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Amelioration of Quinalphos-Induced Oxidative Stress by *Emblica officinalis* Fruit Extracts in Rats

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Abstract

The fruit of *Emblica officinalis* (commonly known Amla) contain potent antioxidants and is most commonly used in the Ayurveda for the treatment of various diseases. Quinalphos- a organophorus insecticide is known to alter antioxidant defense system in mammals. With this premise, experiment was planned with objective to study the effect of amla fruit extract supplementation on quinalphos induced oxidative stress. Twenty-four Sprague-Dawley rats were homogenously divided into six groups of four animals each. Group I was kept as control. Group II was orally given quinalphos at the dose rate of 2 $mg.kg^{-1}.day^{-1}$. Group III and V were orally gavaged with amla in sugar and honey bases respectively at the dose rate of 50 $\mu g.kg^{-1}.day^{-1}$ whereas group IV and VI were orally dosed with quinalphos along with amla in sugar and honey bases respectively at above mentioned dose rates from day 16-45. The various indices of antioxidant status were analyzed in major organs of treatment groups *viz*. Liver, kidney and brain. Sub chronic quinalphos administration produced oxidative stress in rats as evidenced by increase in lipid peroxidation, glutathione peroxidase and decline in superoxide dismutase. Oral supplementation of amla extract in sugar and honey bases provided partially amelioration against altered antioxidant status following sub chronic quinalphos exposure. Results of histopathological study were also in corroboration with blood antioxidant status validating amla in either bases was unable to provide full protection against quinalphos induced toxicity in rats.

Keywords:

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Introduction

Animals are more prone to insecticide toxicity because their sheds are frequently dusted with the same for controlling insect pests. In India, buffaloes are considered as the prime icon in livestock sector since contribute to the major portion of milk and beef industry. Cereals, paddy and leaves of common vegetables are often included in feed of buffaloes. Since the above vegetation is often sprayed with pesticides, chance of exposure of pesticides through feed cannot be ruled out. In addition toxicity may not always be acute in animals rather it could be sub chronic or chronic as this vegetation contains only small quantity of insecticides[1].

Quinalphos (QP) - a synthetic organophosphorus pesticide acts by inhibiting acetyl cholinesterase (AChE) enzyme in the blood as well as central and peripheral nervous systems of humans, vertebrates and insects[2,3]. The pesticide prevents AChE from breaking down acetylcholine and result in high concentration of parasympathomimetics in the nervous system leading to continuous stimulation and uncontrolled, rapid movement of some muscles leading to paralysis, convulsions and even death. Quinalphos is used in the control of pests on vegetables, fruits, cereals, rice, wheat, maize, coconut, tobacco, coffee, tea, sugarcane, jute and cotton[4]. The extensive use of this pesticide can produce a health hazard to animals and humans because

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of its persistence in the soil and crops[5,6]. Residues of this pesticide have been found in different vegetables and fruits above prescribed MRL indicating its persistence in plants[7].

Many effects of quinalphos have been described including oxidative stress[8,9], compensatory induction of antioxidant enzymes[8,9,10] along with decrease in the radical scavengers[11].

Various phytochemicals and its suppression of reactive oxygen species by natural antioxidants from tea, spices and herbs have been documented[12]. *Emblica officinalis* (commonly known Amla or Indian gooseberry) is regarded as "one of the best myrobalans (plants that exhibit various therapeutic properties) in the Ayurveda: an Indian traditional medicinal science. Amla extract contains several antioxidants such as emblicanin A and B, gallic acid, ellagic acid, ascorbic acid that are responsible for its strong antioxidant activity. According to *in vitro* studies, *E. officinalis* fruit extract (EFE) possesses antioxidant and nitric oxide (NO) scavenging activity.[13,14] proved that amla extract supplementation reduced the blood oxidative marker along with improved total antioxidant status. Aqueous extracts of this plant have potency to improve endogenous antioxidant defenses of the body through modulation of basal oxidative markers [16]. Presence of abundant vitamin C along with low molecular weight hydrolysable tannins imparts this medicinal plant a good antioxidant property[17].

