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# Beneficial Effects of Some Nutraceuticals Containing Glucosamine and Antioxidant against $CCL_4$ Induced Brain Injury in Rats

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## Abstract

The present study is performed to investigate the effect of two different glucosamine containing drugs: Drug 1 and Drug 2 (D1 and D2) against $CCl_4$ induced brain damage in male albino rats. Liverin (AM) was employed in the current study as an antioxidant reference drug. $CCl_4$ administration caused a significant elevation in the levels of MDA and NO of brain tissue, in association with a significant decrease in the antioxidant defense system (GSH, SOD and GPX) that indicated the induction of oxidative stress in brain tissue. $CCl_4$ administration induced brain injury as manifested by the obtained changes in neurotransmitter parameter (norepinephrine (NE), Dopamine (DA), Serotonin (5-HT), and Acetylcholinesterase AChE). The tested nutraceuticals and the antioxidant drug displayed a significant improvement against the undue effect of $CCl_4$ via decreasing the brain tissue content of MDA, NO with the elevation of GSH content. Also, the significant increase in SOD and GPX enzymatic activity was obtained when compared to $CCL_4$ group. In addition AM, D1, and D2 have an ameliorative effect on neurotransmitter parameter NE, DA, 5HT, and AChE. Results of this study suggest that both antioxidant drugs and tested nutraceuticals palliate the brain injuries through anti-oxidative effect, with the elimination of the deleterious effect of toxic metabolites of $CCl_4$ on brain tissue.

## Keywords

$CCL_4$, Glucosamine, Antioxidant and Neurotransmitter

## 1. Introduction

Glucosamine (GlcN) has been reported to have anti-tumor (Zahedipour *et al.*

[1]), anti-oxidant (Xing *et al.* [2], Jamialahmadi *et al.* [3]), and anti-allergic activity (Jung *et al.* [4]). Other pharmacological properties of GlcN including protective effects against multiple sclerosis and encephalomyelitis (Zhang *et al.* [5]), learning and memory impairment (Jamialahmadi *et al.* [6]), colitis (Yomogida *et al.* [7]), and ischemic brain injury (Hwang *et al.* [8]) have been investigated. Recent studies further suggest that OGlcN Acylation is involved in the regulation of inflammation and exerts protective effects against inflammation-induced tissue injury, both in the brain and peripheral system (Hwang *et al.* [9], He *et al.* [10] and Zhang *et al.* [11]). Hwang *et al.* [12] showed that GlcN protects against lethal septic shock and sepsis-induced lung inflammation and injury in mouse model, and examined the potential underlying mechanism. The brain is highly vulnerable to Oxidative stress (OS) than other organs of the body in view of the unusually high rate of oxygen consumption, being rich in Polyunsaturated fatty acids (PUFA), and low levels of antioxidant enzymes coupled with high amount of non-haem iron (Chong *et al.* [13] and Somani *et al.* [14]). Neurotoxic compounds generate OS by inducing lipid peroxidation and altering the antioxidant defenses in the brain (Verma and Srivastava [15] and Srivastava and Shivanandappa [16]). In view of this, we hypothesized that brain could be a vulnerable target organ for the action of $CCl_4$. Reports on the effects of $CCl_4$, on the brain are sketchy (Szymonik-Lesiuk *et al.* [17]).

Carbon Tetrachloride ($CCl_4$) is widely used to induce hepatotoxicity in experimental animals.$CCl_4$ hepatotoxicity is characterized by hepatocellular necrosis with fat deposition. At Acute toxic doses of $CCl_4$, when hepatocellular necrosis exceeds the regenerative capacity of the liver, fatal liver failure often ensues. High Doses of $CCl_4$ results in non specific toxicity, including central nervous system depression and respiratory failure resulting in death (Recknagel *et al.* [18]). The Free radicals generated from $CCl_4$ and the parent molecule by itself, damage endoplasmic reticulum (ER), which leads to lipids peroxidation, reduced protein synthesis and mixed-function oxidases activity (Weber *et al.* [19]). $CCl_4$ belongs to the class of hepatotoxins, which act after metabolic activation. Oxidative stress resulting from the increased production of free radical after $CCl_4$ intoxication may play an important role in the degenerative processes in the tissues (Karadeniz *et al.* [20]).

