

Potential hazards of glyphosate on rabbit retina

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Background/aim

Retinal degeneration is a leading cause of vision loss. Exposure to pesticides is a high risk factor of retinal degeneration. The aim of the work is to investigate the molecular structural changes of retina associated with the topical application of glyphosate (GLP), an organophosphorus compound pesticide.

Materials and methods

Twenty rabbits were randomly categorized into four groups. Group I served as control ($n=5$, 10 eye balls) and the other three groups (each composed of five rabbits) received topical instillation of 100 μ l twice a day of GLP in three concentrations of 0.12, 0.97, and 7.8 mg/kg, respectively. After 1 week, eyes were enucleated and retinae were removed and prepared for analysis by infrared spectroscopy (Fourier-transform infrared spectroscopy).

Results

The regions of NH–OH spectra indicated a significant decrease ($P<0.05$) in wavenumber and restriction of two components related to $_{str}O-H$ and OH_{asym} band after application of GLP. The protein secondary structure components in terms of β sheets, alpha helices and β turns were changed in groups III and IV. Also the fingerprint regions indicate changes in the surrounding environment after all doses of GLP.

Conclusion

Certain retinal structure modifications may lead to the degeneration of retina after topical exposure to GLP in rabbits. So this herbicide should be handled with caution. National governments must introduce a GLP reduction program because of its damage effect on retina function.

Keywords:

Fourier-transform infrared spectroscopy, glyphosate, pesticides, rabbit, retina

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Introduction

Pesticides are important elements in agriculture and extensively used to control harmful pests and prevent crop yield losses or product damage. Owing to its high biological activity and long persistence in the environment, pesticides may cause undesirable effects to human health and to the environment [1].

Pesticides enter the human body by four ways: ingestion, inhalation, by skin contact and through ocular exposure. Regular and occupational exposures in the agricultural sector include pesticide applicators, farmers, and other agricultural workers [2]. Handling of pesticides and subsequent hand-to-eye contact increases the probability of ocular exposure in workers. This may also be caused by improper practices as the lack of washing hands. Aerial spraying of pesticides into the environment over farms also increases the risk of ocular exposure in workers on the ground. Ocular exposure to pesticides can occur in nonoccupational accidental circumstances in the general population [3].

Glyphosate [*N*-(phosphonomethyl) glycine] (GLP) is an organophosphorus compound pesticide that is used as an active ingredient of many herbicides and plant growth regulators and found in more than 750 products of them [4]. Glyphosphate was commercially introduced in 1974 since that its use increased rapidly. When it had been registered, it was classified as safe for vertebrates as these herbicides affect the shikimate pathway through the inhibition of specific enzymes responsible for aromatic amino acid metabolism and this pathway did not exist in animals, but the scientific researcher observed that these herbicides cause hepatic, renal, reproductive and neural toxicities [5]. Treatment of albino mice with GLP induce hepatotoxicity, nephrotoxicity, lipid peroxidation, and genotoxicity from significant increase in serum aspartate aminotransaminase, alanine aminotransferase, blood urea nitrogen, creatinine levels, and malondialdehyde levels of

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the liver and kidney tissues [6]. Histopathological examination has shown that there was degeneration in hepatic cells, neural cells, pancreatic acinar cells, stomach cells, and tubular necrosis of rats subchronically exposed to GLP [7]. Hernández-Plata *et al.* [8] have evaluated the effects of three intraperitoneal doses of GLP (59, 100, 150 mg/kg body weight) on the nervous system in male Sprague-Dawley rats for a period of 2 weeks. They reported decreases in locomotor activity, binding to D1 dopamine receptors in the nucleus accumbens, extracellular dopamine levels in the striatum, which mean that GLP affects the dopaminergic system. In a vivo study by Dai *et al.* [9] in rats after administrating GLP at doses of 5, 50, 500 mg/kg once a day for 5 weeks, the results explained that GLP has low reproductive toxicity.

In industrialized countries, retinal degeneration is a leading cause of decreased visual acuity and loss of central vision among older adults [10]. Exposure to pesticides was considered recently as a major risk factor and may increase retinal degeneration [11–13]. This research aimed to demonstrate retinal structure modifications that may lead to retinal degeneration after topical exposure to GLP in rabbits.

Materials and methods

Chemicals

The chemicals utilized were obtained from Sigma Company (St. Louis, Missouri, USA) with the highest purity commercially available.

