrease in MDA and NO compared to the $CdCl_2$ group, while the GSE2+ $CdCl_2$ group revealed a significant decrease when compared to GSE1+ $CdCl_2$. At the end of the 5th week of age, the same result was recorded as the 3rd week of age.

Table 5: The effect of cadmium chloride ($CdCl_2$), grape seed extract by the first dose (GSE1) and grape seed extract by the second dose (GSE2) on some brain oxidative stress markers at the 3rd and the 5th weeks age of chickens.

| Parameters / Treatments | SOD (U/g) | CAT (U/g) | MDA(Mmol / g) | NO ( µM ) |
|---|---|---|---|---|
| The third week of age | | | | |
| Control | 2.44 ± 0.02 [a] | 7.47 ± 0.07 [a] | 0.65 ± 0.02 [d] | 35.22 ± 0.54 [d] |
| GSE1 | 2.45 ± 0.01 [a] | 7.44 ± 0.04 [a] | 0.63 ± 0.02 [d] | 36.75 ± 1.03 [d] |
| GSE2 | 2.47 ± 0.01 [a] | 7.51 ± 0.06 [a] | 0.61 ± 0.03 [d] | 36.19 ± 0.56 [d] |
| $CdCl_2$ | 0.91 ± 0.01 [d] | 5.17 ± 0.07 [d] | 1.86 ± 0.04 [a] | 82.36 ± 1.42 [a] |
| GSE1+ $CdCl_2$ | 1.31 ± 0.02 [c] | 5.40 ± 0.04 [c] | 1.65 ± 0.03 [b] | 72.99 ± 0.18 [b] |
| GSE2+ $CdCl_2$ | 1.61 ± 0.03 [b] | 5.94 ± 0.06 [b] | 1.43 ± 0.04 [c] | 62.21 ± 0.36 [c] |
| The fifth week of age | | | | |
| Control | 2.34 ± 0.03 [a] | 7.46 ± 0.02 [a] | 0.64 ± 0.02 [d] | 36.19 ± 0.32 [d] |
| GSE1 | 2.42 ± 0.03 [a] | 7.48 ± 0.02 [a] | 0.59 ± 0.03 [d] | 35.07 ± 0.16 [d] |
| GSE2 | 2.44 ± 0.03 [a] | 7.01 ± 1.53 [a] | 0.56 ± 0.02 [d] | 35.34 ± 0.16 [d] |
| $CdCl_2$ | 1.19 ± 0.02 [d] | 5.60 ± 0.06 [c] | 1.75 ± 0.03 [a] | 57.28 ± 0.61 [a] |
| GSE1+ $CdCl_2$ | 1.51 ± 0.04 [c] | 6.66 ± 0.07 [b] | 1.42 ± 0.04 [b] | 47.96 ± 0.59 [b] |
| GSE2+ $CdCl_2$ | 1.89 ± 0.03 [b] | 7.10 ± 0.08 [a] | 1.12 ± 0.03 [c] | 41.64 ± 0.66 [c] |

Values are expressed as mean (Mean ± SE). Means with the same letter in the same column are non-significant at P<0.05. GSE1= grape seed extract 1st dose. GSE2= grape seed extract 2nd dose. $CdCl_2$ = cadmium chloride .

## Reverse transcriptase PCR (RT-PCR)

Comparing the GSE1 and GSE2 groups to the control group, **Fig. 1** illustrates that there was no discernible change in expression. The mRNA expression of GST and GPx was downregulated upon cadmium administration. After receiving previous GSE treatment, the expression level improved. The $CdCl_2$ group's expression significantly decreased.

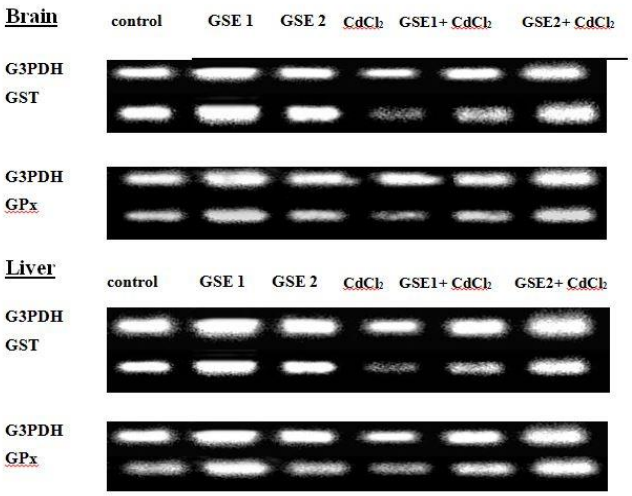


**Fig. 1:** RT-PCR densitometric analysis of GST and GPx expression in brain and liver tissue after administration of cadmium (Cd) and grape seed extract (GSE).