Administration of *Emblica officinalis* aqueous extract has been shown to cause significant amelioration in the ochratoxin-induced lipid peroxidation in liver and kidney of mouse[18]. Reddy et al[14] demonstrated that supplementation of *Emblica officinalis* fruit extracts protected rats from alcohol induced oxidative stress and also brought altered plasma enzymes activities towards normal. Daily supplementation with *Emblica* fruit powder has been reported to improve antioxidant status in fluoride made hepato and renal toxicosis[19]. Co-administration of amla fruit extract lessened the oxidative stress produced by cadmium in wistar rats[20].

Experimental work on several medicinal plants has been carried out to evaluate its efficacy against chemically induced quinalphos toxicity[21]. The researchers have also proposed that phytonutrient rich plant extracts are more useful in reducing oxidative stress than nutrient derived antioxidant[20]. Animal body is naturally gifted with various enzymatic and non-enzymatic antioxidants to take care of reactive oxygen species produced due to oxidative insult following pesticide exposure. In this case exogenous supplementation of antioxidants might improve antioxidant status and thus the oxidative stress induced by these chemicals. The literature regarding effect of *E. officinalis* fruit extract on toxicity produced by quinalphos is not available so the present investigation was undertaken to study the effect of same against quinalphos induced alterations in various antioxidants and histoarchitecture in rats.

Materials and Methods

Chemicals

Quinalphos (Vazra 25% EC, Cheminova, Denmark) was purchased from local pesticide dealer. Amla fruits were dried in the shade for one week, weighed then soaked overnight in saturated sugar solution and honey separately and finally mixed thoroughly in a mincer so as to produce uniform suspension. It was then centrifuge at 3000 rpm for 10 min so as to obtain clear supernatant for administration.

Experimental design

Rats of Wistar albino stock were procured from small animal house of GADVASU and acclimatized in the laboratory condition of the department for 15 days. The rats were homogenously divided into six groups having four animals each. These were weighed weekly throughout the experiment for necessary correction in dose if any. All groups except control were drenched amla extracts at the dose rate of 50 $\mu g.kg^{-1}$ B.W. daily for 45 consecutive days. Dose of amla extract was extrapolated from an average human dose of 4 g (1 tsf) for adult 70 kg. Three groups of rats were exposed to quinalphos at the dose rate of 2 $mg.kg^{-1}.day^{-1}$ ($1/100^{th}$ of oral LD_{50}) orally from day 16 onwards up to 45^{th} day. At the end of treatment period the animals were sacrificed and tissue samples *viz*. liver, kidney and brain were collected in a separate vial for each animal. Half of the portions were transferred to a vial containing 10% neutral buffer formalin for tissue fixation and further hiastopathological examination. Remaining tissues were homogenized in phosphate buffer saline (PBS) for analysis of various antioxidant parameters.

Technical Programme

Group	Study conducted	Treatment
I	Control	-
II	Quinalphos	2 $mg.kg^{-1}.day^{-1}$, orally from day 16-45
III	Amla fruit extact (Sugar base)	50 $\mu g.kg^{-1}.day^{-1}$, orally for 45 days
IV	Amla fruit extact (Sugar base) + Quinalphos	50 $\mu g.kg^{-1}.day^{-1}$, orally for 45 days 2 $mg.kg^{-1}.day^{-1}$, orally from day 16-45
V	Amla fruit extact (honey base)	50 $\mu g.kg^{-1}.day^{-1}$, orally for 45 days
VI	Amla fruit extact (honey base) + Quinalphos	50 $\mu g.kg^{-1}.day^{-1}$, orally for 45 days 2 $mg.kg^{-1}.day^{-1}$, orally from day 16-45

Then 10% of the tissue homogenates were prepared in PBS (pH- 7.4) for the estimation of catalase (CAT)[22], superoxide dismutase (SOD)[23] and glutathione peroxidase (GPx) (Hafeman et al., 1974) activities. Lipid peroxidation (LPO), i.e., the malondialdehyde (MDA) level, were analysed in 33% packed erythrocyte prepared in PBS buffer according to the method described by Shafiq-Ur-Rehman[24].