Animals

Rabbits from both sexes (2–2.5 kg) were haphazardly chosen from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The protocol of the experiment was approved by the local ethics committee, The Association for Research in Vision and Ophthalmology.

Glyphosate application

GLP was dissolved in deionized water in order to prepare the three concentrations, 0.12, 0.97, and 7.8 mg/kg, that will be applied topically to the animal's eyes. These GLP solutions were freshly prepared each day. Twenty rabbits were randomly allocated into four groups, group I as the control ($n=5$, 10 eye balls) and the other three GLP-treated groups (each composed of five rabbits) that received topical instillation of 100 μ l twice a day of the above-mentioned three concentrations. The experiment proceeds for 1 week.

Fourier-transform infrared spectroscopy technique

After decapitation of the animals, eyes were enucleated, and the removed retina was mixed with KBr powder to prepare a disk to be used in the analysis. The disk was

placed in a metallic holder and pressed under vacuum at 3 tons for 2 min thus obtaining IR-KBr disks [14]. Measurements were done using infrared spectrophotometer (ThermoFisher Scientific Inc., Waltham, Massachusetts, USA). The resolution was set at 2 cm^{-1} and dry N_2 gas was used to remove interference. Correction of the baseline of the spectra was done and then smoothing to remove noise and finally Fourier transformation was used.

Statistical analysis

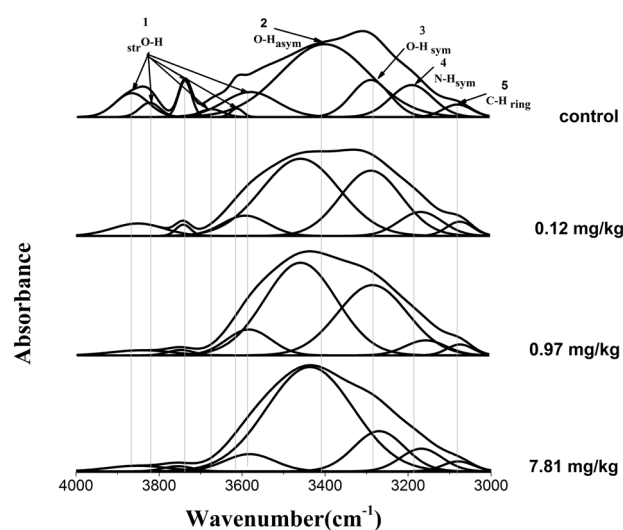
Results were displayed as the mean $\pm$ SD. To obtain a comparison between groups, investigation of fluctuation was done by using the commercially available software program (SPSS-11 for windows, SPSS Inc., Chicago, Illinois, USA), where the significance level was set at $P < 0.05$. All the spectral were averaged using OriginPro9 program (Origin Lab Corporation, Northampton, Massachusetts, USA).

Results

The spectra results from Fourier-transform infrared spectroscopy to retinal tissue extracted from control rabbits and all groups received topically GPL cover in the range 4000–400 cm^{-1} . All the spectra were analyzed in three frequency regions, 4000–3000, 3000–2800, and 1600–900 cm^{-1} , corresponding to NH–OH, C–H, and the fingerprint region, respectively. Amide I (1700–1600 cm^{-1}) that is related to the fingerprint region was discussed separately due to its importance in protein secondary structure.

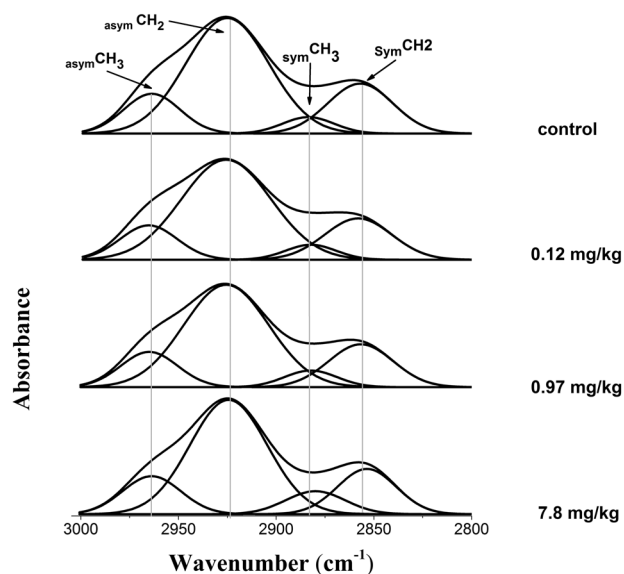
Figure 1 shows the spectral range 4000–3000 cm^{-1} related to stretching NH–OH function group to

Figure 1



Spectral region (4000–3000 cm^{-1}) related to NH–OH of control retina and all groups receiving topical glyphosate.