## Histopathological results

### Brain

Microscopic examination revealed that the cerebral cortex of control, GSE1 and GSE2 groups of broiler chickens showed normal histological structure in the 3rd and 5th weeks of age (**Photo 1 (a, b and c) and Photo 2 (a, b and c)**, respectively.

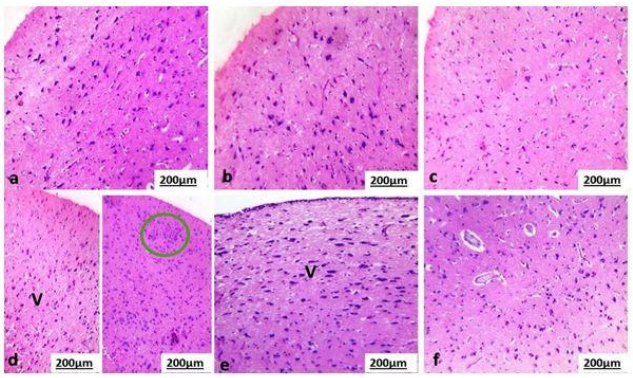


Photo 1: Photomicrograph of the cerebral cortex in the 3rd weeks of age of (a) control, b) GSE 1 group and (c) GSE 2 group showed normal histological structure of the cerebral cortex. (d) $CdCl_2$ group showed vacuolated cell in the three layer (V) and infiltration of inflammatory cells (circle). (e) GSE1+ $CdCl_2$ showed vacuolated cell in the three layers (V). (f) GSE2+ $CdCl_2$ showed normal histological structure of the cerebral cortex. (H&E, 100X).

The external granular layer, the internal pyramidal layer, the external pyramidal layer, the multiform layer, and the molecular layer make up the six layers of the cerebral cortex. In contrast, the $CdCl_2$ group's cerebral cortex tissue showed damage as a result of the histological examination in the 3rd and 5th weeks of age **(Photo 1d and Photo 2d**, respectively).

The cerebral cortex tissue from this treatment showed infiltration of inflammatory cells and vacuolated cells, especially in the first three layers. The cerebral cortex of GSE1+ $CdCl_2$ and GSE2+ $CdCl_2$ showed improvement in the histological structure in the 3rd and 5th weeks of age **(Photo 1 (e&f) and Photo 2 (e&f)**, respectively.

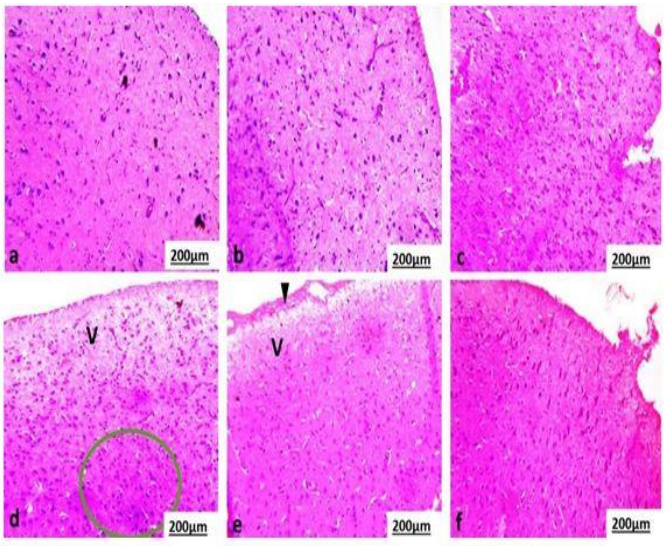


Photo 2: Photomicrograph of the cerebral cortex in the 5th weeks of age of (a) control, (b) GSE 1 group and (c) GSE 2 group showed normal histological structure of the cerebral layer. (d) $CdCl_2$ group showed vacuolated cell in the outer three layers (V) and infiltration of inflammatory cells (circle). (e) GSE1+ $CdCl_2$ showed vacuolated cell in the outer three layers (V) and thickened of the pia matter. (f) GSE2+ $CdCl_2$ showed normal histological structure of the six cerebral cortex layers. (H&E, 100X).