The tissues were processed by acetone benzene method to get paraffin blocks. 5 µm thick paraffin sections were obtained on glass slides. The sections were stained with Haematoxylin and Eosin for histopathological evaluation[25].

Results and Discussion

Effect of amla in sugar and honey bases and their individual co-administration with Quinalphos (2 $mg.kg^{-1}$ B. W.) on activity of various antioxidants eg. lipid peroxidation (LPO) superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase and in rats are presented in table 1, 2, 3 and 4 respectively.

Lipid peroxidation is oxidation of polyunsaturated fatty acids generating hydroxyl radicals and damaging cell membrane. Determination of extent of lipid peroxidation indirectly reflects the degree to which cell lipid membranes are attacked by free radicals[26].

There was significant increase in the lipid peroxidation in the liver of rats after quinalphos treatment (4.8±0.04 nmol MDA/gm) than control (2.92±0.15 nmol MDA/gm) similar increment in malondialdehyde were reported in rats as reported by previous workers[9,27,28]. The increase in lipid peroxidation might be due to production of free radicals after quinalphos administration. Co-treatment of both amla in sugar base (9.60±0.29 nmol MDA/gm) as well as honey base (4.38±0.40 nmol MDA/gm) were unable to protect from pesticide induced increased lipid peroxidation in liver tissues. The results of the present study were contrary to that were observed by Shivananjappa et al.[16] where they found decrease in the levels of lipid hydroperoxide and reactive oxygen species (ROS) using a hepatocyte cell line (HepG2) exposed to *E. officinalis* extract and also by Reddy et al 2009, 2010 in alcohol intoxicated rats. Similar findings were also reported in liver and kidney of mice induced with ochratoxin toxicity[18]. Amla have been shown to reduce thiobarbituric acid reactive substances during renal dysfunction produced oxidative stress[29]. Similar to liver tissues there was significant increase in levels in kidneys of pesticide administered rats (10.9±0.81 nmol MDA/gm) as compared to sham control (3.7±0.07 nmol MDA/gm). Similar to liver supplementation of amla in sugar base (11.2±1.91 nmol MDA/gm) as well as honey base (13.9±0.07 nmol MDA/gm) were unable ameliorate augmented lipid peroxidation in kidneys after insecticide exposure. There was also significant enhancement in lipid peroxidation following pesticide exposure (44.4±1.09 nmol MDA/gm) in the brain of animals. Amla in either

Table 1: Effect of Amla (in sugar and honey base) on lipid peroxidation and its antioxidant role in quinalphos (2 mg.kg^{-1} B. W.) induced oxidative stress in rats.

Sr.No.	Organ / Group	Liver	Kidney	Brain
I.	**Sham Control**	2.92±0.15^{b}	3.7±0.07^{a}	5.3±0.15^{a}
II.	**Insecticide Control**	4.8±0.04^{c}	10.9±0.81^{b}	44.4±1.09^{cd}
III.	**Amla (Sugar base)**	0.51±0.22^{a}	5.20±0.07^{a}	8.8±0.74^{a}
IV.	**Amla (Sugar base) + Quinalphos**	9.60±0.29^{d}	11.2±1.91^{b}	77.7±1.76^{e}
V.	**Amla (honey base)**	2.28±0.07^{b}	4.12±0.33^{a}	9.6±1.09^{a}
VI.	**Amla (honey base) + Quinalphos**	4.38±0.40^{c}	13.9±0.07^{c}	45.8±1.43^{d}

Values given are expressed as nmol MDA formed/ gm tissue.
Values lacking a common superscript in a given column differs significantly from each other ($p<0.05$).

Table 2: Effect of Amla (in sugar and honey base) on superoxide dismutase activity and its antioxidant role in quinalphos-induced (2 mg.kg^{-1}) oxidative stress in rats.

