Ginger extract ameliorates phosphamidon induced hepatotoxicity

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Received 01 April 2014; revised 18 July 2014

Organophosphorus (OP) compounds are commonly used as pesticides in agriculture but are also used in medicine and industry. OP pesticides exert toxicity by inducing oxidative stress (OS) that causes cellular damage. Owing to their lipophilic character, OPs directly interact with the cell membrane and cause lipid peroxidation (LPO). LPO generates potential free radicals such as superoxide anions, H$_2$O$_2$, OH, etc., which affects the structure of proteins, lipids and DNA and disturb metabolism leading to tissue injury and even death. Deleterious effects of LPO include damage of membrane integrity and loss of activity of membrane bound enzymes like acetylcholine esterase, alkaline phophatase, Na$^+/K^+$-ATPase, Mg$^{2+}$-ATPase and Ca$^{2+}$-ATPase. The end product of LPO i.e., malondialdehyde (MDA) is reported to be genotoxic and mutagenic.

Phosphamidon (PHO) [0,0-Dimethyl-0 (2-chloro-2 (N,N Diethylcarboryl)-1-methylvinyl) phosphate] is a broad spectrum OP insecticide extensively used in India to control many chewing, sucking, and mining insect pests. Its widespread use causes health hazards in domestic animals and humans. Researchers have reported induction of OS, LPO of membrane, alteration of superoxide dismutase (SOD) activity, redox imbalance, chromosome aberration and induction of apoptosis as the principal damages which occur in PHO toxicity.

Ginger (Zingiber officinale Roscoe; Zingiberaceae) is a common spice consumed as dietary condiments throughout the world. It contains large amount of phytoceuticals such as flavonols, flavonone, anthocyanin, xanthin, flavonoids, tannin, etc. Apart from cooking, it is widely used as a culinary medicine in ayurveda, candy, digestive juices and also used as an effective antiemetic to prevent vomiting, nausea, etc. Traditionally, ginger is used in the treatment of cold, headache, toothache, nausea, rheumatism, and to improve blood circulation to the limbs. Ginger is rich in bioactive polyphenolic compounds and till date as many as 115 bioactive constituents have been identified from ginger. However, major highlight has been given on the essential oil (1-3%) and pungent compounds that include gingerols, shogaols, 6-paradol, [10]-gingerdione, hexahydrop-curcumin, tetrahydro-curcumin, gingerenone A, sesquipellandrene, beta-bisabololene, etc. Important pharmacological properties like antioxidant, antibacterial, antifungal, anti-parasitic, anti-inflammatory, antiangiogenic, anticancer, immunomodulatory, cardioprotective, chemoprotective and radioprotective activities of ginger polyphenols have been reported previously. Ethanol or aqueous ethanol is widely used for

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extraction of polyphenols from ginger and such extracts showed high antioxidant activity compared to synthetic ones\textsuperscript{6,10}. Here, we studied the protective role of polyphenol rich ethanolic extract of ginger against phosphamidon (PHO) induced hepatotoxicity by ameliorating oxidative stress (OS) and apoptosis in rats.

Materials and Methods

Animals—Adult male Wistar albino rats (160±10 gm) were housed in the animal house of the Department of Zoology, Visva-Bharati University, India. Animals were maintained under standard laboratory conditions (two animals/cage; 12 h light:dark cycle) and provided with food and water ad libitum. All the animal related studies were carried out under strict supervision of Institutional Animal Ethical Committee of the University.

Plant material—Fresh rhizomes of Zingiber officinale Roscoe were collected locally and authenticated by a pharmacognist of the Department of Botany, Visva-Bharati University. The samples were washed, air-dried at 37°C, ground to make powder for use.