**The BAX protein,** which is expressed in all cytoplasmic components of cells, was significantly less expressed in the control broiler chicken at five weeks of age **(Photo 3a),** but the nuclei were not immunostained. The cerebral cortex of the $CdCl_2$ group showed a marked increase in BAX immunoreactivity **(Photo 3b).** The GSE2+ $CdCl_2$ group experienced a significant decrease in this increase when compared to the $CdCl_2$ group; nonetheless, the expression remained more prominent than that of the control animals **(Photo 3c).**

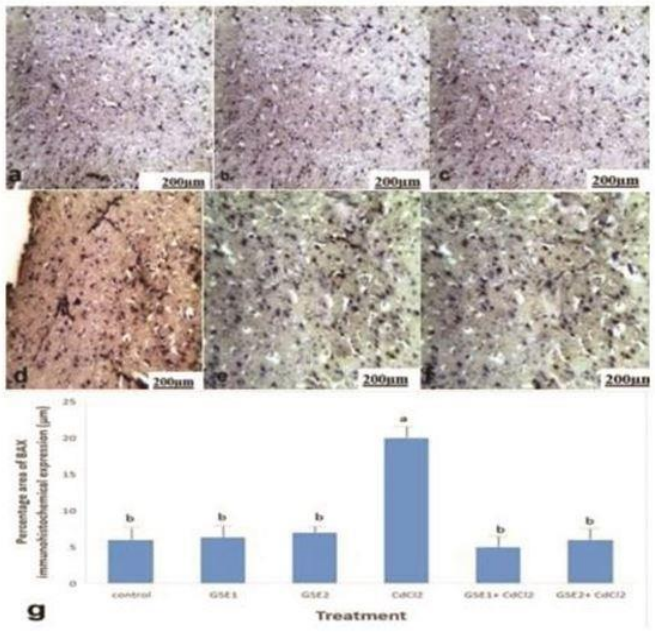


Photo 3: Photomicrograph of the immunohistochemical expression of BAX in the cerebral cortex in the 5th weeks of age of (a) control, (b) GSE1, (c) GSE2, (d) $CdCl_2$ (e) GSE1+ $CdCl_2$ (f) GSE2+ $CdCl_2$. (g) Histogram of the mean percentage areas of BAX in the cerebral cortex of different groups ($n$ = 5). Different superscript letters denote significant differences at $P \leq 0.05$.

## DISCUSSION

Cadmium (Cd) is a common and hazardous heavy metal found in modern environments. It is not well absorbed, transferred to plasma, bound to albumin, and primarily accumulated in the kidney and liver (**Kaya *et al.*, 2002**). Cadmium is a cumulative toxin with a long biological half-life that is mainly distributed to the liver and subsequently redistributed to the kidney (**Cinar, 2003**). The importance of grapes is due to their high polyphenol content. These phenolic compounds are mostly known for their antioxidant properties (**El Gengaihi *et al.*, 2014**). Research has shown that extracting procyanidins may have biological effects, sparing other antioxidants and boosting tissue's overall antioxidant status while shielding biomolecules from potential oxidative damage during digestion (**Brenes *et al.*, 2016**). Gallic acid, which is regarded as a strong antioxidant and antitumor agent, can be found in GSE (**Kaur *et al.*, 2009**). Many substances known as polyphenols are found in it, including dimers, trimers, and other oligomers of epicatechin and catechin, as well as their gallate derivatives, which are known as proanthocyanidins and which have anti-inflammatory, anticancer, and oxidative stress-protective properties (**Hayden *et al.*, 2015**). It has been discovered that the antioxidant properties of GSE are significantly stronger than those of vitamins C and E. (**Ariga, 2004**). According to reports, GSE significantly reduces the risk

of free radical damage compared to vitamins C, E, and A. Free radicals cause less DNA damage, lipid peroxidation, free radical scavenging, chelation, and inhibition of polyphenol oxidase (**Xu *et al.*, 2015**).

All liver cells are damaged by Cd; the inflammatory response caused by Cd results in phagocytic cell activation and infiltration, which produces additional inflammatory mediators, such as cytokines (**Arroyo *et al.*, 2013**). Inflammation is closely linked to cadmium hepatotoxicity. After an acute exposure, polymorphonuclear neutrophils (PMN) frequently infiltrate the injured liver. These neutrophils, along with Kupfer cell damage, increase inflammatory mediators and encourage necrosis, which further exacerbates the hepatotoxicity. Hepatotoxicity is caused by two pathways: the first is the direct result of the metal deposit, and the second is the result of the inflammatory process (**Rikans and Yamano, 2000**). Our study showed that the $CdCl_2$ group revealed a substantial rise in TNF-α and IL-1β, but IL-10 showed a significant decrease when compared to control group. The co-administration of GSE showed a significant recovery.