Sr.No.	Organ / Group	Liver	Kidney	Brain
I.	**Sham Control**	1.4±0.12^{b}	1.2±0.05^{a}	1.2±0.07^{a}
II.	**Insecticide Control**	0.84±0.11^{a}	1.27±0.25^{a}	0.85±0.09^{a}
III.	**Amla (Sugar base)**	1.31±0.23^{ab}	0.98±0.06^{a}	2.34±0.19^{a}
IV.	**Amla (Sugar base) + Quinalphos**	1.28±0.11^{ab}	1.15±0.15^{a}	2.22±0.22^{a}
V.	**Amla (honey base)**	1.16±0.13^{ab}	1.14±0.07^{a}	2.65±0.31^{a}
VI.	**Amla (honey base) + Quinalphos**	1.17±0.10^{ab}	1.32±0.19^{a}	1.35±0.01^{a}

Values given are expressed as amount of enzyme causing 50% inhibition of auto-oxidation of pyragallol and represent the Mean ± SE of values obtained from four animals.
Values lacking a common superscript in a given column differs significantly from each other ($p<0.05$).

Table 3: Effect of Amla (in sugar and honey base) on glutathione peroxidase activity and its antioxidant role in quinalphos (2 mg/kg. B. W.) induced oxidative stress in rats.

Sr.No.	Organ / Group	Liver	Kidney	Brain
I.	**Sham Control**	9.9±0.16[a]	14.7±1.82[a]	3.4±0.09[a]
II.	**Insecticide Control**	31.18±1.38[c]	34.0±0.92[c]	6.20±0.04[bc]
III.	**Amla (Sugar base)**	23.7±3.25[b]	27.2±1.77[b]	13.4±1.29[d]
IV.	**Amla (Sugar base) + Quinalphos**	37.7±1.76[d]	26.73±1.12[b]	4.44±0.16[ab]
V.	**Amla (honey base)**	38.9±0.85[cd]	25.6±1.85[b]	8.20±1.19[c]
VI.	**Amla (honey base) + Quinalphos**	34.5±1.18[cd]	25.9±1.09[b]	3.88±0.02[ab]

Values given are expressed as EU/g of protein and represent the Mean ± SE of values obtained from three animals. Values lacking a common superscript in a given column differs significantly from each other ($p<0.05$).

Table 4: Effect of Amla sugar and honey and their individual co-administration with Quinalphos (2 $mg.kg^{-1}$ B. W.) on catalase activity in rats.

	Group	Liver	Kidney	Brain
I.	**Sham Control**	10.9±0.5[a]	12.3±0.6[a]	1.2±0.01[a]
II.	**Insecticide Control**	9.45±0.71[a]	10.9±1.79[a]	6.75±0.46[a]
III.	**Amla (Sugar base)**	13.4±3.01[a]	9.41±0.89[a]	1.82±0.05[a]
IV.	**Amla (Sugar base) + Quinalphos**	11.2±0.53[a]	11.7±0.67[a]	5.73±1.11[a]
V.	**Amla (honey base)**	11.2±1.89[a]	12.6±0.89[a]	4.54±1.39[a]
VI.	**Amla (honey base) + Quinalphos**	13.2±1.14[a]	12.4±0.45[a]	7.88±0.04[a]

Values given are expressed as µmole H_2O_2 decomposed /min/mg protein and represent the Mean ± SE of values obtained from three animals. Values lacking a common superscript in a given column differs significantly from each other ($p<0.05$).

bases (amla in sugar=77.7±1.76 nmol MDA/gm, amla in honey =45.8±1.43 nmol MDA/gm) could not be able to protect brain from peroxidative damage when administered along with quinalphos. Results in the current investigation were different from Bhattacharya et al[30] where they reduction in the peroxidative damage of brain after administration of active tannoids in rats.

Superoxide dismutase is copper-zinc containing enzyme that dismutases superoxide ions produced after oxidative insult with resulting in formation of H_2O_2. The H_2O_2 produced is then either reduced in a reaction catalyzed by GPx or decomposed by catalase[26].