## 2. Materials and Methods

### 1) Animals and Diet

Male Wistar albino rats weighed 200 - 260 g body wt. were purchased from animal house of NODCAR. Animals were acclimatized to the laboratory conditions 1 week before the start of experiment and caged in temperature (23˚C ± 4˚C). Rats had free access to water and standard rat diet. The experiments were conducted in accordance with ethical guidelines for investigations in laboratory animals.

### 2) Drugs

**Drug 1 (D1):** Move free advanced, which is a coated tablet contains Gluco-

samine Chondroitin plus MSM (methylsulphonylmethane). D1 was purchased from CHIFF Nutrition Group Inc. (USA). Active ingredients: GlcN 1500 mg, vit. D3 2000 IU, MSM 750 mg, uniflex 216 mg, CS 200 mg and hyaluronic acid 3.3 mg.

**Drug 2 (D2):** Glucosamine Plus Vit. D3, the drug was purchased from CHIFF Nutrition Group (USA). Active ingredients: GlcN 1500 mg, vit. D3 400 IU, hyaluronic acid 3.3 mg.

**AM**: Liverin was purchased from Western Pharmaceutical industries (Egypt). Active ingredients: Milk thistle (silymarine) 140 mg, DL. Methionine 110 mg, Inositol 83 mg, vit. B1 3.06 mg, vit. B2 3 mg, Nicotinamide 10 mg, vit. B6 2 mg.

**$CCl_4$**: purchased from SIGMA (sigma-Aldrich, USA).

**3) Experimental Design**

Animals were allocated into eight experimental groups (10 rats each) as the following:

Group I: served as control group, rats of this group were orally received 1 ml /kg body wt. of olive oil twice a week and a daily 1 ml/kg body wt. of 0.25% Carboxymethyl Cellulose (CMC).

Group II: served as Liverin (AM) administered group, the rats of this group were received a daily oral dose of 1 ml/kg body wt. of AM as a suspension in 0.25% CMC

Group III: the rats were received a daily oral dose of 1 ml/kg body wt. (D1) as a suspension in 0.25% CMC.

Group IV: rats of this group were received a daily oral dose of 1 ml/kg body wt. of D2 as a suspension in 0.25% CMC.

Group V: rats were orally received 0.5 ml/kg body wt. of $CCl_4$ that was diluted 1:1 with olive oil, this group served as $CCL_4$ group.

Group VI: the rats of this group were received in addition to $CCl_4$ dose a daily oral dose of 1 ml/kg body wt. of AM as a suspension in 0.25% CMC, this group served as $CCL_4$ + AM group.

Group VII: received in addition to $CCl_4$ dose a daily oral dose of 1 ml/kg body wt. D1 as a suspension in 0.25% CMC, this group served as $CC_4$ + D1 group.

Group VIII: received in addition to $CCl_4$ dose a daily oral dose of 1 ml/kg body wt. D2 as a suspension in 0.25% CMC this group served as $CC_4$ + D2 group.

**4) Doses mg/kg Body wt**

Supplement doses AM, D1 and D2 equivalent to human daily dose (2 tablets/day) and calculated according to Paget and Barnes [21].

**5) Tissue Samples**

At the end of the experiment period (eight weeks), rats were anesthetized with 50 mg/kg body weight ketamine and intramuscularly injected with 5 mg/kg body weight xylazine, brain tissues were removed quickly, washed with cold isotonic saline and stored −80˚C for biochemical examination.

Brain homogenate preparation: all brain tissues were maintained at 4˚C, 1 gm of brain tissue was homogenized in iced 10% potassium chloride using an elec-

tric homogenizer to prepare 10% w/v homogenate. The homogenate was centrifuged at 5000 rpm for 20 minutes and then the supernatants were kept at −20˚C for subsequent use for biochemical analysis.