**El-Boshy *et al*., (2015)** found that rats given 40 mg/L of cadmium in their drinking water had significantly higher levels of TNF-α and IL-1β but significantly lower levels of IL-10. **Chen *et al*., (2017)** also agreed to these results, as they reported that IL-1β and TNF-α significantly increased with $CdCl_2$ while IL-10 decreased. For **Turksoy *et al*., (2019),** there was a statistical difference observed in the levels of the cytokines TNF-α and IL-10 between the groups exposed to Cd and the control group. The significant reduction in IL-10 observed as a result of a result of Cd has a detrimental effect on the bird's immune system. The hypooxidative state that releases reactive oxygen species (ROS) and causes organ damage may be the cause of the decrease in IL-10 levels in cadmium-group chickens (**Ognjanovic *et al.*, 2008**). On the other hand, the administration of strong antioxidants increased the IL-10 decline. According to **Alkhedaide *et al.* (2016),** grape seed reduces oxidative stress, which lessens the effects of Cd toxicity on IL-10 in albino rats. **Hashem *et al.*, (2019)** reported a significant reduction in IL-10.

According to **Lag *et al.*, (2010**), pro-inflammatory cytokines are well-known traditional indicators of inflammation. They not only encourage the induction of acute-phase proteins, but their overproduction can result in chronic inflammation and/or autoimmunity. According to **Akdis *et al.*, (2016)**, IL-10 is an anti-inflammatory interleukin that has the ability to suppress the production of numerous pro-inflammatory cytokines and chemokines. In cases of inflammation and carcinogenesis, IL-1β and TNF-α can stimulate the production of IL-6. According to **Xie *et al.*, (2018)**, tumor necrosis factor-α, a cytokine released by activated macrophages, is a necessary and sufficient mediator of both local and systemic inflammation. It also functions in concert with IL-1β to initiate inflammatory processes that in turn stimulate the production and release of other cytokines (**Lag *et al.*, 2010**). According to **Alkedaide *et al.*, (2015)**, animals that received both $CdCl_2$ and GSE concurrently showed a significant decrease in their serum TNF-α level. Additionally, GSE significantly reduced the DNA damage that $CdCl_2$ caused in lymphocytes. Likewise, according to **Sehirli *et al.*, (2008)**, GSE may reduce organ damage by controlling the release of inflammatory mediators and balancing the oxidant-antioxidant status. Proanthocyanidins have an antioxidant effect on experimental ration contamination with $CdCl_2$. Accordingly, according to **Lingyu *et al.* (2018)**, its mechanism is related to scavenging oxygen-free radicals and inhibiting the production of inflammatory cytokines. In our study, we examined the powerful protective and ameliorative effects of grape seed extract, either by itself or in conjunction with cadmium chloride, in mitigating the oxidative damage and inflammatory cascade in serum and brain tissue caused by broiler ration contamination.

Prior research has demonstrated the detrimental pro-oxidative and pro-inflammatory properties of $CdCl_2$, as well as its ability to generate a range of reactive oxygen species (ROS), including O2•, $H_2O_2$, and OH. This could potentially be the primary mechanism underlying the cellular toxicity caused by this heavy metal (**Jin *et al.*, 2016**). It can take the place of iron and copper in a variety of cytoplasmic and membrane proteins, including ferritin. This increases the concentration of these free redox-active metals, which in turn increases the Fenton reaction's ability to produce hydroxyl radicals. Numerous risks, such as DNA damage and mutations, protein oxidation, and lipid peroxidation (LPO), which can change the lipid composition of cellular membranes and their functionalities, can be brought on by oxidative stress (**Bertin and Averbeck, 2006**). In this regard, studies have demonstrated that elevated ROS concentrations in cortical neurons and the liver cause CdCl2 to induce LPO (**Toppo *et al.*, 2015**). Current data suggests that at the end of the 3rd and 5th weeks of age, GSE1 and GSE2 serum antioxidants (GSH, SOD, CAT and MDA) revealed no discernible change in comparison to the control group. For $CdCl_2$ treated group, GSH, SOD and CAT revealed a substantial drop in comparison to the control group, while MDA showed a significant increase. The co-administration of GSE showed a significant improvement. The outcomes of **Iqbal *et al.*, 2015, Alkhedaide *et al., (2016),* and Abu Hafsa and Ibrahim (2017**) aligned with these findings.