Figure 2



The second analysis region (3000–2800 cm^{-1}) due to C–H vibrations for control rabbits and the three groups receiving topical glyphosate.

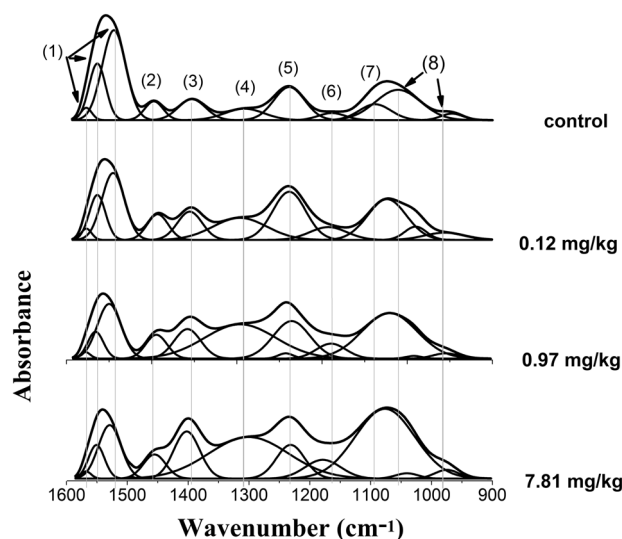
Table 2 C–H region (3000–2800 cm^{-1}) for control rabbits' retina and all groups receiving topically glyphosate

	asymCH ₃	asymCH ₂	symCH ₃	symCH ₂
Control	2964±3	2926±4	2884±2	2857±3
group I	34±1	50±5	31±4	39±5
Group II (0.12 mg/kg)	2966±2	2926±2	2882±2	2858±2
	34±1	54±2	30±5	40±2
Group III (0.97 mg/kg)	2965±2	2925±3	2881±2	2856±1
	34±3	51±2	31±4	39±1
Group IV (7.8 mg/kg)	2963±2	2924±2	2880±3	2853±2
	35±4	46±3	35±7	35±5

First line in each cell indicates the vibrational frequency, while second line reflects the bandwidth.

and 1524 ± 3 corresponding to (1) amide II ($\text{N}=\text{H}_{\text{bend}}$); bands at frequencies 1458 ± 1 , 1396 ± 1 , 1309 ± 1 , 1236 ± 2 , 1166 ± 1 , and 1095 ± 3 corresponding to (2) CH_2 bend, (3) COO^- , (4) CH_3 def, (5) PO_2^- , (6) $\text{strCOOC}_{\text{asym}}$, and (7) PO_2^- , respectively, and two components at 1057 ± 3 and 968 ± 1 corresponding to (viii) strCOC . The wavenumber changes for each band to all groups receiving GLP compared with normal and their bandwidths are illustrated in Table 3. For all doses of GLP, no effect was observed to amide II wavenumber or bandwidth to its three components. A statistically significant decrease ($P < 0.05$) in the absorbance of CH_2 bend was observed in group II and group III which is accompanied by splitting of the band in group IV. In contrast in COO^- , a statistically significant increase ($P < 0.05$) in its wavenumber was observed. CH_3 def and $\text{strCOOC}_{\text{asym}}$ suffered an increase ($P < 0.05$) in their width after all doses of GLP and fluctuating changes in wavenumber. A splitting in PO_2^- band was detected after 0.97 mg/kg of GLP (group III). A decrease ($P < 0.05$) in wavenumber

Figure 3



The fingerprint region (1600–900 cm^{-1}) of the retinal tissues for all groups receiving glyphosate compared with control: (1) amide II ($\text{N}=\text{H}_{\text{bend}}$), (2) CH_2 bend, (3) COO^- , (4) CH_3 def, (5) PO_2^- , (6) $\text{strCOOC}_{\text{asym}}$, (7) PO_2^- , and (8) strCOC .

was observed in PO_2^- and a component of strCOC but in the same time an increase ($P < 0.05$) in wavenumber was observed to the second component of strCOC after GLP.

