Beneficial Effects of Some Nutraceuticals Containing Glucosamine and Antioxidant against CCL4 Induced Brain Injury in Rats

Sawsan A. Nasr¹, Omer O. A. Ahmed-Farid¹, El-Shaymaa A. Zein-El-Abedeen²

¹Physiology Department, National Organization for Drug Control and Research, Giza, Egypt
²Biochemistry Department, National Organization for Drug Control and Research, Giza, Egypt

Email: sawsnasnar2@gmail.com

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 CCL4 induced brain damage in male albino rats. Liverin (AM) was employed in the current study as an antioxidant reference drug. CCL4 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. CCL4 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 CCL4 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 CCL4 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 CCL4 on brain tissue.

Keywords

CCL4, Glucosamine, Antioxidant and Neurotransmitter

1. Introduction

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

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[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 CCl₄. Reports on the effects of CCl₄ on the brain are sketchy (Szymonik-Lesiuk et al. [17]).

Carbon Tetrachloride (CCl₄) is widely used to induce hepatotoxicity in experimental animals. CCl₄ hepatotoxicity is characterized by hepatocellular necrosis with fat deposition. At Acute toxic doses of CCl₄, when hepatocellular necrosis exceeds the regenerative capacity of the liver, fatal liver failure often ensues. High Doses of CCl₄ 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 CCl₄ 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]). CCl₄ belongs to the class of hepatotoxins, which act after metabolic activation. Oxidative stress resulting from the increased production of free radical after CCl₄ 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-
sarine 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.

**CCl4:** 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 CCl4 that was diluted 1:1 with olive oil, this group served as CCL4 group.

Group VI: the rats of this group were received in addition to CCl4 dose a daily oral dose of 1 ml/kg body wt. of AM as a suspension in 0.25% CMC, this group served as CCL4 + AM group.

Group VII: received in addition to CCl4 dose a daily oral dose of 1 ml/kg body wt. D1 as a suspension in 0.25% CMC, this group served as CCl4 + D1 group.

Group VIII: received in addition to CCl4 dose a daily oral dose of 1 ml/kg body wt. D2 as a suspension in 0.25% CMC this group served as CCl4 + 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 Vallen-tine [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, CCL₄ or Their Combination with CCL₄ 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 CCL₄ on MDA, NO and GSH. CCL₄ 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 CCL₄ with AM, D1 and D2 resulted in a significant reduction in MDA, NO and significant elevation in GSH when compared to CCL₄ group. This result confirms the antioxidant activity of AM, D1 and D2.

2) Effect of AM, D1 and D2, CCL₄ or Their Combination with CCL₄ 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 CCL₄ on SOD and GPX. CCL₄ 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 CCl₄ or their combination with CCl₄ on MDA in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. ᵃSignificantly different from control group (P < 0.05), ᵇsignificantly different from CCl₄ treated group (P < 0.05).

**Figure 2.** The Effect of AM, D1, D2 and CCl₄ or their combination with CCl₄ on NO in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. ᵃSignificantly different from control group (P < 0.05), ᵇsignificantly different from CCl₄ treated group (P < 0.05).

**Figure 3.** The Effect of AM, D1, D2 and CCl₄ or their combination with CCl₄ on GSH in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. ᵃSignificantly different from control group (P < 0.05), ᵇsignificantly different from CCl₄ treated group (P < 0.05).
The effect AM, D1, D2 and CCL₄ or their combination with CCL₄ on SOD activity in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. *Significantly different from control group (P < 0.05), †significantly different from CCL₄ treated group (P < 0.05).

Figure 5. The Effect of AM, D1, D2 and CCL₄ or their combination with CCL₄ on GPX activity in male rats. Each bar represents a Mean value ± S. E. for 10 rats group. *Significantly different from control group (P < 0.05), †significantly different from CCL₄ treated group (P < 0.05).

treatment of AM, D1 and D2 with CCL₄ group exerted a significant increase in both SOD and GPX when compared to CCL₄ group. This result indicates that AM, D1 and D2 diminish the toxicity of CCL₄ in brain tissue.

3) Effect of AM, D1 and D2, CCL₄ or Their Combination with CCL₄ on NE, DA, 5-HT and AChE

Figures 6-9 show 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 CCL₄ on NE, DA, 5-HT and AChE. CCL₄ treatment elevates NE and DA significantly and reduces AChE and 5HT significantly, while combined administration of CCL₄ and AM, D1 and D2 reversed the effect of CCL₄ on neurotransmitter parameter and AChE due to their antioxidant activity.
Figure 6. The Effect of AM, D1, D2 and CCl₄ or their combination with CCl₄ on NE in male rats. Each bar represents a Mean value ± S. E. for 10 rats /group. *Significantly different from control group (P < 0.05), †significantly different from CCl₄ treated group (P < 0.05).

Figure 7. The Effect of AM, D1, D2 and CCl₄ or their combination with CCl₄ on DA in male rats. Each bar represents a Mean value ± S. E. for 10 rats. *Significantly different from control group (P < 0.05), †significantly different from CCl₄ treated group (P < 0.05).

Figure 8. The Effect of AM, D1, D2 and CCl₄ or their combination with CCl₄ on 5-HT in male rats. Each bar represents a Mean value ± S. E. for 10 rats/group. *Significantly different from control group (P < 0.05), †significantly different from CCl₄ 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 CCl₄ is primarily metabolized in the liver, its determinant effects on the brain are well documented (Sanzgiri et al. [31]). The present study showed that CCl₄ 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 CCl₄ administration affected brain tissue. Melin [32] reported that CCl₄ 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 CCl₄-induced toxicity, mediated by the generation of free radical metabolites of CCl₄ 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 CCl₄ toxicity played a crucial role in brain oxidative damage. Meanwhile, the administration of AM, D1 and D2 showed a significant impairment 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 CCl₄ significantly decreased NO level in the brain tissue when compared to CCl₄ group. The attenuating effect of the examined treatment against the deleterious effect of CCl₄ 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 CCl₄ were significantly increased compared with CCl₄ group (D1 > D2 > AM).

In the present work, the co-administration of AM, D1, and D2 with CCl₄ 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 CCl₄ displayed a limited ameliorative effect on the obtained decrease in brain GPX activity as a consequence of CCl₄, the most pronouncing ameliorative effect was induced by the co-administration of AM with CCl₄ followed by D2 and then D1. Our study showed that CCl₄ 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 CCl₄ 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 CCl₄ exerts neurotoxicity by altering signal transduction pathways in the brain or the inhibition of different complexes from mitochondrial respiratory chain. Combined treatment of CCl₄ and AM, D1, D2 reversed the effect of CCl₄ due to antioxidant activity of these drugs.

The efficiency of the combination between AM, D1 and D2 with CCl₄ was the same for NE, DA and 5-HT. The magnitude of the efficiency on were AM, D1 and D2; in descending order.

Administration of CCl₄ 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 CCl₄ 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 CCl₄ 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 CCl₄-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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