Reduced glutathione (GSH) is an endogenous antioxidant that is found in large quantities in cells. It serves a variety of purposes, including the storage and transportation of cysteine, the preservation of proteins' and thiols' reduced states, and the detoxification of numerous oxidative toxicants, including heavy metal ions and drugs. Furthermore, glutathione-S-transferase and glutathione peroxidase are detoxification enzymes that GSH serves as a substrate for (**Wu *et al.,* 2004**). When free $CdCl_2$ ions are present, GSH is the main target. It scavenges $CdCl_2$ to stop it from interacting with important cellular targets. Consequently, extended exposure to $CdCl_2$ caused the reduced GSH pool to be depleted, which upset the redox balance and created an oxidative environment (**Liu *et al.,* 2008**). Consequently, extended exposure to $CdCl_2$ caused the reduced GSH pool to be depleted, which upset the redox balance and created an oxidative environment (**Liu *et al.,* 2008**). So according to **Lopez *et al.,* (1998)**, cadmium can also induce stress by raising lipid peroxidation or altering the level of intracellular glutathione (GSH). Reduced glutathione and protein sulfhydryl groups bind covalently to cadmium **(Anke, 2023).**

One important antioxidant enzyme that shields cells and other organisms from the harmful effects of superoxide anion is called superoxide dismutase (SOD). Superoxide dismutase and peroxidases work together to lessen the negative effects of reactive oxygen species (ROS). Apart from superoxide dismutase found outside of cells, two other known superoxide dismutase forms exist: an intracellular form, Cu+2/Zn+2SOD, and a mitochondrial form, Mn+2 SOD **(Filomena *et al.,* 2008)**. Superoxide dismutase is extremely sensitive and inactivated by cadmium treatment. It is known to be expressed in cerebellar granule neurons (CGNs) and is likely Cu/ZnSOD **(Okabe *et al.,* 2000)**. It was hypothesized that cadmium could substitute zinc in the catalytic region of the enzyme, resulting in a decrease in superoxide dismutase activity (**Casalino *et al.,* 2002**). The exchange of essential metals from the active sites of some enzymes through interactions with SH groups or the modification of amino acid chains due to radical mediating reactions are other plausible mechanisms in the depletion of antioxidant enzymes induced by cadmium. Inactivation and loss of protein function are the ultimate consequences of these harmful alterations (**Sharma *et al.,* 2014).**

Almost every living thing that comes into contact with oxygen contains the enzyme catalase (CAT). According to **Chelikani *et al.,* (2004)**, it serves as a catalyst for the breakdown of hydrogen peroxide into water and oxygen. As an antioxidant enzyme, it is one of the defense mechanisms that contribute to the preservation of equilibrium in the human body and has one of the highest conversion numbers among all enzymes **(Jones, 1982)**. **(Stipek *et al.,* 2000)**. It is likely that direct Cd binding to the enzymes' active sites is the mechanism causing the Cd-induced decrease in enzyme activities **(Dukic-Cosic *et al.,* 2020).**

Cell membrane damage is indicated by malondialdehyde (MDA), according to **Jacob and Burri (1996)**. MDA is a byproduct of lipid peroxidation brought on by reactive oxygen and free radicals. Lipid peroxidation rises in RBC membranes exposed to cadmium toxicity. Indicators of oxidative damage to the brain and other organs are found in MDA **(Stohs *et al.,* 2001)**. Upon peroxidation, polyunsaturated fatty acids in cells are transformed into MDA. Cell oxidative damage is indicated by an excess of MDA produced by free radicals **(Moitra *et al.,* 2014)**. By inducing oxidative stress, CD can exacerbate negative effects in different organs. An increase in MDA production indicates that peroxidation of membrane lipids due to dietary excess Cd generates free radicals, which in turn damage the liver and kidneys **(Nemmiche, 2017).** Thiobarbituric acid reactive substance, or TBARS, is a measurable indicator of lipid peroxidation and a significant byproduct of this reaction that is measured as MDA **(Bo and Yanli, 2013).** Proanthocyanidins and procyanidins, two types of polyphenols found in GSE, have been shown to have antioxidant and free radical-scavenging properties. The enzyme systems that produce free radicals are inhibited by GSE **(Bagchi *et al.,* 2014).** Also, referred to as sustained-release antioxidants, they differ mechanistically from other water-soluble antioxidants in that they can exert their antioxidant effects in the plasma and tissues for a maximum of 7–10 days **(Hagerman *et al.,* 1998)**. In line with this, concurrent administration of GSE and CdCl2 resulted in a noteworthy decrease in MDA levels within the splenic tissues, as well as an increase in CAT, SOD activities, and GSH content.