There was significant decrease in the activity of SOD in the liver of quinalphos administered rats (0.84±0.11 EU/ gm) as compared to control (1.4±0.12 EU/ gm). This might be due to inactivation of the enzyme by quinalphos induced lipid peroxidation or its inhibitory effect on important cofactors (copper and zinc) necessary for activity of SOD[14,20]. The activity of the enzyme was restored after co-administration of amla in sugar base (1.28±0.11 EU/ gm) and honey base (1.28±0.11 EU/ gm). The results in the present investigation corroborates with previous studies where increment in the SOD levels were observed after co-administration with amla with cadmium[14]. The *in vitro* data also suggest increased activity of the enzyme in the liver cells upon incubation with *E. officinalis* extract[16]. Sharma et al[31] also demonstrated that amla extract produced significant improvement in the SOD activity during arsenic induced hepatopathy. The activity of SOD was decreased in pesticide treated rat brains whereas there was increasing trend in rest of the groups but the difference was not statistically significant in comparision to control group. Previous studies also suggests increasing trend in frontal cortical and striatal SOD levels in the brain of rats after administration with active tannoids of amla[30]. The enzyme levels remained unaltered in kidney tissue of the treated animal as compared to sham control.

Glutathione peroxidase is a glycoprotein having selenium present in mammalian erythrocytes. GPx reduces lipid hydroperoxides to the corresponding alcohols and free hydrogen peroxide to water[26].

There were significant alterations in the GPx activities of the enzyme in treatment groups. The levels rose significantly in the liver of quinalphos treated rats (31.18±1.38 EU/g of protein) as compared to sham control (9.9±0.16 EU/g of protein). The increase in the blood levels of the enzyme after quinalphos administration may act as adaptive response to reduce H_2O_2 formed through GSH dependent mechanism as seen by declined SOD levels along with augmented lipid peroxidation in this group. Similar results were also reported in the liver and testicular tissues of rats following quinalphos dosing[9,10]. There was also further increase in the levels in groups where amla in honey base (34.5±1.18) and sugar base (37.7±1.76) were co-administered along with the pesticide. Similar findings were also reported by[16] following amla treatment in Hep-G2 cells and by Sultana et al[32] after pretreatment with *E. officinalis* in carbon tetrachloride intoxicated rats. Similar increasing trend was also seen in kidney tissues of treated rats compared to control one in the present study. The only difference in this tissue was that the enhancement was significantly less in groups where amla was co-administered along with quinalphos as compared to insecticide group (34.0±0.92) indicating some protective effect by amla extracts in kidney. There was also increment in the activity of the enzyme in brain tissues of treated rats except in groups given amla in sugar base (4.44±0.16) and honey base (3.88±0.02) where it was non-significant as compare to sham control (3.4±0.09). Bhattacharya et al[30] demonstrated significant increase in brain GPx activity following active amla tannoids exposure in rats.

Catalase protects cells from H_2O_2 produced in the cell during oxidative stress. It is of equal or greater importance than GPx in protecting red blood cells against H_2O_2 as reported by Daundkar and Rampal[26]. There was no any significant alterations produce after administration of amla through either bases or their individual co-administration with quinalphos in liver, kidney and brain of rats. This might be due to upper hand in reduction of H_2O_2 by GPx catalysed pathway following dismutation of superoxide radicals than decomposition by catalase. Although there was increment in the catalase activity in the brain of treatment groups except of amla sugar, it was not significant with sham control might be because of more standard error values.

Results of histopathological study of major organs *viz.* liver, kidney also in accordance with blood antioxidant status demonstrating amla in either bases was unable to provide full protection against quinalphos induced toxicity in rats.

Conclusion

In the present investigation, sub-acute quinalphos administration at the selected dose rate induced oxidative damage through generation of reactive oxygen species and thus modifying the biological activities of antioxidant enzymes, like SOD, GPx and LPO in the liver, kidney and brain in rats. Oral supplementation of amla extract (in sugar as well as honey bases) provided partially amelioration against altered antioxidant status. Histopathological study also supported the results of blood antioxidant status in treated rats. Further study is required to investigate the exact mechanism happening at molecular levels by amla extracts against quinalphos induced toxicosis using advanced biotechnological tools and techniques.

Conflict of Interest

None Declared

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