Figure 4 showed that the curve enhancement procedure resolved the contour of the normal amide I band into four compositional bands that were at 1688 ± 3 and 1674 ± 8 cm^{-1} (β -turns), 1650 ± 7 cm^{-1} (α -helix), and 1625 ± 22 cm^{-1} (β -sheet). In groups III and IV which are characterized by an increase of GLP dose, the protein secondary structure was characterized by the presence of a structural band discernible at 1636 ± 10 and 1639 ± 8 cm^{-1} which was assigned as the β -sheet structure.

Table 4 indicates that the distribution of normal protein secondary structure components that were calculated as the area percentage were $21.5 \pm 2.3\%$ for β -turns, $66.5 \pm 3\%$ for α -helix and $11.7 \pm 4\%$ for β -sheet. After 0.12 mg/kg of GLP (group II), there were no significant changes in the area percentage for protein structure components. In groups III and IV the relative distribution of the structural component differs. The β -turns and α -helix content significantly decreased ($P < 0.05$). On the other hand, the content of β -sheet significantly increased.

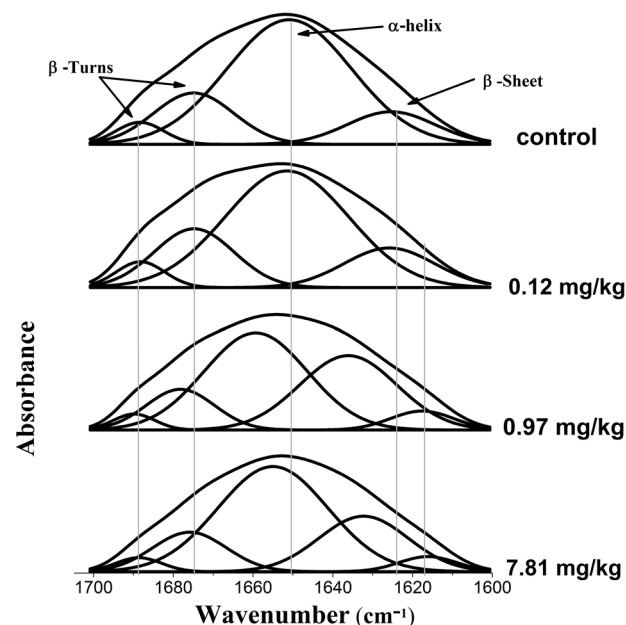
Discussion

Organophosphorus pesticides poisoning is widely investigated, and a growing number of evidence

Table 3 Fingerprint region (1600–900 cm⁻¹) of all the studied groups receiving glyphosate compared with control

	(1) Amide II (N=H _{bend})	(2) CH ₂ bend	(3) COO ⁻	(4) CH ₃ def	(5) PO ₂ ⁻	(6) str COOC _{asym}	(7) PO ₂ ⁻	(8) str COC		
Control group I	1569±2 21±2	1551±1 32±2	1524±3 45±1	1458±1 35±5	1396±1 52±6	1309±1 75±3	1236±2 59±2	1095±3 59±3	1057±3 87±8	968±1 42±2
Group II (0.12 mg/kg)	1570±2 20±4	1551±1 31±5	1526±2 43±2	1452±2 [†] 40±1 [†]	1399±1 [†] 47±3	1317±2 [†] 104±9 [†]	1236±3 63±2	1075±1 [†] 74±4 [†]	1028±1 [†] 42±3 [†]	980±4 [†] 77±7 [†]
Group III (0.97 mg/kg)	1568±1 19±3	1551±1 30±5	1529±2 46±2	1451±2 [†] 41±6	1400±1 [†] 51±1 [†]	1303±3 [†] 138±14 [†]	1239±3 29±3	1068±1 [†] 105±3 [†]	1028±1 30±5 [†]	975±2 [†] 49±3 [†]
Group IV (7.8 mg/kg)	1567±1 19±4	1550±1 31±6	1528±5 43±5	1493±2 11±2	1452±1 [†] 43±1	1300±1 [†] 153±4 [†]	1231±1 [†] 50±3 [†]	1076±1 [†] 114±2 [†]	1040±1 [†] 44±6 [†]	974±1 [†] 46±2 [†]

First line in each cell indicates the vibrational frequency, while the second line reflects the bandwidth; [†]Statistically significant.

Figure 4

Amide I region (1700–1600 cm⁻¹) for control and all groups receiving topical glyphosate.