Preparation and partial characterization of GE—Polyphenols rich ethanolic extract of ginger rhizome was prepared following the optimized extraction protocol described previously\textsuperscript{6}. In brief, powdered air-dried roots of gingers were extracted with 75% aqueous ethanol (1:2 w/v) for 60 min at 40°C with continuous stirring at 400 rpm. After completion of extraction, the mixture was subjected to ultrafiltration using 0.4 μm membrane, and the filtrate was collected as crude extract or GE. The extract was concentrated by evaporating under reduced pressure in a rotary evaporator (Eyela, Japan).

Total polyphenol content (TPC) of GE was measured by Folin–Ciocalteu reagent and antiradical activity was determined by DPPH as given in Mukherjee et al.\textsuperscript{6}. The antioxidant and antiradical activities of the extract were compared in vitro with commercially available antioxidants \textit{viz.}, BHT (Butyl Hydroxy Toluene) and ascorbic acid. The IC\textsubscript{50} value for GE was calculated from the linear regression of the % antiradical activity (against DPPH) vs. extracts concentrations ranging from 10 μg/ml-10 mg/ml in methanol.

The compound profiling of GE was studied by high performance thin layer chromatography (HPTLC). GE was separated by thin layer silica gel 60 F\textsubscript{254} immobilized on aluminium TLC plates (20 × 10 cm and 10 × 10 cm; Merck, Germany) using two different solvent systems (mobile phase) \textit{viz.}, solvent system-1; n-hexane: ethyl acetate: methanol: formic acid (10:8:1:1 (V/V/V/V)) and solvent system-2; toluene: ethyl acetate: formic acid (55:40:5 (V/V/V)) separately in two Twin Trough Chamber (20×10 cm) previously saturated with mobile phase solvent (10 min at 25°C). Gallic acid (phenolic acid), catechin (flavonoid), rutin (glycosylated flavonol), resveratrol (stilbenoid), quercetin (flavonol), ferulic acid (hydroxycinnamic acid), curcumin (natural phenols), ursolic acid (pentacyclic triterpene) and acaciaide-A (triterpenoid trisaccharide saponin) was used as standards, simultaneously spotted (5 μg) and ran with GE. Samples were applied to the plates as 4 mm bands using Camag (Switzerland) Linomat V applicator fitted with a microsyringe. After run, plates were dried and scanned by a Camag TLC scanner 3 in UV range. Initial HPTLC parameters were calibrated by applying different amounts GE (5-25 μg/spot) and scanning the plate in different wavelength (220-280 nm). After development of the plates, compounds in the GE were formally identified by means of retention factor (RF) with respect to the standard. Peak height, peak area and concentration of individual compound were calculated by linear regression analysis provided by the software winCATS Planar Chromatography Manager.

Sample preparation—Desired treatment concentrations were prepared by dissolving GE in 1.1% aqueous ethanol (11 ml 99% ethanol in 1000 ml sterile distilled water).

Screening of effective dose and treatment schedule—Experimental rats were divided into nine groups (Group I-IX) of six animals (n=6) each. The acute oral toxicity (LD\textsubscript{50}) of PHO in rat is 10 mg/kg body wt.\textsuperscript{11}. About 2 mg/kg body wt. of PHO was mixed with saline (1:1, v/v) and administered intraperitonealy (i.p.). For determination of effective dose, five group of rats (n=6) pre-treated with PHO, were force fed with GE at doses of 0.25, 0.5, 1, 2 and 5 mg/Kg body wt. along with one control group. LPO was monitored by estimating MDA from serum.

