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SCHOLARENA

Journal of Environmental Toxicology and Analytical Research

RESEARCH ARTICLE

ISSN: 2768-1076

Neuroprotective Effect of Chlorogenic Acid on Glyphosate Base Roundup Inducing Cognitive and Memory Impairment in Rats by Enhancing the Anti-Inflammatory and Antioxidant Functions

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Citation: Olfa Rebai, Malek Ben Tmessek, Mohamed Amri, Sami Fattouch (2024) Neuroprotective Effect of Chlorogenic Acid on Glyphosate Base Roundup Inducing Cognitive and Memory Impairment in Rats by Enhancing the Anti-Inflammatory and Antioxidant Functions, J of Environ Toxicol and anal Res 5: 102

Abstract

Industrialization contributes to an elevation in environmental contamination, with industrial air, commercial pollutants, and combustion of organic chemicals posing numerous health risks. Exposure to organic chemicals causes acute and chronic toxicity, imbalance of various metabolisms and physiological functions in the body leading to degenerative diseases. Epidemiological studies and case reports have suggested and provided evidence for an association between and exposure to pesticides and brain neurodegeneration in particular Parkinson's disease and Alzheimer diseases. Roundup formulations are herbicides whose active principle is glyphosate. Glyphosate N-(phosphonomethyl)glycine), Recent research indicates that glyphosate can be toxic to the human, which warrants further investigation. . The acute effects of glyphosate herbicide (100 mg kg^{-1} BW) for a period of 15 days revealed that glyphosate may be neurotoxic and alter enzymatic and biochemical parameters in the brain. Chlorogenic acid (CGA) at a dose of 10mg mg kg^{-1} bw appears is to exerts anti-inflammatory and anti-oxidant activities by modulate NO production and decreasing MDA levels in the plasma.Effects of Glyphosate on animal behavior, learning, memory and cognitive function were assessed . The results of the study would provide insights into the potential protective properties of the CGA and its impact on neurobehavioral outcomes in animals subjected to acute Glyphosate toxicity. Rats were subjected to behavioral testing with the open field (OF) and the elevated maze.

Keywords: Glyphosate; neurotoxicity; memory and cognitive function; chlorogenic acid; biochemical parameters; Antioxidant enzyme system

Abbreviations: b.w: Body weight; i.p : Intra-peritonial; SOD: Superoxide dismutase; LDH: Lactate dehydrogenase; PD: Parkinson's disease; POD: Peroxidase; CRP: C-reactive protein; OF: Open field; EPM : The Elevated Plus Maze; Dentate gyrus (DG); Molecular layer (ML); Granule cell layer (GCL) polymorphic layer (PL); GLY: Glyphosate

Introduction

Herbicides are environmental contaminants that have attracted a more attention due to the potential hazards they pose to human health. Glyphosate is the active ingredient in glyphosate-based herbicides (GBHs), such as Roundup™ the most widely used herbicides in the world and is a popular chemical herbicide used on lawns, farms, and in gardens across the world. Today, over 113 million kilograms of glyphosate are utilized agriculturally each year across the United States [1]. Glyphosate has also been found to cause reproductive damage in animals. These adverse effects include disruption of key regulatory enzymes in androgen synthesis, alteration of estrogen and testosterone levels in the blood, damage to reproductive tissues, and impairment of gametogenesis. Also there is some evidence that exposure to agricultural pesticides, especially organophosphate pesticides, may increase the risk of autism. Previous research has shown that subacute exposure to formulation herbicides (0.05–250 mg/kg glyphosate) can result in infammation of the peripheral body in adult rats [2].

Recent research indicates that glyphosate can be toxic to the human [3], which warrants further investigation. The acute effects of glyphosate based herbicide have been studied, Neurobehavioral assessment and memory and cognitive disorders yet has not been studied extensively.

However, following recent studies that link Roundup to Parkinson's and other diseases, GBHs exposure has been associated with an increased risk of Parkinson's Disease and death of neurons in the *substantia nigra*. Other studies have shown the effects of GBHs on synaptic transmission in animal and cellular studies. The major mechanism of action appears to be oxidative stress, accompanied by mitochondrial dysfunction. The recent rise in glyphosate application to corn and soy crops correlates positively with increased death rates due to Alzheimer's disease and other neurodegenerative disorders. Additionally, reports have shown Glyphosate has been shown to cross the blood–brain barrier in *in vitro* and in *vivo* models an that glyphosate exposure increases pro-infammatory cytokines in blood plasma, particularly TNFα [4, 5].

Glyphosate toxicity could be a model of choice for investigating preclinical lesions, elucidating mechanisms of brain vulnerability, and testing agents for neuroprotection against acute glyphosate toxicity. The human neurological disease for which there is the greatest concern over exposure to glyphosate and GBHs is Parkinson's disease. Parkinson's Disease is a result of the death of dopamine-containing neurons in the *substantia nigra* and is the fastest growing neurological disorder in the world. Previous research has shown that subacute exposure to formulation herbicides (0.05–250 mg/kg glyphosate) can result in inflammation of the peripheral body in adult rats [2]. Ait Bali et al. (2017) exposed one-month-old mice to Roundup™ (250 or 500 mg/kg/day). There were no obvious effects of acute exposure. They report that the animals exposed sub-chronically (6 weeks) or chronically (12 weeks) showed a reduction in locomotor activity in an open field test and an increase in anxiety- and depression-like behavior on the basis of an Elevated-Plus-Maze test. There was an increase in immobility and a decrease in grooming time [6].

In the recent years, interests for natural substances with beneficial activity to human have sharply risen. In fact, there is a significant increase in nutraceuticals and pharmaceutical products, based on natural compounds. The main interest has been observed for natural substances with strong antioxidant activity, because oxidative stress induced by multiple factors is the main cause of many pathological conditions such as inflammation, cancer, coronary heart disease and even skin aging. In addition, there has been a significant consumer interest in health enhancing role of specific foods or physiologically-active food components. Epidemiological data suggest that a high intake of extracts enriched in various flavonoids, including berry ANC (the glycosylated form of anthocyanidins) and PAC, is associated with lower risk of PD [7].