**6) Biochemical Analysis**

**a) Oxidative Stress Parameters Were Determined by Spectrophotometer:**

- Malondialdehyde (MDA) according to the method of Ohkawa *et al.* [22].
- Nitric oxide according to the method of Montgomery and Dymock [23].
- Glutathione reduced (GSH) according to the method of Beutler *et al.* [24].
- Superoxide Dismutase (SOD) according to the method of Nishikimi, *et al.* [25].
- Glutathione Peroxidase (GPX) according to the method of Paglia and Vallentine [26].

All of the mentioned above parameters were determined using the corresponding diagnostic kits of biodiagnostic.

**b) Determination of Neurotransmitter Parameter by HPLC**

Brain monoamines (NE, DA and 5-HT) and AChE were detected by HPLC according to the methods described by Pagel *et al.* [27] and Gorun *et al.* [28] respectively.

**7) Statistical Analysis**

The data obtained from the biochemical analysis of different groups are represented in figures as mean ± S. E. The significance of difference between groups was calculated by one-way analysis of variance (ANOVA) followed by Duncan and Dunnett (2-slided) at $p < 0.05$ using the SPSS-PC computer software package version 17.

## 3. Results

**1) Effect of AM, D1 and D2, $CCL_4$ or Their Combination with $CCL_4$ on MDA, NO and GSH**

Figures 1-3 show that the sole administration of AM, D1 and D2 displayed a non-significant change in MDA, NO and GSH. Figures 1-3 also show the effect of $CCL_4$ on MDA, NO and GSH. $CCl_4$ exhibited significant elevation ($p < 0.05$) in MDA, NO and a significant decrease in GSH as compared with the control group. The combined administered of $CCL_4$ with AM, D1 and D2 resulted in a significant reduction in MDA, NO and significant elevation in GSH when compared to $CCL_4$ group. This result confirms the antioxidant activity of AM, D1 and D2.

**2) Effect of AM, D1 and D2, $CCL_4$ or Their Combination with $CCL_4$ on SOD and GPX**

Figure 4 and Figure 5 show that the sole administration of AM, D1 and D2 displayed a non-significant change in SOD and GPX enzymatic activity. Figure 4 and Figure 5 also show the effect of $CCL_4$ on SOD and GPX. $CCL_4$ administration caused a significant decrease ($p < 0.05$) of SOD and GPX enzymatic activity in comparison with the control non-treated group. While the co-administered

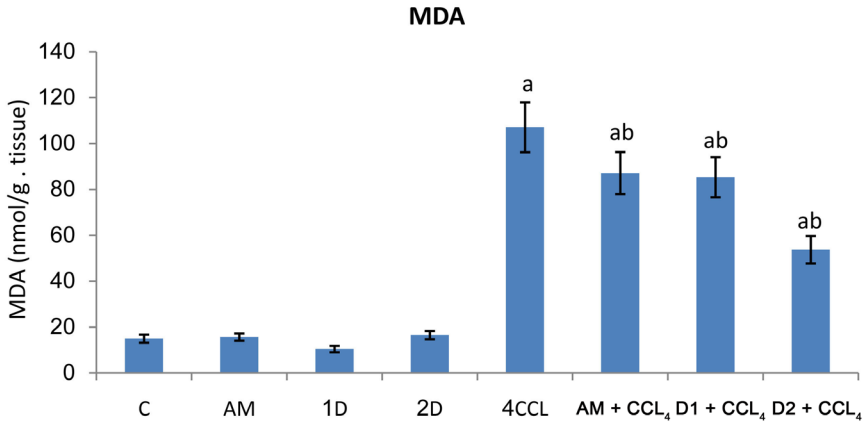


**Figure 1.** The Effect of AM, D1, D2 and $CCl_4$ or their combination with $CCL_4$ on MDA in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