Group I served as vehicle (only saline) treated control. Group II received 2 mg/kg body wt. of PHO by i.p. for only 2 days where as Group III received same dose for 10 days; Groups IV and V received for 15 and 20 days, respectively. Groups VI, VII, VIII, and IX were given the same dose of PHO for same days, but were orally fed with GE (1 mg/kg body wt.) regularly during the treatment period.
Determination of biochemical and oxidative biomarkers—Liver was perfused with cold phosphate buffered saline (PBS; 10 mM and pH 7.4) and homogenized in a known volume (10 ml of ice-cold PBS for 1 gm of tissue). The homogenate was centrifuged at 9050×g for 20 min at 4°C and the resultant supernatant was used in the study. Malondialdehyde (MDA) was estimated from serum and liver tissue extract. Level of MDA was expressed in moles/gm of protein. From serum, apurate aminotransferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP) activities were assayed. Activity of transaminases (AST and ALT) was expressed in U/L whereas serum ALP activity was determined by K.A. units. Similarly, catalase activity was determined from the liver tissue extract and expressed in U/mg protein. Activity of glutathione-S-Transferase (GST), reduced glutathione (GSH) and glutathione peroxidase (GPx) were also assayed from the tissues extract. GST and GPx activities were expressed in U/mg protein while GSH was determined by ‘n’ mole/mg protein. Activity of SOD was estimated from the liver homogenates using SOD assay kit (Cayman Chemical Company, Ann Arbor, USA) following the manufacturer’s instructions. Activity of SOD was expressed in U/mg protein. Additional biochemical parameters such as protein, nucleic acids (DNA and RNA), total lipid, phospholipids and cholesterol levels were estimated from the liver tissue. According to the manufacturer’s protocol, glucose level was estimated from the liver tissue extract by using Glucose Oxidase (GOD)-Peroxidase (POD) assay kit (KEE GAD Biogen, India). 

Determination organophosphate induced genotoxicity and apoptosis—In order to study chromosome condensation, Hoechst-33342 staining was performed following the method of Cicchetti and Argentin. To study DNA fragmentation, genomic DNA was isolated, electrophoresed on 2% agarose gel, stained with 1% ethidium bromide and visualized in UV transillumination.

SDS-PAGE and immunoblotting—Total protein (70 µg) from liver homogenates was resolved by 12.5% SDS-PAGE and immunoblotted with different primary antibodies namely B-cell lymphoma-2 (Bcl-2), bcl-2 associated X protein (BAX), Apaf-1, Cytochrome-C, p53, NF-kB, nuclear factor erythroid 2-related factor 2 (Nrf2) and β-actin. Expression of each protein band was recorded after reaction with 5-bromo-4-chloro-3-indolyl-phosphate-nitro blue tetra-zolium and densitometry was performed using Quantity One software (Bio-Rad, USA).

Assay of caspase activity—Caspase-3 activity was assayed colorimetrically from tissue homogenate using the CaspACE™ Assay System (Promega, USA) in a 96-well plate reader. For substrate, p-nitroaniline labeled DEVD (Asp-Glu-Val-Asp) was used and Z-VAD-FMK served as an inhibitor.

Assessment of side effects—Side effects were monitored by feeding 100 mg/kg of GE to a group of rat (n=6) for 30 days on regular basis. Haematological parameters included determination of hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC). Liver function was diagnosed through the determination of SGPT, SGOT and ALP as demonstrated previously. Similarly, oxidative parameters like MDA, GST, GSH, SOD and catalase level were also estimated (mentioned in previous section) from serum.

Statistical analysis—The data were expressed as Mean ± Standard deviation (SD). Difference among the data was analyzed by Student’s t-test using GraphPad Prism 6.0 software. Values of P < 0.05 (*), P < 0.001(***) were considered as statistically significant.

Results—In this study, we explored the hepatoprotective effect of GE on PHO induced OS and apoptosis. Our experimental data revealed that GE exerts its protective role by targeting OS and apoptosis, the two major consequences of PHO toxicity.

GE is enriched with polyphenolic antioxidants—GE showed adequate antioxidant activity as evident from its scavenging effect over DPPH. The antioxidant activity of the extract was compared with the two commercially available antioxidants like vitamin C and BHT (Fig. 1A). The radical scavenging activity of GE on DPPH was recorded up to 100 µg/ml concentration. The radical scavenging activity of GE on DPPH was compared with the two commercially available antioxidants like vitamin C and BHT (Fig. 1A). The radical scavenging activity of GE on DPPH was recorded up to 100 µg/ml concentration. The antioxidant activity is inversely proportional to IC_{50} value. In this study, the IC_{50} value for antiradical activity GE based on DPPH scavenging activity was 0.83 mg/ml which is quite high for a crude plant extract. In addition, the total polyphenols content was 16.03 mg GAE/g of dried powder which corroborates with previous findings.