Chlorogenic acid (CGA) is a naturally occurring non-flavonoid polyphenol found in green coffee beans, teas, certain fruits, and vegetables that exerts antiviral, antitumor, antibacterial, and antioxidant effects. The antioxidant effects of CGA have been reported in the extracts of various plants. It has been discerned that hydroxyl groups (OH) on phenolic acids act as positive moi-

eties for their antioxidant properties, which is associated with the number of hydroxyl groups as follows: tri-hydroxy phenolic acids > di-hydroxy (catechol) > mono-hydroxy [8]. The caffeic acid CA (a metabolite of CGA) has also been reported to have anti-inflammatory and antioxidant activities. The anti-inflammatory mechanism of CGA and CA is elucidated which inhibited the activation of the IL-8 and PKD-IKKNFκB signaling pathway by scavenging intracellular ROS. In the study of Shine et al(2017), they reported that the anti-inflammatory properties of CA and CGA were associated with their catechol groups. Hence, it is believed that CGA, CA, and other compounds possessing the catechol group might be helpful in contributing to preventing inflammatory diseases [9].

Several *in vivo* and *in vitro* studies have demonstrated that CGA can protect against toxicities induced by chemicals as pesticides by preservation of cell survival via reducing overproduction of nitric oxide and reactive oxygen species and suppressed pro-apoptotic signaling. CGA antioxidant effects mediated through the Nrf2-heme oxygenase-1 signaling pathway were shown to enhance the levels of antioxidant enzymes such as superoxide dismutase, catalase, glutathione-*S*-transferases, glutathione peroxidase, and glutathione reductase as well as glutathione content. Also, CGA could suppress inflammation via inhibition of toll-like receptor 4 and MyD88, and the phosphorylation of inhibitor of kappa B and p65 subunit of NF-κB, resulting in diminished levels of downstream inflammatory factors including interleukin (IL)-1 β, IL-6, tumor necrosis factor-α, macrophage inflammatory protein 2, cyclooxygenase-2, and prostaglandin E2. Moreover, CGA inhibited apoptosis by reducing Bax, cytochrome C, and caspase 3 and 9 expression while increasing Bcl-2 levels [10].

Young et al, (2019), demonstrate that neuroprotective properties of chlorogenic acid-rich Solanum melongena extracts (SM extract) in rotenone-induced PC-12 cell death and by prevention of apoptosis, decreasing ROS, increasing ATP production in the cells and upregulating SOD and catalase activities in the cells [11].

Ishida et al. investigated the effect of chlorognic acid (CGAs) on the prevention of cognitive dysfunction in APP/PS2 transgenic mouse model of AD in which animals received either a control or a CGA diet. The results indicated that chronic ingestion of CGA ameliorated cognitive deficits and prevented Aβ deposition and neuronal loss in these mice. CGA enhanced the gene expression of hippocampal low-density lipoprotein (LDL) receptor-related protein 1, which has a key role for Aβ clearance and cognitive function maintenance, and restored the perivascular localization of aquaporin 4, which facilitates Aβ clearance [12a].

Ishida et al. (2020) found that CGA increased the viability and decreased apoptosis of hippocampal neurons from newborn Sprague–Dawley rats treated with $A\beta_{25\text{-}35}$. CGA decreased activities of lactate dehydrogenase and the malondialdehyde (MDA) levels, and raised contents of superoxide dismutase (SOD) and glutathione peroxidase (GSH)-Px in $A\beta_{25\text{-}35}$-treated cells, suggesting that CGA restrained the apoptosis of $A\beta_{25\text{-}35}$-induced hippocampal neurons by improving the anti-oxidant capacity, mitochondrial injury, and the state of ER stress in cells [12b].

A study using an experimental protocol that combines NMR spectroscopy and atomic force microscopy showed that green and roasted coffee extracts, CGA, and melanoidins can inhibit Aβ aggregation and toxicity in human neuroblastoma SH-SY5Y cells [13, 14] found that when SH-SY5Y cells were incubated with 10 μM Aβ along with 20 μM CGA, the cells became more viable compared to SH-SY5Y cells incubated without CGA. The results of an animal experiment showed that the administration of CGA improved spatial learning and memory in SAMP8 mice, which are senescence-accelerated-prone mice that exhibit age-related deterioration in learning and memory having plaques resembling AD like depositions of Aβ.

Another study conducted by Oboh [15] demonstrated that CGA inhibited AChE activity in rat brain homogenates in a dose-dependent manner, suggesting its beneficial effect on AD, since inhibition of AChE represents the primary treatment modality against the cognitive impairment observed in AD [16] Molecular docking studies revealed that CGA has the most significant binding affinity towards AChE [17]. As an additional model of learning and memory impairment like AD, scopolamine has

been used to induce cognitive impairment in rode. Kwon [18] demonstrated that CGA improved the impairment of short-term or working memory and cognitive impairments induced by scopolamine.

This work focuses on the features of the presented *in vivo* model with the intent of highlighting mechanisms of likely relevance to the role of CGA in neuroprotective potential against glyphosate exposures and improving cognitive memory impairments.

Materials and Methods

Reagents and Chemicals

The Glyphosate used in this study has a commercial name Roundup plus H.029-11 and was purchased from the company ATLAS AGRICOLE, Tunisia. Chlorogenic acid CGA (1, 4, 5-Trihydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate) is purchased from sigma Aldrich Germany . Formaldehyde, bovine liver catalase, DL-epinephrine, Trichloroacetic acid (TCA) and guaiacol were acquired from Sigma Aldrich (St. Louis, MO, USA) Calcium (Réf. 20051), total proteins (Réf. 20161) .

Animals

Male Wistar rats weighing about 210–250 g were purchased from the Central Pharmacy of Tunisia (SIPHAT, Tunisia). Animals were housed in a controlled environment (22 ± 3 °C, 54–56% humidity, a 12 h light/dark cycle).The animals had free access to commercial pellet diet and water *ad libitum*. The handling of the animals was approved by the Tunisian Ethical Committee for the Care and Use of Laboratory Animals. Animals were used to perform a behavioral test of motor skills in order to evaluate the effect on the motor behavior of rats. Immediately after behavioral assessments, the animals were killed by decapitation.

Experimental Design

Rats were randomly divided into four groups of six animals each: Group 1 received a standard diet (control). Group 2 received an injection (i.p., intraperitoneal) of glyphosate-based solution (100 mg kgG^{-1} b.w). Group 3 received an injection of CGA (10 mg kgG^{-1} b.w) (i.p., intraperitoneal) and finally and group 4 received an injection of Glyphosate and CGA. The rats were treated daily for two weeks. The battery of behavioral tests included: Open field (OF) test and Plus Maze (PM) test. Each behavioral test was separated at least by 1 day. At the end of the treatment time, the animals were anaesthetized with Ketamine and then sacrificed; the blood was collected from the jugular vein. And the brain was collected, homogenized and processed for biochemical and histological parameters.

