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Flavonoid Treatment for Mustard Agents' Toxicity

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1. Introduction

The weapons of mass destruction, chemical, biological and nuclear warfare are the most brutal created by the humans. They kill and incapacitate not only the armed forces but also the innocent public, without any mercy. The Chemical Weapons Convention prohibits the production, storage and use of toxic chemicals during warfare. In fact, the use of "Any chemical which through its chemical action on life processes can cause death, temporary incapacitation or permanent harm to humans and animals" as a method of warfare is discouraged by the Chemical Weapons Convention and many of such toxic chemicals are listed in its three Schedules for verification purpose (OPCW). The chemical warfare agents are extremely toxic chemicals. They act in very small quantities and very rapidly, and death may occur in minutes, like the nerve agents and the blood agents (Somani, 1992). Some of them like the blistering agents, though may not cause immediate lethality, but are highly incapacitating (Dacre & Goldman, 1996). The nerve agents are organophosphorous compounds that include tabun, sarin, soman and Vx. They inhibit acetylcholinesterase enzyme resulting in the accumulation of acetylcholine leading to muscarinic and nicotinic receptor stimulation (Bajgar, 2004). The blood agents include the cyanides. They inhibit cytochrome oxidase enzyme leading to cellular hypoxia (Way, 1984). Though the nerve agents and the blood agents are immediately lethal, specific antidotes are available for use in the field as First Aid Kit (Vijayaraghavan et al, 2011). For nerve agent poisoning the recommended antidotes are atropine sulphate and pralidoxime chloride that are administered by autoinjectors (Friedl, 1989; Vijayaraghavan et al, 2007). For cyanide poisoning the recommended antidotes are amyl nitrite inhalation, and sodium nitrite and sodium thiosulphate injection (Chen & Rose, 1952; Bhattacharya & Vijayaraghavan, 2002).

The blistering agents are the sulphur mustard (SM) and the nitrogen mustards (NM). They cause severe toxicity with delayed clinical symptom. In the biological system they undergo an intramolecular cyclisation and produce highly reactive electrophiles that have strong affinity for a variety of macromolecules. They are extremely toxic to rapidly dividing cells, resulting in multiorgan failure (Papirmeister et al, 1991). Unlike the nerve agents and the blood agents no specific treatment is available for the mustard agents. A wide variety of molecules are being evaluated as antidote for mustard agent toxicity. Antidote against mustard agents require few major characteristics: (a) molecules should be strong neucleophiles because mustard agents are highly reactive electrophiles, (b) molecules may

be effective only prophylactically and may be difficult or less effective after exposure due to the cascade of events following mustard agent exposure, (c) molecules should have delayed clearance and low protein binding as the chain reactions following mustard agent poisoning is long-lasting and fast, and (d) as free drug concentration in the blood is required for a long time, molecule should be highly safe and non toxic even at higher doses. At present decontamination by physical adsorption and by chemical degradation is the main methodology adopted for reducing the toxicity of the mustard agents.

The effects of SM are more prominent on the eyes, skin and lung. Toxic effects of the mustard agents (SM and NM) also occur in bone marrow, the central nervous system and the gastrointestinal tract. Oxidative stress is one of the many causes of the mustard agents' toxicity and antidotes directed against them have proven to be beneficial. Antioxidants can enhance survival time, protect liver and lung from oxidative damage and also reduce accumulation of purine metabolites in SM toxicity (Kumar et al, 2001; Vijayaraghavan et al, 2009). Among the various types of nucleophiles, the flavonoids appear to be very promising. They are polyphenolic compounds present in several plants, inhibit lipid peroxidation and also act as a free radical scavengers. The flavonoids are in current choice of drug for various disease conditions like cardiovascular disease, diabetic nephropathy, hypertension, colorectal cancer and aging, as an adjunct to other drugs (Narayana et al, 2001).