HPTLC based fingerprinting of GE showed the presence of different groups of phytochemicals such as curcumin, gallic acid, catechin and rutin which were...
identified from the respective standards (Fig. 1D & E). Figures 1B and 1D demonstrate the chemoprofiling of GE resolved by solvent system-1. It demonstrated abundance of polyphenols among which curcumin, gallic acid and catechin were the major polyphenols with peak area of 12.51%, 3.0% and 2.37%, respectively identified from the chromatogram (Fig. 1D). The specific proportions of curcumin, catechin and gallic acid were 4.800, 3.103 and 1.543 μg, respectively in 20 μg of GE. Although, we used the phenolic standards only, 6-shogaol could be identified with an Rf value of 0.65 that closely corroborate with an earlier report27. After separation with solvent system-1 (Fig. 1B), the two terpenoids, acasiacide-A and ursolic acid, were retained in the base whereas curcumin and ferulic acid were resolved with same Rf. Therefore, we changed the solvent system (toluene: ethyl acetate: formic acid-55:40:5) by making it more polar. Separation of GE by solvent system-2 (toluene: ethyl acetate: formic acid-55:40:5) also showed curcumin (17.55%) as major reference polyphenol in GE whereas other polyphenols like rutin and ferulic acid were also evident (Fig. 1C & E). Chemoprofile of GE constituted three similar peaks of gallic acid (0.26), rutin (0.43) and curcumin (0.51) and were identified with reference to standards simultaneously ran with GPE (Fig. 1E). Interestingly, resveratrol like compound was evident in the chromatogram while scanning at 260 nm (data not shown).

GE served as a natural remedy to PHO induced oxidative damage and apoptotic death of hepatocytes—GE was found to ameliorate PHO induced hepatotoxicity at a dose of 1 mg/kg body wt. (Fig. 1F). Rats treated with only PHO (groups IV and V) showed drastic reduction in mobility at the end of treatment period; whereas PHO+GE treated rats (groups VI-IX) were quite normal. The group IV that had the PHO treatment for longest duration showed signs of reduced motor activity, altered gait aggressive behaviour and frequent scratching movements including mortality (16.6%). But, such changes were not noticed in any of the PHO+GE treated group which indicated towards the hepatoprotective action of GE.

GE reduced LPO level and membrane damage—Level of serum and hepatic MDA were increased significantly ($P < 0.001$) in groups II-V (Fig. 2A) which indicated induction of LPO in rat liver after PHO treatment. However, such elevation in MDA...
level was not significant in the groups VI-IX which had PHO treatment but continuously fed with GE (Fig. 2A). Significant depletion of total lipid, phospholipid and cholesterol also indicated towards induction of LPO (groups II-V) and extent of such depletions were observed less in the GE treated groups (groups VI-IX) (Table 1). These experimental outcomes indicated that dietary consumption of GE could inhibit LPO.

**GE maintained the normal level of principal biomolecules after PHO intoxication**—Significant ($P <0.001$) alteration in the level of hepatocytic biomolecules such as protein, lipids, cholesterol and nucleic acids were noticed after 10, 15 and 20 days (groups III-V) of PHO intoxication. However, administration of GE restored the normal level of the hepato-cellular biomolecules in the PHO intoxicated rats (groups VII-IX) (Table 1). The ameliorating effect of GE was more pronounced after or from 10 days of administration.