Measurement of LDH Cytotoxicity

Measurement of LDH activity in culture medium was determined using a coloromertic method, the CytoTox96 ®nonradioactive Cytotoxicity assay. Neurons were seeded onto 96-well plates (2 × 104 cells/well). The CytoTox96 assay quantitatively measures lactate deshydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis. Release LDH is measured with a 30-minute coupled enzymatic assay that results in the conversion of a tetrazoliumm salt (INT) into a red formazan product. The results were expressed as a percentage of total LDH release from neurons. Data are expressed as the mean ± SEM from three independent experiments performed in quadruplicate. The results were expressed as a percentage of total LDH release from homogenate.

Determination of NO Production

NO levels in the culture medium were directly measured using Total Nitric Oxide Assay Kit (Biomaghreb, Tunisia). Brain homogenate (50 µl) were mixed with 100 µl Griess reagent and incubated for 3 min at room temperature. The absorbance of each

reaction was measured at 540 nm on a microplate spectrophotometer.

Protein Quantification

Total Protein was performed according to the method described by Hay [19]. At acidic pH, a blue-colour complex of soluble proteins with copper was quantified by spectrophotometry at 546 nm.

Free Iron Determination

Free iron was determined according to Leardi et al. (1998) using a commercially available kit from Biomaghreb (Ariana, Tunisia). Briefly, at acidic pH 4.8, all Fe3+ released from transferrin were reduced by ascorbic acid into Fe2+, which constitutes with ferrozine a colourful purple complex measurable at 560 nm. Heart extract was added to 250 µl of reaction mixture containing ascorbic acid (5 g/L) and ferrozin (40 mM), and incubation was performed at 37°C for 10 min [20].

Calcium Determination

Serum ionizable calcium was determined using a commercially available kit from Biomaghreb, Tunisia. At basic pH, calcium was constituted with cresolphtalein, a purple-colored complex measurable at 570 nm. Briefly, 50 µl of sample was added to the reaction mixture containing 2-amino-2-methyl 1-propanol buffer (500 mmol L−1), cresolphtalein (0.62 mmol l−1), and hydroxy-8 quinoleine (69 mmol l−1). Incubation was carried out at room temperature for 5 min assuming the complex was stable during 1 h.

H_2O_2 Determination

The amount of H_2O_2 acumulation in the plasma was determined according to Chance et al. (1979) using a commercially available kit from Biomaghreb (Tunisia) [21].

Determination of Liver Lipoperoxidation

The amount of lipid peroxidation in the brain was carried out with the MDA measurement method according to De Las Heras Rosa [22]. An aliquot of brain homogenate was mixed with a BHT-TCA solution containing 1% BHT and 20% TCA. After centrifugation, the supernatant was combined with a second solution containing 0.5 N HCl and TBA (120 mmol mL) and heated at 80EC for 10 min. After cooling, the absorbance of the resulting chromophore was measured at 532 nm using a double UV-visible spectrophotometer. Malondialdehyde contents were represented as millimoles of MD per milligram of protein with an extinction coefficient of 1.56105 mol LG-^{1} cm.

Catalase (CAT) assay Catalase activity was assayed by measuring the initial rate of H_2O_2 disappearance at 240 nm. The reaction mixture contained 33 mM H_2O_2 in 50 mM phosphate buffer pH 7.0 and 5 µL of sample. CAT activity was calculated using the extinction coefficient of 40 mM*1 cm−1 for H_2O_2. One unit of catalase activity is defined as the amount of enzyme catalyzing the degradation of 1 mmol of H_2O_2 per minute at 37 °C and specific activity corresponding to transformation of substrate (in mmol) (H_2O_2) per minute per milligram protein.

Peroxidase (POD) assay Peroxidase activity was measured at 25°C using guaiacol as hydrogen donor. The reaction mixture contained 9 mM guaiacol, 19 mM H_2O_2 in 50 mM phosphate buffer pH 7, and 10 µL samples in 1 mL final volume. The reaction was initiated by the addition of H_2O_2 and monitored by measuring the increase in absorbance at 470 nm each 30 s for 3 min. Peroxidase activity was expressed as nanomoles of guaiacol oxidized per minute with a molecular extinction coefficient of 26.2 mM−1 for calculation.

Superoxide dismutase (SOD) Superoxide dismutase (SOD) activity was determined by using the modified epinephrine assay. At alkaline pH, superoxide anion (O_2^-) causes the auto-oxidation of epinephrine to adenochrome. One unit of SOD is defined as the amount of extract that inhibits the rate of adenochrome formation by 50%. Samples were added in 2 mL reaction mixture containing 10 µL bovine catalase (0.4 U µL−1), 20 µL epinephrine (5 mg mL−1), and 62.5 mM sodium carbonate/sodium bicarbonate buffer pH 10.2. Changes in absorbance were recorded at 480 nm each 30 s for 3 min.