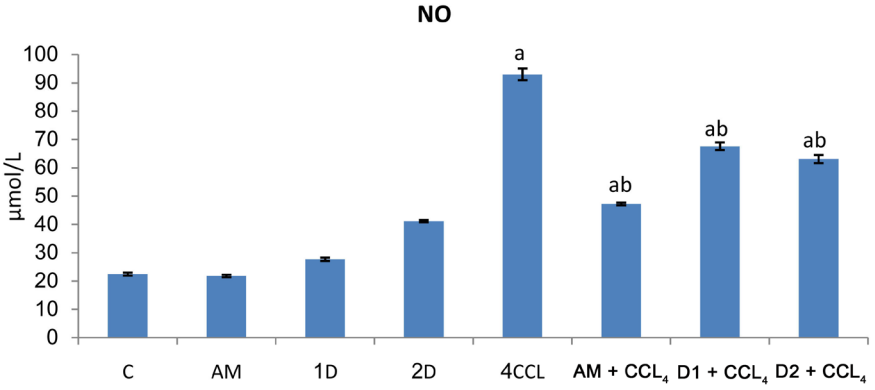


**Figure 2.** The Effect of AM, D1, D2 and $CCl_4$ or their combination with $CCL_4$ on NO in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

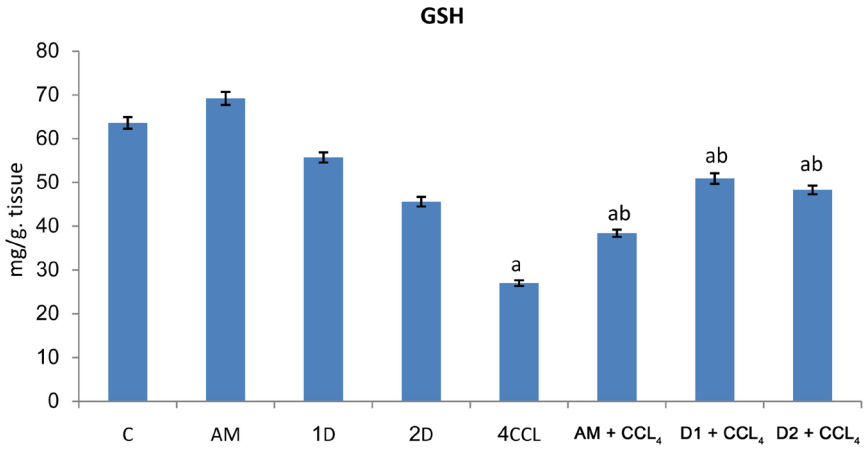


**Figure 3.** The Effect of AM, D1, D2 and $CCl_4$ or their combination with $CCL_4$ on GSH in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

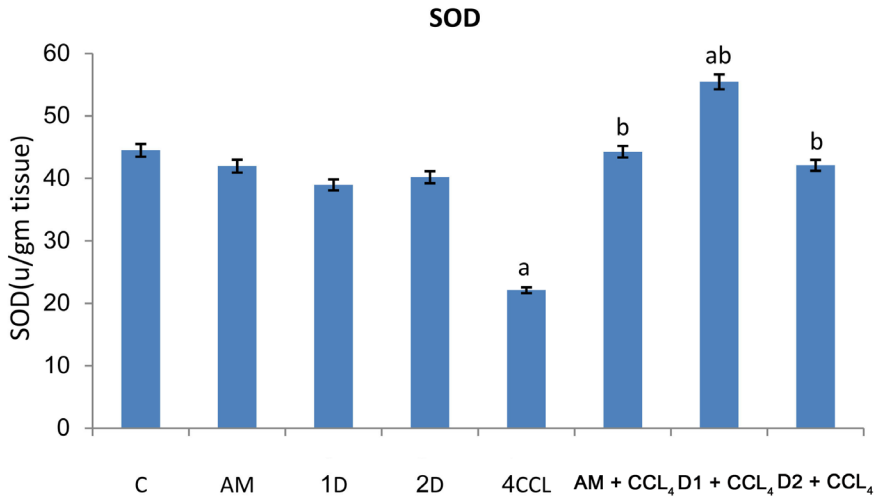


**Figure 4.** The effect AM, D1, D2 and $CCL_4$ or their combination with $CCL_4$ on SOD activity in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