**GE restored liver function**—Serum transaminases are the sensitive indicators of liver injury. Significant elevation ($P <0.0001$) in AST (S.G.P.T) and ALT (S.G.O.T) activities were recorded after PHO intoxication (groups II-V). However, such elevations in transaminase activities were not significant in the groups (VI-IX) that were fed with GE indicating towards its hepatoprotective role (Fig. 2B). The elevated level of ALP from the liver homogenate of PHO treated rats (groups II-V) indicates distortion of membrane resulted from LPO (Fig. 2C). A complete reverse observation in ALP activity in GE treated groups also indicated towards hepatoprotective role of GE (Fig. 2C).

GE attenuated PHO induced OS and restored the level of key antioxidants—Alteration in the level OS markers is an indicator of ROS generation and subsequent cellular imbalance. In this study, catalase activity was significantly ($P <0.05$) reduced after 15 and 20 days of PHO intoxication (groups II-V) (Fig. 2D). In contrast, GE treated groups restored normal activity which suggested that indicated protective role of GE in the amelioration of ROS and maintenance of normal catalase activity (Fig. 2D). Any alteration in GSH or intercellular GSH/GSSG ratio indicates onset of oxidative stress. Significant depletion ($P <0.001$) in GSH level was recorded in groups II-V that were intoxicated with PHO (Fig. 2E). During sustained exposure to PHO along with GE (groups VI-IX) showed gradual restoration in GSH level with duration of treatment (Fig. 2E). Marked decrease in GST activity (U/mg protein) was found during PHO exposure. Groups II-V showed 0.85, 0.51, 0.54 and 0.50 fold increased GST activity, respectively compared to control (Gr. I). Interestingly, GST activity in PHO intoxicated rats were tend to normal level after 10 days after continual treatment with GE indicating towards its hepatoprotective role (Fig. 2F). The elevated level of ALP from the liver homogenate of PHO treated rats (groups II-V) indicates distortion of membrane resulted from LPO (Fig. 2C). A complete reverse observation in ALP activity in GE treated groups also indicated towards hepatoprotective role of GE (Fig. 2C).

### Table 1—Changes in the level of biomolecules of liver tissue of albino rats in different experimental groups.

<table>
<thead>
<tr>
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<th>Group I (Control)</th>
<th>Experimental animals in groups (n=6)</th>
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<tr>
<td></td>
<td>II</td>
<td>III</td>
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<tr>
<td>Protein (mg/g)</td>
<td>7.01±</td>
<td>6.99±</td>
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<tr>
<td>Lipid (mg/g)</td>
<td>29.16±</td>
<td>28.66±</td>
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<tr>
<td>Phospholipids (mg/g)</td>
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<td>9.93±</td>
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<tr>
<td>Cholesterol (mg/g)</td>
<td>2.18±</td>
<td>1.88±</td>
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<tr>
<td>DNA content (mg/g)</td>
<td>1.43±</td>
<td>1.20±</td>
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<tr>
<td>RNA content (mg/g)</td>
<td>0.76±</td>
<td>0.65±</td>
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Values expressed as Mean ± Standard deviation. Level of significance: *, <0.05; **, <0.001; and ***, <0.0001.
with sustained PHO exposure in groups II-V showing enhancement of 1.4, 2.35, 2.67 and 2.46 fold, respectively in reference to control group (Fig. 2G). Interestingly, when GE was administered along with PHO (groups VI-IX), elevation in GPx activity were less (1.35, 1.59 and 1.02 fold, respectively for groups VI-VIII). Moreover, GPx activity in group IX was similar to control with only 1.009 fold increment (Fig. 2G). Thus, GE could restore normal GPx activity by eliminating the peroxides. SOD is an antioxidant enzyme that causes dismutation of ROS, especially superoxides, to protect the cell from oxidative damage. In this study, significant elevation of SOD activity was observed in PHO treated experimental rats viz., 9.58, 9.78, 9.35 and 9.74 fold, respectively for groups II-V (Fig. 2H). Whereas continuous GE treatment (10 days onward) along with PHO exposure ameliorated superoxide thereby inhibited the enhancement of SOD activity. Group IX showed an unaltered SOD activity as that of control (Fig. 2H). Therefore, GE with its antioxidant activity could maintain normal level of oxidative parameters and intracellular redox homeostasis by mitigating the hepatotoxic effect of PHO.