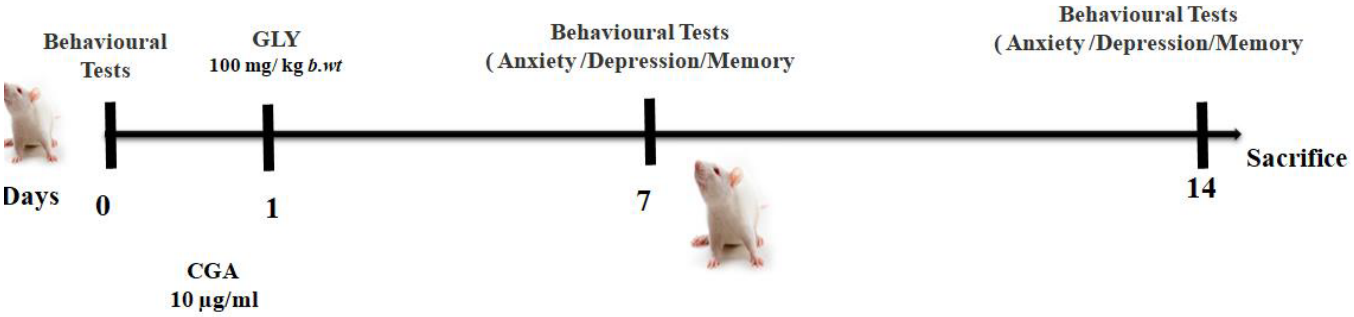
Behavioral Tests

Open field (OF) test

Behavior in the OF test is used to assess locomotor activity as well as emotionality. Individual rats were released in the corner of a square (50 cm × 50 cm) OF arena. Rats were left undisturbed and recorded with a camera mounted above the center of the OF arena for 15 min. At the end of the test, mice were returned to their home cage. After each animal was removed from the test area, its floor was carefully cleaned with a piece of cloth soaked with a 10% ethanol solution. Typically, when rats are introduced into an OF, they explore mainly the peripheral zone of the OF. This tendency to remain close to the walls, called thigmotaxis, decreases gradually during the first minutes of exploration, entering to the central zone of the arena during the following time intervals. The preferential exploration of the peripheral zone in the OF is considered an index of anxiety. Thigmotaxis was determined in the OF, virtually divided in a peripheral and a central zone, determining the ratio of time spent along the periphery relative to time spent in the center over each 5-min interval. The analysis of distance travelled was performed with the automated software program recently described in Patel et al. (2024). Grooming episodes (face washing, forepaw licking and head stroking) and rearing episodes (the mouse lifts both of its forefeet off the floor [23].

Elevated Plus Maze Test

All parts of the apparatus were made of dark polyvinyl plastic. The maze was elevated to a height of 50 cm and had two open (30 × 5 cm) and two closed arms (30 × 5 × 15 cm), arranged in a manner that the arms of the same type were opposite to each other and connected by an open central square (5 × 5 cm). To prevent rats from falling of the open arms, a rim (2.5 mm high and 8 mm deep) surrounded the perimeter of the open arms. At the beginning of the 5-min test session, rats naive to the apparatus and with no previous drug treatment were placed individually in the central square of the maze, facing one of the closed arms. An entry in the arm was counted when the animal placed all four paws into the arm. The total number of visits to the open arms, the total number of visits to the closed arms and the cumulative time spent in the open arms were recorded. The results were expressed in number of entries into closed arms, percentage of time spent in the open arms and percentage of entries into open arms. After each test, the apparatus was cleaned with a 10% ethanol solution. Drugs were administered intraperitoneally 30 min before testing. Ten animals per group were used for each drug as described in section.Experimental design.



Statistical Analysis

Statistical analysis: The data were represented as mean± Standard Error of the Mean (SEM) and were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, which was performed with GraphPad Prism software version 6 (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant when the p level was less than 0.05.

Results

Protective Effects of Chlorogenic *acid* against Toxicity Induced by Glyphosate Exposure

Serum LDH and CRP activities were determined to evaluate the protective effect of chlorogenic acid CGA at a dose of 10mg/kg /bw against damage caused by glyphosate .As shown in Figure 1 LDH activity obviously increased in rats group treated with a single dose of glyphosate 100 mg/kg/bw compared with group control or non treated group($p<0,05$), moreover, compared with the group of rats co- treated with glyphosate and CGA had shown significantly a decrease of and LDH release ($p<0,05$).

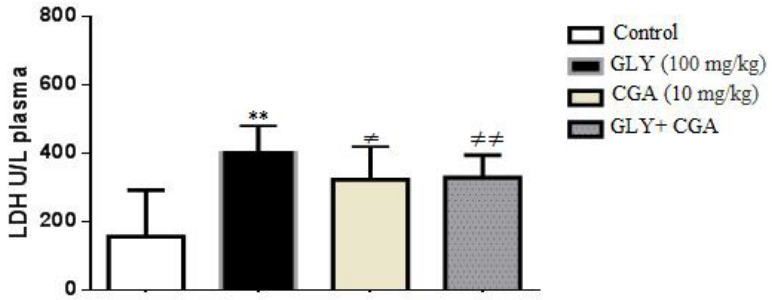
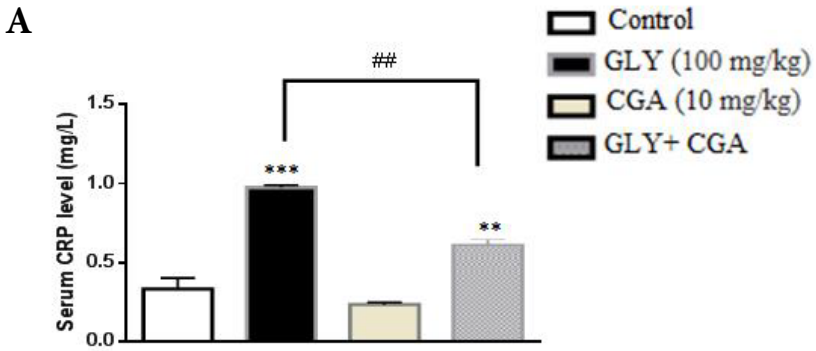


Figure 1: Protective effect of CGA against Glyphosate –induced toxicity. Plasmatic LDH levels expressed in nmol/mg of protein. *Results are expressed as mean ± SD (n = 5). Asterisk P < 0.05 vs control, number signs P < 0.01 vs pesticide treated rats.*

Assessment of Inflammation by Determining the Level of Nitric Oxide (NO) and CRP levels

Figure 2 demonstrate that CGA modulate NO production and CRP levels in plasma as inflammatory mediators compared with the group of rats treated with glyphosate.