Table 4 Protein secondary structure changes of retina expressed as percentage area of β-turn, α-helix, and β-sheet for control and all groups receiving topical glyphosate

	β-Turn	α-Helix	β-Sheet
Control group I	21.5±2.3	66.5±3	11.7±4
Group II (0.12 mg/kg)	23.5±3.7	62.7±2	13.7±1.8
Group III (0.97 mg/kg)	14.9±1.6 [†]	46.8±0.8 [†]	38.3±2.7 [†]
Group IV (7.8 mg/kg)	15.5±2 [†]	55.5±2 [†]	28.8±3 [†]

[†]Statistically significant.

indicates its effects to cause ocular lesions, but the mechanisms of its ocular effects are not well elucidated. In our study the effects of organophosphorus pesticide GLP on rat retina were investigated.

Accidental splashes of pesticide exposure resulted in the direct entry of these chemicals into the eye tissue. As a result, these chemicals are absorbed through the eye tissue and enter the circulation, leading to systemic effects. Radioactive studies using β radiation had confirmed the transport of a carbamate insecticide from the cornea, through the aqueous humor and vitreous humor to the retina [15].

A vibrational spectroscopy technique – especially Fourier-transform infrared spectroscopy – can be used to probe the molecular structure changes that accompany diseased tissues. By assignment of functional groups, bonding and molecular conformations in spectral bands, accurate information about the biochemical and biophysical features of the oxidative stress damage in the retina can be detected.

In the present study, the assignment of the C–H bands has been previously mentioned by Severcan *et al.* [16]. CH stretching modes of retina did not change after all doses of GLP application. The peak assignments appeared in normal bands to NH–OH after deconvolutions were as previously mentioned by Dovbeshko *et al.* [17]. The NH–OH region changes revealed unusual interface/binding mechanism that is related to different surrounding environment due to GLP application. O–H mode changes after GLP suggested different conformations. The band assignments of the fingerprint region had been indicated previously by Jung [18]. The noticeable fluctuation changes in the fingerprint region recognized to an oxidative stress after topical application of GLP and increase the stress with the dose. The splitting in the PO_2^- band is a response to disorder in the lipid molecule and changes in the genetic material or phospholipids that involved in the structure of retinal tissues which is highly specialized layers.

Amide I absorption is allied with protein amide C=O vibrations, and it is fitting to establish the special secondary structures of proteins and polypeptides. The types of secondary structure types include the α -helices and β -sheets that let the amides to hydrogen bond very powerfully with one another. In the α -helix the polypeptide backbone is coiled in a right-handed helix where the hydrogen bonding occurs between following turns of the helix. In β -sheets, the strands of the polypeptide are expanded and be positioned either parallel or antiparallel. Also, secondary structures include β -turns which are sharp turns that connect the neighboring strands in an antiparallel β -sheet [19]. After topical application of 0.12 mg/kg of GLP, the protein secondary structure has not been affected. But there was a change in the α -helix, β -sheet, and β -turn contents to the other two doses of GLP. As the β -sheet content increased, the protein insolubility increased [20]. β -sheet formation increases the disordered structure of the helical structure, and then the disordered chains aggregate to form β -sheet structure. So the two doses 0.97 and 7.8 mg/kg of GLP had supported the aggregation of proteins as they became more folded and insoluble.

The mechanism of GLP damage to retina may be due to its action on dopamine as GLP decreases the extracellular level of dopamine. GLP may affect dopamine synthesis and interfere with the phosphorylation of tyrosine hydroxylase for the preservation of catecholamine levels resulting in a decrease in high-potassium-induced dopamine release [8].

Dopamine is released by amacrine cells and activates D1 and D2 dopamine receptors dispersed throughout the retina. Dopamine also has various trophic roles in retinal function related to circadian rhythmicity, cell survival, and eye development. Any decrease in retinal dopamine results in reduced visual contrast sensitivity and affects the function of the retina [21].

Moreover, another reason explaining the mechanism of GLP damage is the oxidative stress due to its action on reduced glutathione and lipid peroxidation levels of animals as an index of antioxidant status and oxidative stress [22].

Conclusion

GLP causes damage to the retinal tissue on the basis of molecular structure. So this herbicide should be handled with caution. National governments must introduce a GLP reduction program due to its damage effect on retina function. All food processors should minimize their customer's exposure to GLP residues by specifying GLP-free products from their suppliers. They should extend their internal pesticide monitoring program and include GLP in their regular testing.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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