For tissue antioxidants, at the end of the 3rd and 5th weeks of age, GSE1 and GSE2 showed no significant change compared to the control group. Brain SOD and CAT showed a significant decrease in the CdCl2-treated group, while brain MDA and NO showed a significant increase. The co-administration of GSE showed a significant improvement in all previously measured parameters. For mRNA expression, the GSE1 and GSE2 groups revealed no discernible change in expression when compared to the control group. The mRNA expression of GST and GPx was downregulated upon cadmium administration. After receiving previous GSE treatment, the expression level improved. The CdCl2 group showed a significant decrease in expression.

Furthermore, GSE (100 and 200 mg/kg) dramatically decreased liver MDA contents and raised

liver GSH content in a dose-dependent manner, according to **Sehirli *et al.*, (2008).** Furthermore, **Somaia *et al.*, (2015)** discovered that administering GSE at doses of 100 and 200 mg/kg resulted in a noteworthy reduction in elevated liver MDA and a noteworthy elevation in GSH levels when contrasted with the positive control group. GSE (150 mg/kg b. wt.) pretreatment caused a considerable change in MDA, inflammatory cytokines, antioxidant enzymes, and apoptotic markers. Furthermore, due to its potent anti-inflammatory, strong antioxidant, powerful free radical scavenging, and effective anti-apoptotic properties, it also significantly reduced lung GSH and SOD levels (Hanaa et al., 2015). **Cheng *et al.*, (2007)** findings support **Baiomy, (2016)** findings that grape seed treatment significantly reduced oxidative stress in rat groups by lowering lung tissue MDA and increasing antioxidant enzyme activity in lung tissues. Sodium valproate (VPA) was used to create the rat model of autism **(Rinaldi *et al.*, 2007)**. In the cerebellum of the offspring rats treated with VPA, there was a significant decrease in the levels of CAT, SOD, and GSH-Px, along with a reduction in the number of purkinje cells and nucleus size. Following GSE treatment, the activities of SOD, CAT, and GSH-px dramatically increased, indicating that GSE's antioxidative qualities may have had a neuroprotective effect on the cerebellum **(Arafat and Shabaan, 2019)**. neurotoxic markers in animals **(Zarzecki *et al.*, 2014)**. The brain tissue exhibited a significant reduction in the activities of SOD, CAT, and GPx. It was discovered that GSE had considerably changed their course of action. Furthermore, it demonstrated neuroprotective effects by lowering iNOS mRNA expression and MDA content **(Abdou and Wahby, 2016)**.

When Cd was added to the chicken diet, the kidney's MDA level increased significantly, the hepatic GSH-Px level decreased in the Cd-treated groups, and the chickens' blood CAT activity significantly decreased in comparison to the control group's enzyme activity **(Vasiljeva *et al.*, 2018)**. Similar to this, Cd has the potential to harm nerves **(Liu *et al.*, 2001)**. These outcomes are similar to those reported by **El-Tarras *et al.* (2016)**, who found that while GSE normalized the expression of GST and GPx, Cd downregulated their expression. In some parts of the brain, exposure to cadmium also caused a reduction in the GSH/GSSG ratio. There were reports of increased GSSG levels, reduced thiol and GSH, and elevated ROS production. Activities for SOD, CAT, GST, and GPx decreased. These findings point to oxidative impairment. According to **Wardani *et al.* (2018)**, administering cadmium chloride led to a significant decrease in SOD and GPx levels as well as an increase in MDA levels in comparison to the control group. **Omotoso *et al.*, (2019)** It was also reported that, when compared to control rats, rats given cadmium showed a notable increase in the levels of SOD and GPx in their brain tissue as well as a decrease in their serum activity. In comparison to the normal group, cadmium also dramatically raises the activity of glutathione peroxidase in the brain tissue. Following hypoxic-ischemic encephalopathy (HIE) in neonates, it was discovered that the proteins Bcl-2 and BAX were involved in the regulation of neurons **(Tu *et al.*, 2019)**. By hindering the expression of caspase-3 and BAX, GSE may prevent cell apoptosis. This would enhance brain function by limiting the amount of BAX protein and expanding the amount of Bcl-2 protein **(Fang *et al.*, 2018)**. Similar to this, cadmium (Cd) has the potential to harm nerves (Liu et al., 2001). GSE was discovered to lessen the neurotoxicity brought on by Cd, increase mRNA expression of GST and GPx,