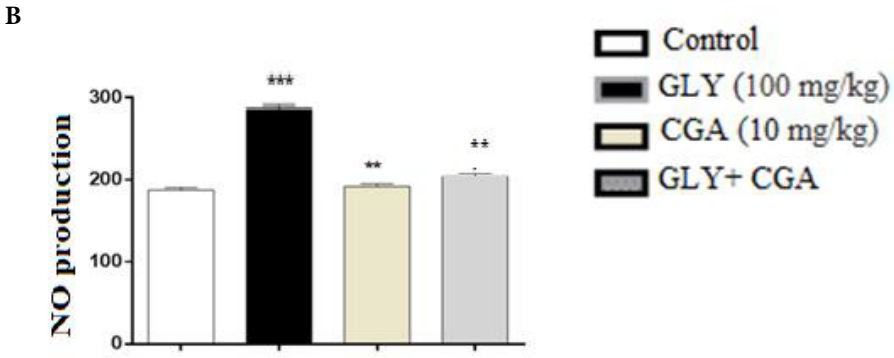


Figure 2: CGA attenuate inflammatory markers: CRP and NO production. (A) CGA attenuates Glyphosate-induced increased levels of serum CRP. The results are expressed as mg/L. (B) CGA restores NO overproduced by Glyphosate exposure. *Results are expressed as mean ± SD (n = 5). Asterisk P < 0.05 vs control, number signs P < 0.01 vs pesticide treated rats*

CGA Extract Modulate the Activity of Plasmatic Antioxidant Enzymes

Glyphosate affects plasmatic antioxidant enzyme system in response to pesticide Roundup –based glyphosate neurotoxicity. CAT and POD activities were increased but decreased the levels of SOD activity. CGA administrated in co-treatment with pesticide decreased both CAT and POD activities in the glyphosate-treated group and abolish the deleterious effects caused by pesticide for SOD activity to be decreased to value near the control grourp (Table 1)

Table 1: Effect of Glyphosate sub-acute exposure and CGA treatment on the endogenous antioxidant enzymes system: Superoxide dismutase (SOD); Peroxidase (POD) and Catalase (CAT). Wistar rats were administered i.p. with CGA (10 mg/Kg/ bw), Glyphosate 100 mg kg−1 b.w, or pesticide plus CGA. Values are expressed as mean ± SEM (n= 5). One asterisk P < 0.05Compared with vehicle-treated group; two number signs P < 0.01 compared with glyphosate -treated group.*p< 0.05; **p< 0.01 (one-way ANOVA followed by Dunnett's test).

	Control	Glyphosate GLY (100mg/kg-1 b.w)	Chlorogenic acid CGA (10mg/kg-1/b.w)	GLY+CGA
SOD (U/mL)	13.29 ± 0.16	19.01 ± 0.58	12.89 ± 0.46	11.30 ± 0.28*
CAT (U/mL)	28.33 ± 1.61	38.66 ±3.12	29.51 ±1.52	32.46 ± 0.60*
POD (U/L)	128.20 ± 27.37	200.70 ± 38.64	109.00 ± 37.64	140.01 ± 14.75

CGA Modifies Intracellular and Biochemical's Mediators in Plasma after Sub-Acute Exposure to Glyphosate

Ionized Calcium free iron and H_2O_2 accumulation in the plasma were investigated, Glyphosate provoked an increase in levels of free iron (Table 2), and ionisable calcium. Whereas, co-treatment of rats with a single dose of Glyphosate at the concentration of 100ppm for 15 days, and CGA significantly decreased these mediators when compared to the control group or none treaded group. Sub -acute glyphosate treatment significantly ($p < 0.05$) increased plasmatic lipid peroxidation, as indicated by a raise in plasma MDA levels compared to the vehicle-treated group. Administration of CGA (10 mg/kg) significantly ($p < 0.05$, $p < 0.01$) attenuated MDA levels.

Table 2: Modulation of the intracellulaire biochemicals parameters at the plasma level. Wistar rats were administered i.p. with CGA (10 mg/Kg/ bw), Glyphosate 100 mg kg−1 b.w, or pesticide plus CGA. Ca_2^+, H_2O_2, free iron and MDA levels were assessed.Values are expressed as mean ± SEM (n= 5). One asterisk P < 0.05Compared with vehicle-treated group; two number

signs P < 0.01 compared with glyphosate -treated group.*p< 0.05; **p< 0.01 (one-way ANOVA followed by Dunnett's test).

Groups	[Ca2+] (mmol/mg prot)	[H2O2] (mmol/mg prot)	Free iron(nmol/mg prot)	MDA (nmol/mgprot)
Control	0,002 ±0,01	8.9±0.27	2.3±0.50	28.62 ± 3.26
GLY100mg/kg/bw	0,007 ±0,0009	11.8±0.65	7.8±1.75	35.05 ± 1.04
CGA100mg/kg/bw	0,003±0,03	9.01 ±0.25	5.0±0.57	24.38 ± 7.00**
GLY 100mg/kg/bw +CGA 10mg/kg/bw	0,004 ±0,0044	10.23±0.42	4.20 ±0.72	25.7 ± 2.88

*N=6 *P<0.05, **P<0.05 VS vehicule*

Effects of Glyphosate and CGA on Animal Behaviour, Learning, Memory and Cognitive Function

Motor activity, which is considered to be a test of nervous system function, shows the integrated output of the sensory, motor and associative processes of the nervous system in case of absence of systemic toxicity. The use of the elevated plus maze as an assay of Anxiety is a widely used behavioural assay for rodents and it has been validated to assess the anti-anxiety effects of pharmacological agents and steroid hormones, and to define brain regions and mechanisms underlying anxiety-related behaviour and then examined the learning and memory abilities of each group.

In control rats this test reveals that the time spent in the center is an average of 33.20 ± 17.64. Whereas in rats treated with Glyphosate, the time spent in the Center reached 19.60 ± 11.48 seconds, the minimum recorded being 6 seconds and the maximum 35 seconds (Table 3 A). The statistical analysis shows no significant difference between the means .For the Time spent in closed arms, the time spent in closed bars was found to average 243.20 ± 12.46 seconds with a minimum of 228 seconds and a maximum of 256 seconds for control group Whereas in those treated with Glyphosate, the time spent in closed arms increased and reached an average of 269.20 ± 14.10 seconds, the minimum recorded being 252 seconds and the maximum 290 seconds (Tab.Next). The statistical analysis shows that there are significant differences between the means (Table 3 B).Statistical analysis shows no significant difference between the average times spent in the open arms by control rats and rats treated with Glyphosate When analysing time Time spent in open arms with an average of 11 ± 8.94 seconds with a minimum of zero seconds and a maximum of 21 seconds.

Table 3: Elevated cross maze test: Variation in time spent in the center of the elevated cross maze(s) in control and Glyphosate-treated rats (n=24). (A)Time spent in the center of the elevated cross maze, (B) Time spent in the closed arms of the elevated cross maze,(C) Time spent in the open arms of the elevated cross maze. Values are expressed as mean ± SEM (n= 5). One asterisk P < 0.05Compared with vehicle-treated group; two number signs P < 0.01 compared with glyphosate -treated group.*p< 0.05; **p< 0.01 (one-way ANOVA followed by Dunnett's test).