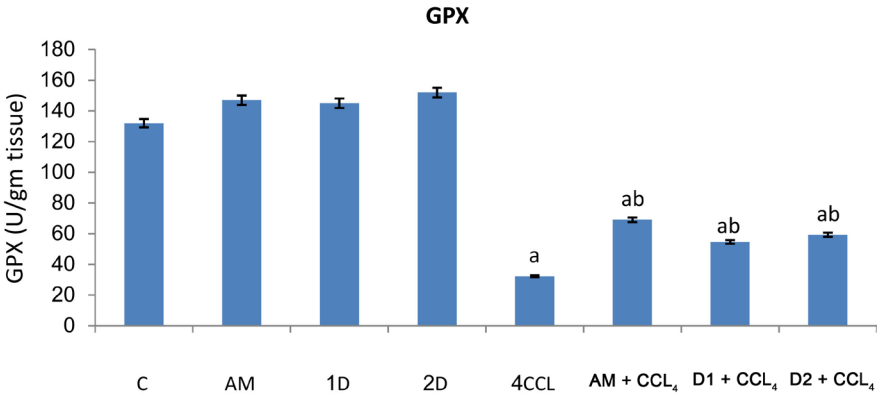


**Figure 5.** The Effect of AM, D1, D2 and $CCl_4$ or their combination with $CCL_4$ on GPX activity in male rats. Each bar represents a Mean value ± S. E. for 10 rats group. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

treatment of AM, D1 and D2 with $CCL_4$ group exerted a significant increase in both SOD and GPX when compared to $CCL_4$ group. This result indicates that AM, D1 and D2 diminish the toxicity of $CCL_4$ in brain tissue.

**3) Effect of AM, D1 and D2, $CCL_4$ or Their Combination with $CCL_4$ on NE, DA, 5-HT and AChE**

Figures 6-9 show that that the sole administration of AM, D1 and D2 displayed a non-significant change in neurotransmitter parameter NE, DA, 5-HT and AChE. Figures 6-9 also show the effect of $CCL_4$ on NE, DA, 5-HT and AChE. $CCL_4$ treatment elevates NE and DA significantly and reduces AChE and 5 HT significantly, while combined administration of $CCL_4$ and AM, D1 and D2 reversed the effect of $CCL_4$ on neurotransmitter parameter and AChE due to their antioxidant activity.

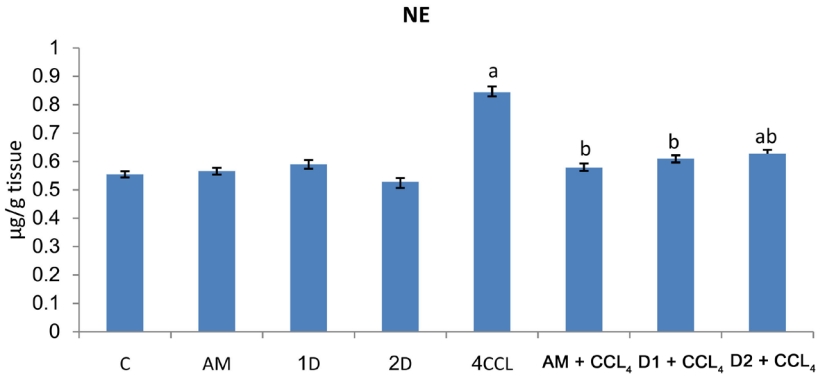


**Figure 6.** The Effect of AM, D1, D2 and $CCl_4$ or their combination with $CCL_4$ on NE in male rats. Each bar represents a Mean value ± S. E. for 10 rats /group. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