Due to its high lipid content, the central nervous system is particularly vulnerable to the damaging effects of free radicals produced during oxidative neurotoxicity. The blood-brain barrier (BBB) prevents foreign molecules from entering the body, but when cadmium is exposed to minimum levels over time, the BBB becomes damaged and the brain is negatively affected **(Mizee and DeVries, 2013)**. This is an essential brain event that causes lipid peroxidation**. El-Tarras *et al.*, (2016)** found that the overproduction of superoxide radicals mediates cadmium-evoked lipid peroxidation, resulting in the production of toxic products. Cadmium's post-BBB entry causes neurotoxicity, exhibiting a variety of clinical symptoms including behavioral abnormalities, neuronal degeneration, and modifications to nerve transmitters **(Renugadevi and Miltonprabu, 2009)**.

Microscopic examination revealed that the cerebral cortex of control, GSE1 and GSE2 groups of broiler chickens showed typical histological shape at the end of the 3$^{rd}$ and 5$^{th}$ weeks of age. The six layers that make up the cerebral cortex are the molecular layer, the external granular layer, the external pyramidal layer, the internal pyramidal layer, and the multiform layer. On the other hand, damage to the cerebral cortex tissue was identified in the histological examination results. of the CdCl2 group at the end of the 3$^{rd}$ and 5$^{th}$ weeks of age. The cerebral cortex tissue from this treatment showed infiltration of inflammatory cells and vacuolated cells, especially in the first three layers. The cerebral cortex of GSE1+ $CdCl_2$ and GSE2+ $CdCl_2$ showed improvement in the histological structure at the end of the 3rd and 5th weeks of age.

The control, GSE1 and GSE2 broiler chicken groups at the end of the 5$^{th}$ week of age showed a significant decrease in expression of BAX (a protein present in the cytoplasm of every cell), but the nuclei lacked immunostaining. There was a notable rise in

BAX immunoreactivity in the cerebral cortex of the $CdCl_2$ group. This increase was significantly reduced in co treated GSE groups compared to the $CdCl_2$ group.

The brain results matched those of **El-Tarras *et al.*, (2016)**, who reported that the control and GSE groups' histological structures showed normal brain structure. The six well-organized layers of the adult male albino rat's gray matter, which is made up of nerve cells with various sizes and shapes, were visible. Neural fibers with uniform staining extending down the cortex comprised the typical white matter pattern. The nerve cells were dispersed throughout the gray matter and came in various sizes and shapes. Congested blood vessels in the meninges and brain were present in the brain tissue of the Cd group. There have been reports of fibrosis in the white matter nerve fibers and degeneration in certain nerve cells with pyknotic nuclei. In the architecture of the brain tissue, numerous tiny vacuoles accumulated as bounded regions that took the place of the nerve cells. The capsular cells surrounding the nerve cells exhibited stelteltosis. There was some blood vessel congestion. Although some lesions persisted, the treated group's brain tissue showed improvement. There was obvious meningeal blood vessel congestion. The arrangement of nerve cells and fibers was determined by the structure of the brain tissue.

Many small vacuoles accumulated as bounded regions in the architecture of the brain tissue, replacing the nerve cells. Telteltosis was seen in the capsular cells that encircled the nerve cells. Some blood vessel congestion was present. The brain tissue of the treated group improved, despite the persistence of some lesions. Meningeal blood vessel congestion was clearly present. The structure of the brain tissue dictated the arrangement of nerve cells and fibers.

Immunohistochemistry in the nerve cells revealed that Cd enhanced the expression of BAX, which raised the incidence of apoptosis. The incidence of apoptosis was significantly reduced by GSE treatment. Due to the permeability transition and loss of the mitochondrial membrane, BAX depolarizes mitochondria and causes the release of cytochrome c through openings in the outer membrane. Through the inhibition of pro-apoptotic proteins, GSE exhibits strong antiapoptotic effects in a variety of tissues **(Chen and others, 2013)**. These results corroborated our findings and provided further evidence for them. According to **Ye *et al.*, (1999)**, GSE significantly increases the growth and viability of normal cells while having a strong cytotoxic effect on gastric, lung, and breast cancer cells. As a result of permeability transition and loss of mitochondrial membrane potential, pro-apoptotic proteins like BAX have been shown to migrate intracellularly, depolarizing mitochondria and causing the release of cytochrome c through openings in the outer membrane **(Green and Reed, 1998)**. GSE's antiapoptotic properties in different tissues, such as its ability to lower apoptotic cell death and suppress proapoptotic proteins **(Chen *et al.*, 2013)**.