A

Time spent in the center of the elevated cross maze				
	Control	**GLY**	**CGA+GLY**	**CGA**
Mean ± SEM	33,20 ± 17,64	19,60 ± 11,48	11 ± 7,31	45,40 ± 27,15
Min	13,00	6,00	1,00	11,00
Max	61,00	35,00	20,00	68,00
tobs	1,45		-2,74	

p	*0,19*	*0,03**

B

Time spent in the closed arms of the elevated cross maze				
	Control	GLY	CGA+GLY	**CGA**
Mean ±SEM	243,20 ± 12,46	269,20 ± 14,10	257 ± 24,40	242,80 ± 26,64
Min	228,00	252,00	238,00	220,00
Max	256,00	290,00	299,00	282,00
tobs	-3,1		0,88	
p	*0,02**		*0,41*	

C

Time spent in the open arms of the elevated cross maze				
	Control	**GLY**	**CGA+GLY**	**CGA**
Mean±SEM	18 ± 24,22	11 ± 8,94	25,80 ± 23,00	15,20 ± 11,43
Min	0,00	0,00	0,00	0,00
Max	59,00	21,00	52,00	28,00
tobs	0,61		0,92	
p	*0,56*		*0,38*	

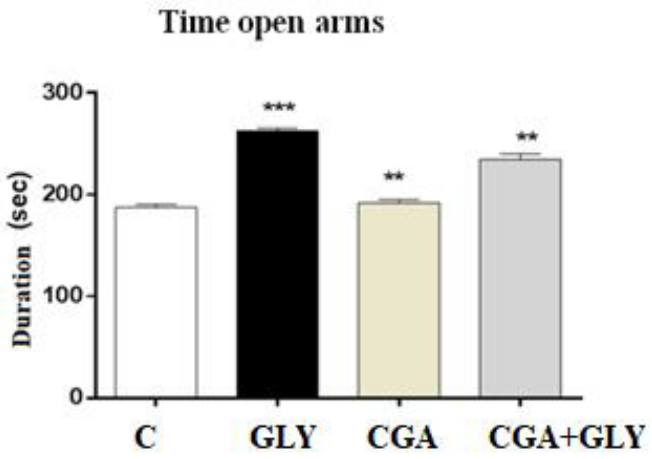


Figure 4: Effects of administration of CGA acid at 10 mg/ kg (CGA), glyohosate at 100 mg/kg (GLY) and administration in combination of both chlorogenic acid (20 mg/kg) and glyphosate (CGA + GLY), compared to non treated group on the behavior of wistar rats in of the Open-Field behaviour test . Data represent mean ± SEM. *p< 0.05; **p< 0.01 (one-way ANOVA followed by Dunnett's test).

Results of Open field test, we observed the voluntary locomotor ability of mice in each group by the open field experiment the vehicle-treated rats with glyphosate traversed 104.0 ± 10.96 squares and showed 19.5 ± 2.6 assisted recoveries. CGA at 10, 1

mg/kg and glyphosate brought about a significant ($P < 0.01$) and dose-dependent increase in the number of squares traversed. The assisted rearing and self rearing was significantly ($P < 0.05$ and $P < 0.01$, respectively) increased by CGA (10mg/kg) and Glyphosate; CGA at 10 mg/kg did not a produce significant effect (Figure 4).Together results suggest that chlorogenic acid can improve the cognitive dysfunction induced by glyphosate in rats.

Discussion

Industrialization contributes to an elevation in environmental contamination, with industrial air, commercial pollutants, and combustion of organic chemicals posing numerous health risks. Exposure to organic chemicals causes acute and chronic toxicity, imbalance of various metabolisms and physiological functions in the body leading to degenerative diseases. In the present day lifestyle, exposure to organic chemicals used in resins, plastics, detergents, dyes, natural gas and medicines, causes amyloid protein aggregation on neuronal cells, suggesting that chemical exposure may cause AD like diseases [16].

It has been shown that pesticides and other organophosphates can be ingested through food and therefore induce a state of oxidative stress. Glyphosate [N-(phosphonomethyl) glycine] (GLP) is an organophosphate and the active ingredient in Roundup® and many other formulations can cause oxidative stress and therefore the production of ROS. According to the INSERM (National Institute of Health and Medical Research) report on the analysis of pesticides on health, there is a 2 times higher risk of Parkinson's disease observed in people exposed to pesticides inducing oxidative stress while the risk is 1.7 times higher if people are exposed to pesticides inducing inhibition of mitochondrial complex I [24].

Previous research on various organs has revealed that glyphosate is capable of triggering dysregulation of numerous metabolic pathways that can contribute to oxidative damage and the generation of reactive oxygen and nitrogen species (ROS), RNS) [25].

Phenolic compound have been well documented to be involved in various bioactive properties and are used for their antioxidant properties in the development of potential neurological therapy. According to several scientific studies conducted on various levels such as cells, animals and people, consuming berry fruits have positive effects on the brain and may help prevent age related memory and neurological disorders in the brain. Due to the high levels of antioxidants found in them, which can alter neuron signalling to protect cells from damage induced by dangerous free radicals and enhance both motor coordination and cognition, inflammation can also be prevented.

Historically, the biological actions of polyphenols have been attributed to their antioxidant properties, either through their intrinsic reducing capacity or through their influence on intracellular redox status.Thus far, few studies have determined whether polyphenols affect the pro-inflammatory polyphenols have been shown to play an important part in suppressing chronic inflammation and oxidative stress damage, as well as to possess anticarcino-genic, antimicrobial, antiviral, anti-obesity and antidiabetic properties [26, 27].

Chlorogenic acid (CGA), esters of caffeic, and quinic acids are hydroxycinnamates that comprise a group of plant polyphenols present in the human diet. Chlorogenic acid (CGA), also known as 5-O-caffeoylquinic acid (5-CQA), is a bioactive polyphenolic compound found in fruits and vegetables which known by their potential antioxidant properties and their CGA content contributes to such activities [28]. Anti-oxidant effect of CGA, via its radical scavenging activity was shown to be potentially mediated via two mechanisms (a) a hydrogen-atom-transfer reaction in which, a free radical abstracts a hydrogen atom from CGA and/or (b) a radical adduct formation in which, a free radical binds CGA and produces a radical intermediate.