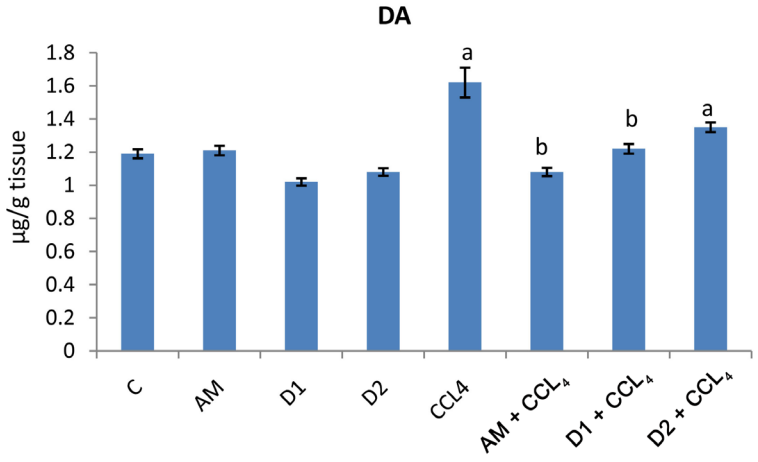


**Figure 7.** The Effect of AM, D1, D2 and $CCl_4$ or their combination with $CCL_4$ on DA in male rats. Each bar represents a Mean value ± S. E. for 10 rats. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

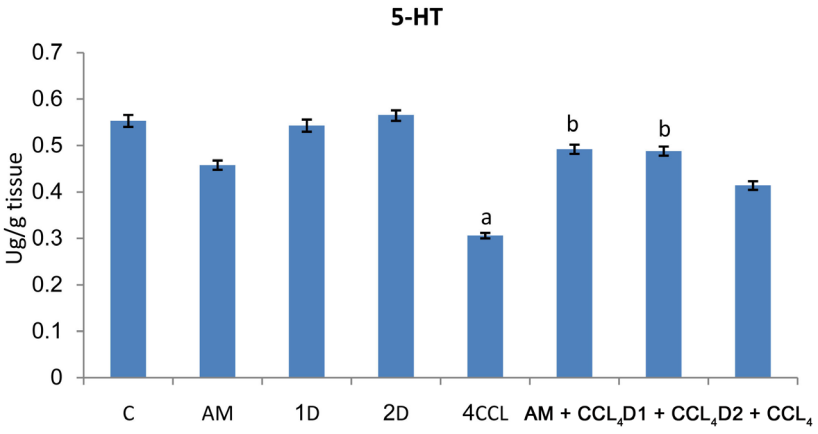


**Figure 8.** The Effect of AM, D1, D2 and $CCl_4$ or their combination with $CCL_4$ on 5-HT in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

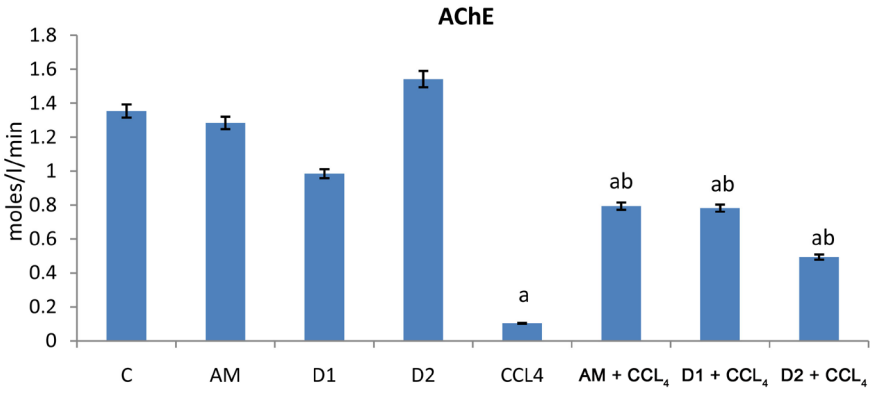


**Figure 9.** The Effect of AM, D1, D2 and $CCl_4$ or their combination with $CCL_4$ on AChE in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. [a]Significantly different from control group ($P < 0.05$), [b]significantly different from $CCL_4$ treated group ($P < 0.05$).