**GE attenuates PHO induced apoptosis**—Chromatin condensation and DF was evident from Hoechst staining and agarose gel electrophoresis primarily revealed the induction of apoptosis of liver cells of the rats treated with PHO (groups III-V) (Fig. 3). However, such alterations were not significant in the groups (VI-IX) that had PHO+GE treatment (Fig. 3A & B). Marked differences in the number of apoptotic cells as well as DF was evident among the PHO treated (groups III-V) and PHO+GE treated (VI-IX) groups as shown in Fig. 3A & B.
Fig. 3—Inhibitory effect of GE on PHO induced apoptosis of rat liver. (A) Micrographs showing changes in Hoechst-33342 positive condensed nuclei (marked with arrows) in PHO treated groups after GE administration. Hoechst stained liver tissue sections were visualized by UV microscopy with 20X magnification; (B) Histogram showing the presence of Hoechst positive condensed nuclei in percentage (%); (C) Histogram is showing the change in caspase activity in the liver tissue extract of different rat groups; (D) Gel electropherogram (2% agarose gel) showing different degrees of fragmentation of genomic DNA in different experimental group; and (E) Immunoblot showing differential expression of redox regulator (Nrf2), pro (p53, BAX, Apaf-1 and Cyt-c) and antiapoptotic (bcl-2) protein. [From upper side, first panel showing the expression of Nrf2 protein in the experimental groups. Second panel is showing the expression pro-apoptotic proteins viz. P53, Apaf-1, Cyt-c and Bax along with the antiapoptotic BCL-2. The last panel is depicting the expression of NfκB protein in the experimental groups. Group I served as vehicle (only saline) treated control. Group II received 2 mg/kg body wt. of PHO by i.p. for only 2 days while Group III received same dose for 10 days; Groups IV and V received for 15 and 20 days, respectively. Groups VI-IX were given the same dose of PHO for same days as Gr II-V, but were orally fed with GE (1 mg/kg body wt.)]
Fragmentation of chromosomal DNA by caspase-activated DNase is one of the major markers of apoptosis. As given in Figure 3D, DF in hepatocytes was found and the intensities of fragmentations were observed maximum in groups IV and V. In contrast, absence of DNA degradation in GE treated rats (group VI-IX) indicated that GE could exert anti-apoptotic effect by lowering DF (Fig. 3C). A gradual course dependant decrease in DNA fragmentation after continuous oral administration of GE to PHO intoxicated rats indicated inactivation of apoptotic signals like DNase activation due to amelioration of ROS.

In this study, PHO treated groups showed over expressed Nrf2 protein (groups II-V) and the upregulation was proportional with the treatment duration (Fig. 3E). But, reverse was noticed in the PHO treated groups continuously fed with GE (Fig. 3E). Due to the scavenging of ROS by GE, the expression of Nrf2 was tending to normal as found in groups VII-IX.

The altered expression of apoptogenic proteins (p53, Apaf-1, Cyt-C, BAX, Bcl-2 and NF-κB) in the liver extract of each experimental group were evident as compared to control (Fig. 3E). Elevation of pro-apoptotic proteins viz., p53, Apaf-1, Cyt-C and BAX and a reduction of anti-apoptotic protein Bcl-2 compared to the control were evident as the duration of exposure with PHO prolonged (groups II-V) while complete opposite result was noticed in the rats continuously fed with GE (groups VI-IX) (Fig. 3E). The results indicated that GE could inhibit PHO induced apoptotic death of hepatocytes by up- and down-regulating the level of anti- and pro-apoptotic proteins, respectively. Meanwhile, upregulation of NF-κB at translational level was observed in groups III and IV (Fig. 3E). In contrast, NF-κB in groups VIII and IX showed almost similar expression compared to the control (group I). Therefore, the induction of apoptosis was less in the GE treated groups. However, the effect of GE in groups VI and VII were not detectable indicating that a continuous treatment up to 10 days could provide significant amelioration of PHO induced apoptosis (Fig. 3E).