Chlorogenic acid and its metabolites are widely distributed in the brain and can easily cross blood brain barrier (BBB) and the high BBB permeability of chlorogenic acid is a great advantage in neuroprotective mechanisms [29]. It exerts neuroprotective

effects against glutamate-induced excitotoxicity and ROS-mediated neurotoxicity [30].

It protects neurons against oxidative stress and alleviates neuronal degeneration [31]. Chlorogenic acid mitigates brain ischemia by controlling inflammatory cytokines, hypoxic factors, and nerve growth factors [32]. Although the neuroprotective effect of chlorogenic acid has been reported, the putative protective mechanism of chlorogenic acid against pesticide is still not yet understated. Thus, we investigated the protective mechanisms of chlorogenic acid against glyphosate toxicity and underlying the neurobiological approach and behavioral observations.

Oxidative stress is major drivers of cellular and molecular events that can lead to the development of ROS accumulation due to glyphosate exposure, Oxidative stress is characterized by accumulation of reactive oxygen species (ROS), which is caused by "an imbalance between oxidants and antioxidants in favour of oxidants". Lactate dehydrogenase**,** an important class of enzymes was found in the present study to be down-regulated in acute toxicity induced by glyphosate. Our results showed that glyphosate induced oxidative stress by altering the enzyme system and increasing levels of lactate dehydrogenase. Administration of a toxic dose of glyphosate of 100mg/kg bw causes production and SOD and CAT and POD activities. In our previous study [30], we confirmed that glyphosate can cause oxidative damage in the brain by exhausting the endogenous antioxidant system, which leads to the decreased activity of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). Furthermore, during acute exposure to glyphosate pesticide, the endogenous antioxidant system is able to revese the oxidative damage once the exposure has ended. Conversely, if ROS reach high concentrations and the exposure period becomes chronic, the antioxidant system is easily overcome, and oxidative damage is disseminated in tissues and organs. During these events, chained oxidative reactions occur through a variety of oxidant metabolites that affect most biomolecules [33, 34] also studying poisoning with rotenone in *vitro* study, demonstrate that CGA-rich *Solanum melongena* extract significantly increased ATP production and SOD and CAT activity in PC12 cells treated with rotenone [33].

Xu et al., 2014 demonstrated also that CGA could protect against tetrachlorobenzoquinone, an organochlorine compound used as a pesticide and a disinfectant inducing hepatotoxicity by reducing ALT and AST, hepatic oxidative stress, and MDA while in - creasing GSH content and selected antioxidant enzymes activity. More recently it has bben shown also that CGA ameliorated toxic effects of paraquat and increased paraquat-suppressed Nrf2, SOD, and GSH levels; given that excessive exposure to paraquat can induce morbidity and mortality in humans. Paraquat may produce neurotoxicity but the lung is the major target organ) CGA significantly repressed oxidative stress in the liver and erythrocytes compared to the controls [35].

Brain is often associated with pronounced oxidative stress that is due, at least in part, to an increased expression of NADPH oxidase, an enzyme generating superoxide anions.

In the present study, Our results are consistent with these results and those with those of (Atiz et al. 2009), which demonstrate that glyphosate can increase also the plasma level of TABARS and even at low doses (10mg/kg) intraperitoneally, when pregnant rats and their fetuses received GLP during the gestation period [25].This protective effect may be due to the trapping of MDA molecules by the active ingredients contained in MA or to the inhibition of mitochondrial chain reactions. MDA countered the increase in calcium levels triggered by the toxic effect of glyphosate. As glyphosate increased H_2O_2 and free iron levels, it could also increase the hydroxyl radical, a toxic radical that could in turn alter calcium homeostasis. Sasaki et al., 2010 show also that CGA and its esters repress lipid oxidation via both free-radical scavenging in the lipid phase and metal chelation in the aqueous phase [36]. Also, free iron can act as a catalyst for autoxidation and mediated oxidation of cysteine residues represents a common mechanism by which H_2O_2 exerts its second messenger role in signal transduction pathways. Moreover, H_2O_2, by inducing the oxidation of sulfhydryl residues, can release intracellular calcium. This oxidation can affect proteins as calcium channels or antioxidant enzymes as catalase or the induction of SOD activity. In this case, glyphosate simultaneously increased free iron and H_2O_2 and CGA enhanced these deleterious effects at the tissue and plasma level.

Growing evidence suggests that natural phenolic compounds play preventative and therapeutic roles in neurodegenerative diseases and inflammatory pathological states. The therapeutic potential of these bioactive compounds is due to their antioxidant and anti-inflammatory properties. CGA has been shown to have potent anti-inflammatory, antigenotoxic, and antioxidant activities [37]. Inflammation should be considered as a target for treatment in clinical trials so as to delay progression of subclinical brain damage and potentially cognitive decline. The Assessment of inflammation triggered by glyphosate by determining the level of C-reactive protein CRP. Specifically, glyphosate exposure resulted in an upregulation of C-reactive protein (CRP) in the liver, and cytokines IL-1β, IL-6, and tumor necrosis factor α (TNFα) in liver and adipose tissue of rats [2].CRP has been suggested also to mirror the inflammatory process in the brain, thus allowing its utility as a potential biomarker [38, 39]. Previous studies on CRP and associated with imaging markers reporting an association between higher CRP levels with WMH, lacunes, and ePVS. These associations may be explained by a combination of following mechanisms: First, elevated CRP levels are a marker for arteriolosclerosis which involves vessel occlusion, altered cerebral autoregulation, and increased vascular permeability giving rise to WMH, lacunes, and ePVS [2]. Then elevated CRP reflects large-vessel atherosclerosis. Carotid atherosclerosis has been associated with CSVD, which are caused by decreased cerebral blood flow and production of inflammatory mediators and free radicals that affect microvascular endothelium [40]. Also NO is a pro-inflammatory mediator that contributes to the pathogenesis of inflammatory disorders [41, 42]. NO is an important inflammatory mediator produced by the body and its excessive production is closely associated with various neurodegenerative diseases. Overproduction of these pro-inflammatory mediators causes inflammation. ROS is a major signaling molecule that can lead to activation of NADPH oxidases (NOX) resulting in increased production of ROS such as hydrogen peroxide (H_2O_2) and superoxide anion (O2•−). Superoxide anion reactswith the vasodilator nitric oxide (NO) forming peroxynitrite (OONO−), thereby reducing NO bioavailability [43].The gaseous molecule NO is generated from l-arginine by the enzyme endothelial NO synthase (eNOS) and diffuses towards the underlying vascular smooth muscle cell layer to dilate blood vessels in a cyclic guanylyl monophosphate-dependent manner. NO can also diffuse towards the lumen to prevent platelet adhesion and activation, and also monocyte adhesion.