## 4. Discussion

The brain is poor in oxidative protection mechanisms and hence is at a greater danger of damage mediated by reactive oxygen species (ROS) resulting in molecular and cellular dysfunction in the brain, a collection of cellular defense systems exists to rebalance the ROS. These include enzymatic and non-enzymatic antioxidants that lesser the concentration of free radical species and restore oxidative cellular damage (Gupta *et al.* [29]).

Carbon tetrachloride is rapidly taken up by the liver and brain (Boer *et al.* [30]). Although $CCl_4$ is primarily metabolized in the liver, its determinant effects on the brain are well documented (Sanzgiri *et al.* [31]). The present study showed that $CCL_4$ administration induced a significant elevation in brain MDA and NO levels in association with a significant reduction antioxidant enzymes defense system (SOD and GPX) as well as reduction in brain GSH. These findings are in the same line with those of Sanzgiri *et al.* [31] and proved that $CCL_4$ administration affected brain tissue. Melin [32] reported that $CCl_4$ metabolized by cytochrome p-450 and the producing metabolite generate a highly reactive free radical that initiate lipid peroxidation of the cell membrane of the endoplasmic reticulum. Results of the present are in the same line with those obtained by Merry *et al.* [33] who reported that, Lipid peroxidation is one of the major reasons of $CCl_4$-induced toxicity, mediated by the generation of free radical metabolites of $CCl_4$. and causes a chain reaction. The brain tissue is highly susceptible to LPO because of its high rate of oxygen utilization, an abundant supply of polyunsaturated fatty acids, a deficient antioxidant defense and a high content of transition metals like copper and iron as described by Singh *et al.*, [34]. The obtained results in the present study confirmed those obtained by Sahar and Ahmed [35] who reported that in the brain the level of cytochrome P-450 and arachidonic acid in the brain is more than those in the liver, indicating that the antioxidant defense system has a limited capacity in the brain. In consistent with the reports of Dani *et al.* [36], Sanzgiri *et al.* [31] and Sahar and Ahmed [35], data of the

present study proved that $CCl_4$ toxicity played a crucial role in brain oxidative damage. Meanwhile, the administration of AM, D1and D2 showed a significant impartment in the redox status of the brain and showed a significant decrease in the production of free radicals as indicated by the obtained decrease in brain MDA level. These findings in the harmony with results of Dani *et al.* [36], Boer [30] and Soliman and Fahmy [37]. In present study, the obtained results revealed that, the co-administration of AM, D2 or D1 with $CCL_4$ significantly decreased NO level in the brain tissue when compared to $CCL_4$ group. The attenuating effect of the examined treatment against the deleterious effect of $CCL_4$ on brain NO is in the following order AM > D2 > D1, this finding suggests that, AM, D1 and D2 have anti-inflammatory effect beside their antioxidant one. In the same manner, Hwang *et al.* [9] reported that GlcN or its derivatives may serve as novel neuroprotective or anti-inflammatory agents. Also, the GSH contents in the groups co-administered AM, D1, and D2 with $CCl_4$ were significantly increased compared with $CCl_4$ group (D1 > D2 > AM).

In the present work, the co-administration of AM, D1, and D2 with $CCl_4$ restored SOD activity to be more than or near the control level and the obtained improvement is in the following order D1> AM > D2. However, results of the current study revealed, that, the co-administration of D1 or D2 with $CCl_4$ displayed a limited ameliorative effect on the obtained decrease in brain GPX activity as a consequence of $CCl_4$, the most pronouncing ameliorative effect was induced by the co-administration of AM with $CCl_4$ followed by D2 and then D1.