Interestingly, significant ($P < 0.001$) upregulation in caspase-3 activities (groups II-VII) upon PHO intoxication were also nullified after continuous oral administration of GE for 15 days (groups VIII and IX) as depicted in Figure 3C.

**GE exerted relatively lower level of side effects**—No considerable alteration in rat liver function was observed after 30 days oral administration of GE and level of hepatic oxidative stress markers were also tend to normal level (Fig. 4A). Similar results were observed for haematological parameters as well (Fig. 4B).

### Discussion

ROS are one of the major byproducts produced during mitochondrial oxidative phosphorylation and progression of several cellular metabolic pathways. Cellular level of ROS is tightly controlled by the antioxidants and any imbalance in antioxidant status can upregulate ROS production that can cause cellular damage or lead to cell death. Exogenous stressors such as UV light, ionizing radiation and chemicals, particularly pesticides, upregulate the ROS production. Increased ROS generation and associated cellular abnormalities are termed as oxidative stress (OS). During OS, levels of cellular antioxidants is upregulated to restore the redox homeostasis and maintain normal cellular functions resulted from the activation of a redox-sensitive gene circuit controlled by Nrf2. GST, SOD, non-enzymatic thiols and reduced glutathione (GSH) are the crucial members of mammalian antioxidant machinery. Generally, cells succumb to OS when endogenously stored antioxidants are used up by the oxidant exposure. In such condition, antioxidants from external source can play a crucial role in scavenging ROS and survivability of the cell. Administration of natural antioxidants, particularly from plant sources, can ameliorate deleterious effect of ROS. In particular, polyphenols have long been recognized for functional antioxidant, anticancer, anti-aging, antimutagenic, antimicrobial activities as well as exerting protection.
in cardiovascular, arthritis, diabetes, hepatic and neurological disorders, asthma, etc.\textsuperscript{6}. Pesticide induced liver injury is a potential complication, since this organ occupies pivotal role in detoxification, biotransformation and metabolic disposition of noxious xenobiotics. Most toxicants cause liver damage by inducing LPO and OS. Moreover, pesticide induced hepatotoxicity is the major cause behind liver failure in developing countries.\textsuperscript{28} PHO toxicity has been characterized by disruption of redox homeostasis, LPO, generation of ROS and imbalance in the levels of cellular antioxidants (SOD, CAT, GSH, etc.)\textsuperscript{29}. In the present study, we noticed an increasing trend in the level of hepatic OS markers and translational expression of Nrf2 during the course of PHO intoxication. Under normal circumstances Nrf2 remains attached to cytosolic Keap1. But under OS, Nrf2 is dissociated from Keap and translocated to the nucleus where it mediates the transcription of antioxidant gene\textsuperscript{30}. The overexpressed Nrf2 might have upregulated the level of SOD and GPx as evident from their elevated levels in the PHO treated rats. Elevated serum or tissue MDA level suggests induction of LPO and damage of liver cell membrane. This is further supported by elevated level of serum transaminases. Increased serum transaminase activity caused by the oxidative damage of hepatic membrane possibly promoted release of transaminases into serum. Antioxidants from GE scavenge ROS and inhibit LPO or membrane damage which restricts the release of transaminases from liver. Such alterations gradually decreased following GE (1 mg/Kg body wt.) treatment. Increased hepatic SOD and GPx revealed occurrence of oxidative stress. In contrast, GST followed a decreasing trend with the treatment duration. However, the altered level of the key antioxidant enzymes such as SOD, GST, GPx and catalase in PHO treated groups was recorded towards normal value (control) after GE administration.