More recently many research focused on glyphosate acute and lon toxicicty among those ,a study by Winstone [5] show that glyphosate is detectable in PBS perfused brain that glyphosate infltrated the brain in a dose-dependent manner and upregulated TNFα in both plasma and brain tissue post-exposure. Notably, glyphosate measures correlated positively with TNFα levels. Glyphosate exposure in APP/PS1 primary cortical neurons increases levels of soluble Aβ40-42 and cytotoxicity. RNAseq revealed over 200 diferentially expressed genes in a dose-dependent manner and cell-type-specifc deconvolution analysis showed enrichment of key biological processes in oligodendrocytes including myelination, axon ensheathment, glial cell development, and oligodendrocyte development [5]. *In vivo* investigations of CGA Anti-inflammatory effects was carried out in many studies. The mechanism by which CGA is beneficial in inflammatory disease states may be due to its immunosuppressive effects; pro-inflammatory factors released by activated microglia may contribute to the progression of neurodegenerative diseases, whereas CGA prevented neurotoxicity caused by microglial activation and ultimately improved survival of dopaminergic neurons [44]. Notably, CGA protects dopaminergic neurons against neuro-inflammatory conditions associated with Alzheimer's disease [17]. CGA may show neuroprotective effects in the case of pro-inflammatory factor-mediated neurodegenerative disorders [44]. Possible mechanisms of immunosuppression in a model of LPS-stimulated primary microglial activation include suppressing: NO production, TNF-α release, and NF-κB translocation. Oral administration of coffee extract and CGA has been reported to protect against retinal degeneration as well [45]. Moreover, CGA metabolites caffeic acid and ferulic acid have also been reported to have anti-inflammatory effects. These findings indicate that the anti-inflammatory effects of CGA are not limited to the parent molecule. The metabolites of CGA appear to contribute to its pain-alleviating mechanisms by interrelated anti-inflammatory mechanisms [46].

Therefore, CGA may have a potential therapeutic effect on cognitive dysfunction caused by acute glyphosate exposure causing neurtoxicity. Altering behaviour and diminishing memory are the major manifestations some neurological disorders. In this

context, it has been reported that CGA was able to improve some measures of cognitive function [47]. In this sudy it is interested in the harmful effects of glyphosate on cognition, learning and memory.

The open field test (OFT) is a common measure of exploratory behavior and general activity in both mice and rats, where both the quality and quantity of the activity can be measured. Principally, the open field (OF) is an enclosure, generally square, rectangular, or circular in shape with surrounding walls that prevent escape. The most basic and common outcome of interest is “movement” ; however, this can be influenced by motor output, exploratory drive, freezing or other fear-related behavior, sickness, relative time in circadian cycle, among many other variables. Distance moved, time spent moving, rearing, and change in activity over time are among many measures that can be tabulated and reported. Some outcomes, particularly defecation, center time, and activity within the first 5 minutes, likely gauge some aspects of emotionality including anxiety. The OFT is also commonly used as a mechanism to assess the sedative, toxic, or stimulant effects of compounds. Thus, the OFT measures a number of facets of behavior beyond simple locomotion. As such, the test has a number of uses and is included in almost every thorough analysis of rodent behaviour. The Elevated Maze (EM) test is used to assess anxiety-related behavior in rodent models of CNS disorders. The EPM apparatus consists of a "+"-shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a center area. As subjects freely explore the maze, their behavior is recorded by means of a video camera mounted above the maze and analyzed using a video tracking system. The preference for being in open arms over closed arms (expressed as either as a percentage of entries and/or a percentage of time spent in the open arms) is calculated to measure anxiety-like behavior.

In the elevated plus-maze, CGA (10mg/kg/bw) significantly increased the exploration of the open arm in similar way to that of glyphosate. At a CGA (10mg/kg/bw) i.p. significantly increased both the time spent in the open arm and closed arms by rats. Further, in the open field test, CGA significantly increased number of squares traversed, all of which are demonstrations of exploratory behavior.

In accordance with our results and more recently, studies Xiong have shown that Xiong showed that mice subjected to LPS stimulation had reduced autonomic activity. The water maze experiment showed that the learning memory ability of mice was decreased after being subjected to LPS. All of the above behavioral changes in mice triggered by LPS were improved in the LPS+CGA group mice [48]. Intraperitoneal injection of LPS leads to the production of IFN-γ and TNF-α in the mouse brain, and these two inflammatory factors have been shown to play an important role in LPS-induced depression-like behaviour [49].

Conclusion

Animal models play a central role in investigation of behavioural and cognitive processes and the underlying biological mechanisms, understanding neurological disorders and development of preventive and curative strategies. Taken together, glyphosate may produce direct effect neurotoxicity through ROS overproduction and lipid damage. Glyphosate also causes depletion of antioxidant enzymes both at the plasma level (SOD, CAT, peroxidase, etc.) and at the brain tissue .CGA extract appears to be able to abrogate this effect which could be attributed to its antioxidant and scavenging activities. On the other hand, the protective effect of the against oxidative stress by modulation of biochemical parameters (Ca_2^+,H_2O_2, free iron) as well as against the plasma level of inflammation triggered by glyphosate (CRP level, figure).Previous studies have shown that higher CRP levels are associated with increased risk of cerebrovascular disease and dementia as CRP increases the permeability of the blood-brain barrier. CGA regulate Nitric oxide (NO) production has been that ache been reported as inflammatory marker, showing a significant increase in NO production in Wistar rats treated with CGA and glyphosate. Animal models play a central role in investigation of behavioural and cognitive processes and the underlying biological mechanisms. Altering behaviour and diminishing memory are the major manifestations some neurological disorders .Herin, CGA was able to improve some measures of cognitive caused by acute glyphosate exposure causing neurotoxicity.While optimistic, the results of these clinical

studies would need to be replicated by independent researchers in order to conclude that CGA is a viable treatment for neurotoxicty caused by pesticides exposure or other degenerative diseases.

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