Immunohistochemically, in comparison to the control group, the specimens treated with $CdCl_2$ had a higher number of BAX-positive cells and necrosis. The rise in cells that are positive for BAX signifies apoptosis. Increased lipid peroxidation and ROS accumulation following $CdCl_2$ exposure can cause both necrosis and apoptosis **(Mohammed *et al.*, 2017)**. By using immunohistochemistry, it was evident that the BAX-positive splenic cells were fewer in this group than in the $CdCl_2$-treated one. The active ingredient in GSE may stimulate the immune system, promote splenocyte proliferation, and cause hyperplasia of the lymphoid tissue in the spleen's white pulp. Experimental research has demonstrated that oral administration of GSE enhances antioxidant activities and inhibits lipid peroxidation, which is consistent with these findings **(Spranger *et al.*, 2008)**. Following hypoxic-ischemic encephalopathy (HIE) in neonates, it was discovered that the proteins Bcl-2 and BAX were involved in the regulation of neurons **(Tu *et al.*, 2019)**. By preventing the contents of BAX and caspase-3, GSE may prevent cell apoptosis. This would improve brain function by reducing the amount of BAX protein and increasing the amount of Bcl-2 protein **(Fang *et al.*, 2018)**.

An important mechanism mediating $CdCl_2$-toxicity in various organs was apoptosis, as demonstrated by elevated lipid peroxidation following exposure to $CdCl_2$ **(Mohammed *et al.*, 2017)**. The current study demonstrated that $CdCl_2$ induced histological and immunohistochemical changes in the spleen in comparison to the control group. These changes included small aggregations of lymphocytic infiltration in the red pulp, congestion, hemolysis, and edema, as well as thick splenic vessels. Splenic follicle depletion was common, which was consistent with **Tarasub *et al.* (2012)** suggestion that $CdCl_2$ is a lymphocyte toxic agent that can induce necrosis or apoptosis. Besides this, the infiltration of subcapsular lymphocytes could indicate a recurrent tearing and wearing of tissues as a reaction to the damage brought on by $CdCl_2$. Compared to the control group, the immunohistochemical analysis of the $CdCl_2$-treated specimens revealed a higher quantity of BAX-positive cells as well as necrosis. The rise in cells that are positive for BAX signifies apoptosis. Increased lipid peroxidation and ROS accumulation following $CdCl_2$ exposure can cause both necrosis and apoptosis **(Fleury *et al.*, 2002).**

**Wahdan *et al.*, (2014)** reported that the brain tissue under examination displayed meningeal blood vessel congestion, degeneration of nerve fibers and cells, and cerebrum and cerebellum fibrosis as a result of a build-up of Cd in the choroidal plexus at concentrations greater than those found in the cerebrospinal fluid (CSF) and brain tissues. In the cerebellum's white matter, cadmium has the ability to kill nerve cells, oligodendrocytes, and nerve fibers. Immunohistochemistry in the nerve cells revealed that Cd enhanced the expression of BAX, which raised the incidence of apoptosis. The incidence of apoptosis was significantly reduced by GSE treatment. Due to the permeability transition and loss of the mitochondrial membrane, BAX depolarizes mitochondria and causes the release of cytochrome c through openings in the outer membrane. Through the inhibition of pro-apoptotic proteins, GSE possesses strong antiapoptotic effects in a variety of tissues **(Ozkan *et al.*, 2012)**. At the synapse level, cadmium interferes with neurotransmission through its interactions with zinc or calcium. Due to the synergistic toxicity these interactions cause in astrocytes, the BBB is disrupted, leading to behavioral dysfunction in rats **(Rai *et al.*, 2010)**.

## CONCLUSION

It could be concluded that, CdCl2 is a harmful ration contaminant that significantly affects broiler health. It significantly decreases pro-inflammatory cytokines and oxidative stress markers. GSE has a favorable effect on broiler health and also ameliorates the side effects of ration contamination. Moreover, the optimum concentration of GSE added to the ration and obtained a desirable effect was 250 mg/kkg diet. So it is recommended to use GSE as a protective agent against ration contamination, growth enhancer, antioxidant and oxidative stress inhibitor.

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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