Another important finding of the study is that GE not only ameliorates the level of ROS but also attenuated the downstream effects of ROS inside the cell. According to the previous researchers, PHO can induce apoptotic death of mammalian cells and organs like peripheral blood mononuclear cells, brain, reproductive organs, liver and kidney.\textsuperscript{31,32} Our experimental data suggested that GE inhibits the progression of PHO induced apoptotic signaling pathway which is initiated by ROS. We observed that GE could reduce the number ($P <0.001$) of apoptotic hepatocytes in PHO (1/10 of LD\textsubscript{50} for 20 days) exposed rats. Moreover, other hepatocyte apoptotic markers viz., DNA fragmentation, upregulated p53, BAX, Apaf-1, cytochrome-C and attenuated BCL-2 in PHO were restored to normal level after 15 days continuous administration of GE.

NF-kB is a key transcription factor that can protect or contribute to apoptosis depending upon the cellular conditions. Here, translational over-expression of p53 and NF-kB in PHO treated groups suggests that p53 may have activated NF-kB triggering the apoptosis inducing ability of p53. This hypothesis corroborates with the previous report\textsuperscript{33}. Furthermore, caspase activity was also decreased gradually as GE administration prolonged. Figure 5 depicts the probable mode of hepatoprotective action of GE.

Ginger has antioxidants that scavenge free radicals.\textsuperscript{6} Aqueous extract, juice and dried powder of ginger have been reported for ameliorating hepatotoxicity, testicular apoptosis and oxidative brain injury of rat resulted induced by OP pesticides and xenobiotics.\textsuperscript{1,8,34-36} Methanolic GE has been reported to influence multiple therapeutic molecular targets of Alzheimer’s disease (AD) and considered as an effective nontoxic nutraceutical supplement for AD.\textsuperscript{37} Ajith \textit{et al.}\textsuperscript{36}, have reported the hepatoprotective role of ethanolic extract of ginger.

![Fig. 5—Probable molecular mode of action of GE on PHO induced hepatotoxicity.](image)
(200 and 400 mg/kg body wt.) in acetaminophen-induced acute hepatotoxicity through the enhancement of hepatic antioxidant status based on biochemical and histological findings only. But, our study depicted the protective role of GE against PHO toxicity through biochemical, histological and molecular investigations. The novelty of our work is based on two important aspects. Firstly, a lower dose-longer duration treatment schedule with GE revealed a better amelioration of oxidative damages. Secondly, the mode of amelioration was studied by a number of key biochemical and molecular parameters in the progression of OS and apoptosis. The ameliorating effect of GE was more pronounced on or after 10 days of continuous feeding. The probable reason is the low level of absorption of active polyphenolic ingredients of GE. Keeping this in view, we selected a continuous low dose treatment instead of single or double bulk dose. Summarizing the experimental findings, it is evident that GE can restore the normal level of oxidative parameters by neutralizing ROS generated from PHO intoxication. In addition, amelioration of ROS by GE also inhibits apoptosis of liver cells. Therefore, GE with its ameliorating action over oxidative stress can be effective to attenuate hepatotoxicity induced by PHO.

Conclusion

Overall, the experimental outcome suggests that PHO toxicity can be attenuated by continuous oral administration of GE. However, considering the effectiveness and cytoprotective function, GE can be considered as an economical and effective plant extract to circumvent PHO toxicity. Partial purification including further downstream processing can improve its efficacy. Care should be taken that the combination of bioactive compounds tested in the GE are unaltered to avoid side effects.

Acknowledgement

University Grants Commission (UGC), New Delhi, Govt. of India is acknowledged for financial support (Grant No. 42-534/2013 (SR) as well as the award of Senior Research Fellowship to authors SM, NM and PS.

Conflict of Interest

The authors declare that there is no conflict of interest.

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