2. Blister inducing chemical warfare agents

The blistering inducing chemical warfare agents are also known as vesicants and are of three types namely the mustards, arsenicals and phosgene oxime. The mustard agents belong to two distinct classes, the sulphur mustard (halogenated thioether) and the nitrogen mustards (halogenated tertiary amines). Chemically, Sulphur mustard is bis (2-chloroethyl) sulphide and generally known as mustard gas (Figure 1). Sulphur mustard was first synthesised in 1822 and has the highest military significance since its first use in 1915 in World War I. It is one of the frequently used chemical warfare agents and the latest use was in the Iran-Iraq war (Kehe & Szinicz, 2005). It is considered as the king of the chemical warfare agents. As a chemical warfare agent, three nitrogen mustards are important (HN-1, HN-2 and HN-3). The nitrogen mustards were synthesised in the 1930s but were not produced in large amounts for warfare. Among the nitrogen mustards HN-3, which is chemically, tris (2-chloroethyl) amine is more toxic than others. HN-2, also known as mechlorethamine is used as a cancer chemotherapeutic agent and has remained the standard drug for many years. Lewisite was synthesised in 1918 for military purpose and no report is available of its use in battlefield.

In the pure form SM is colorless and odorless liquid. With impurities it has a characteristic smell similar to mustard or garlic. It has low volatility. It is soluble in organic solvents readily but very less soluble in water (0.8 $g.L^{-1}$). In pure state the nitrogen mustards are also colorless liquids. They are less volatile, less soluble and more resistant to oxidising agents than SM, but are less stable on storage. Pure lewisite is also colorless liquid with metallic odor and its solubility in water is similar to SM. Lewisite is relatively unstable. Thus, nitrogen mustards and lewisite lack the basic requirement, i.e. storage stability of a chemical warfare agent (Ganesan et al, 2010).

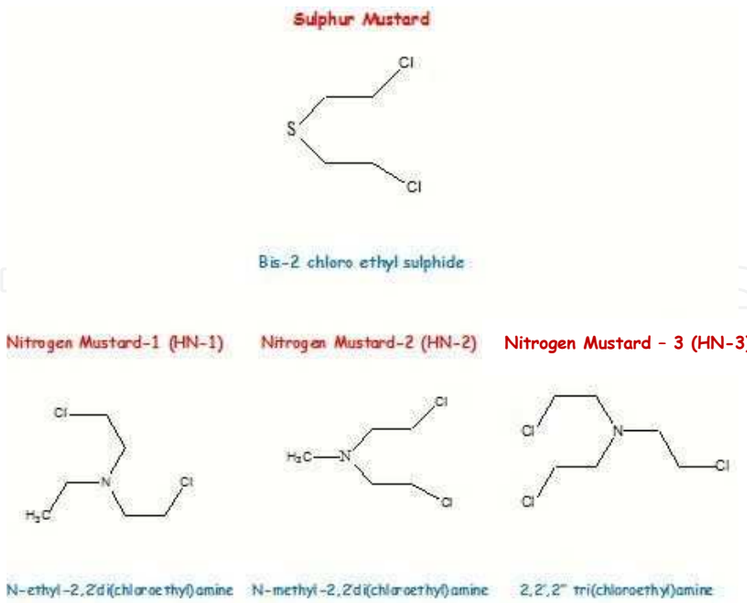

Fig. 1. Structure of sulphur mustard and nitrogen mustards.

The mustard agents are radiomimetic and are extremely toxic to dividing cells. They are highly lipophilic and readily penetrate the skin, and even cotton fabric, latex and rubber. Sulphur mustard cyclises inside the body to a highly reactive sulphonium ion. The most important target for mustard agent is DNA. Mustard agents alkylate the purine bases of DNA and damage them (Papirmeister et al, 1991). They irreversibly alkylate DNA, RNA and protein, causing cell death. At the cellular level mustard agents cause cytostasis and mutation. Death will occur if 4 to 5 grams of SM falls on the bare skin. HN-3 is as toxic as SM. The respiratory lethal dose of SM is estimated to be 1500 $mg.min.m^{-3}$.