Our study showed that $CCL_4$ induced a significant increase in the concentrations of NE, DA and reduced 5-HT level. These results are in a good keeping with the findings of Ritesh *et al.* [38] and [soliman and fahmy [37]. Whereas Ritesh [38] reported that, Although $CCl_4$ is well known as a well-known a hepatotoxic agent, it is equally a neurotoxic chemical that causes oxidative damage to the brain. Moreover, soliman and fahmy [37], Chen *et al.* [39] and Boer *et al.* [30] reported that $CCl_4$ exerts neurotoxicity by altering signal transduction pathways in the brain or the inhibition of different complexes from mitochondrial respiratory chain. Combined treatment of $CCL_4$ and AM, D1, D2 reversed the effect of $CCL_4$ due to antioxidant activity of these drugs.

The efficiency of the combination between AM, D1 and D2 with $CCL_4$ was the same for NE, DA and 5-HT. The magnitude of the efficiency on were AM, D1 and D2; in deseeding order.

Administration of $CCl_4$ in the present work induced a decrease in AChE activity in the brain tissues of intoxicated rats. This result was in the same line with those of soliman and fahmy [37] and Kazi and Ira [40]. This decrease could be due to oxidation of the presynaptic protein thiol groups which subsequently reduced the AchE release Yousef and EL-Demerdash [41]. Escobar [42] indicated that enhanced free radical concentration resulting from oxidative stress condition can cause loss of enzymatic activities. The enzyme is present in the motor end plate, synaptic junction, brain, spinal cord, red blood corpuscles, and blood

serum. Anything which interferes with the action of AChE causes serious disturbance of neurojunctional and neuromuscular activities. Exposure to certain toxic agents leads to convulsion, paralysis and perhaps death (Laurie [43]). AChE is one of the most crucial enzymes of nerve response and function Mirjana *et al.* (44). The co-administration of AM, D1, and D2 with $CCl_4$ displayed a significant improving effect on AChE activity that may be attributed to the antioxidant properties of the examined treatments. This can be indicated from the direct effect of these drugs on the AchE activity in the brain of $CCl_4$ intoxicated rats as they restored the enzyme activity near the effects of the normal values.

Glucosamine was chosen in the present study in two formulation due to it has been reported that GlcN is a naturally occurring amino monosaccharide, that exerts to a certain degree of immunosuppressive effects in vitro and in vivo and is used widely as an alternative therapeutic regimen for rheumatoid arthritis and osteoarthritis as well as because of its anti-inflammatory, antioxidative, and antiapoptotic effects (Geng, *et al.* [45], Gouze *et al.* [46], Chen *et al*., [47] and Reginster *et al.* [48]). GlcN, the main precursor of glycosylation by posttranslational modification, exhibited a regulatory role in the activation of the hexosamine biosynthetic pathway (Marsh and Chatham [49]). The anti-inflammatory effects of GlcN were mainly attributable to its ability to inhibit nuclear factor kappaB (NF-κB) activation (Hwang *et al.* [8]). Glucosamine itself will help manage the stress response and reduce the amount of inflammation and thereby help in limiting the amount of tissue damage (Syed *et al.* [50] and Faezeh *et al.* [51]). The study of Chen *et al.* [52] supports the previous results, findings that GlcN protected retinal ganglion cells (RGCs) from oxidative stress-induced injury via the modulation of protein O-GlcNAc glycosylation. Hwang [12] found that Glucosamine improves survival in a mouse model of sepsis and attenuates sepsis-induced lung injury and inflammation.

## 5. Conclusion

Our data demonstrated that CCl4 induced brain toxicity that might be related to oxidative damage and the co-administration of antioxidant drug AM, as a reference drug and nutraceuticals containing glucosamine D1and D2 with CCl4 decreased the harmful effect of $CCl_4$-induced brain toxicity probably through inhibiting free radical generate. D1 had a more protective effect than D2 when compared with the protection of the reference antioxidant drug Liverin (AM).

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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