3. Toxic effects of sulphur mustard

In the form of vapour or aerosol, SM attacks the eyes, lungs and skin. If the concentration and the duration of exposure are larger than systemic effects will occur. In small laboratory animals SM is highly lethal (Vijayaraghavan et al, 2005). The characteristic of SM poisoning is the delayed appearance of toxic effects. The victim knows about the exposure only after a lapse of 3 to 6 hours, though the damage begins within 1 to 2 minutes after contact. The eye is more vulnerable and sensitive to SM vapour. Exposure to a concentration of 0.001 $mg.L^{-1}$ for one hour can cause conjunctivitis. This concentration is not easily recognisable by odour. The effect of mustard gas on the eye can be classified as mild, moderate and severe. If the exposure is mild there will be itching, lacrimation and a sensation of a dust particle in the eye. This is followed by a burning sensation and photophobia. There will be hyperemia of the conjunctivae. Edema of the lids may also be present. In moderate exposure there will be complete closure of eyes, because of spasm and swelling of the lids leading to blurring of the vision. Edema of the conjunctiva, mild iritis and edema of the cornea will be present. Blepharospasm and edema of the lid will be so severe that the patient cannot open the eyes. If liquid SM directly falls in the eye, the cornea and iris may be affected very severely. In this type the latent period will be very short. Severe edema of the lids, and marked hyperemia

and edema of the conjunctiva will be present. The epithelium and stroma of the cornea will be damaged, and edema will develop later. Iritis and mucoserous discharge will also be present. In severe cases, blindness may occur (Safarinejad, 2001). The development of SM induced ocular lesions in rabbits is similar to the lesions described in human casualties (Kadar *et al.,* 2001). The lesions produced by HN-3 on the eye will be more severe and will appear in a shorter time than SM.

The effects of SM on the skin resemble those of burn injuries. It depends upon the weather condition and the degree of exposure. In a warm humid climate, the effect will be more severe. Similarly, the lesions will be more severe in damp and warm parts of the body like finger folds, groin and axilla. Normally, the symptoms appear after a latent period of 6 - 12 hours. Sulphur mustard is a potent cutaneous vesicant. Sulphur mustard penetrates rapidly through the skin, causing prolonged injuries and leading to severe incapacitation (Kadar et al, 2000). The proliferating basal cells in the skin are metabolically very active and are more sensitive to SM (Ray et al., 2000). Sulphur mustard produces blisters with a severe inflammatory reaction in skin of exposed individuals (Casbohm et al, 2004). The first symptom will be continuous itching at the site of contact. Then erythema will appear gradually followed by vesication. This is due to the necrosis of cells in the lower layers of the epidermis, and exudation of tissue fluids into the spaces so formed. In animal models frank blisters are not seen, but microblisters appear. The blister may rupture in due course with the possibility of infection. The healing will take place with a small scar, except in very severe or infected burns. Sulphur mustard burns usually are followed by a persistent brown pigmentation except at the site of actual vesication, where there may be a temporary depigmentation. The effect of SM on the respiratory tract also depends upon the degree of exposure. If the exposure is mild, swelling and erythema will be present, in the nose, larynx and trachea. This will be followed by sloughing and fibrous exudation. The laryngeal edema and necrosis may lead to respiratory obstruction. If the exposure is severe, bronchioles and alveoli may be damaged. In more severe cases the pulmonary parenchyma shows congestion, mild patchy edema, emphysema and focal atelectasis. There is a danger of bacterial infection of the lungs which will result in bronchopneumonia. The latter may be responsible for the death in humans following SM exposure. SM vapours induces sensory irritation in mice during exposure. The respiratory frequency decreased on subsequent days of exposure depending upon the exposure concentration, and the breathing pattern was characteristic of bronchial obstruction (Vijayaraghavan, 1997). Ingestion of food or water contaminated with liquid SM, may produce nausea, vomiting, pain and diarrhoea. Even exposure to the skin alone can cause malaise, anuria, vomiting and cardiac abnormalities. When the amount approaches lethal dose, injury to the haematopoietic tissues may occur. SM in small animals will cause systemic toxicity distal to the site of exposure. Probably, SM is the only chemical showing more toxicity through percutaneous route compared to subcutaneous route and oral route in animal models (Vijayaraghavan et al, 2005).

4. Treatment of sulphur mustard toxicity

The treatment for SM lesions is similar to that of burn injuries. There is no specific antidote for SM intoxication. A variety of compounds have been evaluated as antidotes using *in vitro* and *in vivo* models but none of them are recommended as standard therapy (Vijayaraghavan

et al, 2009). In the case of eye contamination the eye should be washed with water. If the eye lids are sticky then sterile petroleum jelly can be applied. Photophobia and blepharospasm can be relieved by instilling one drop of atropine sulphate solution (1 %) 3 - 4 times a day. Topically applied steroid treatment and anti-inflammatory drugs are potential therapies, and this can be supplemented with an antibacterial agent like ciprofloxacin eye drops.

Sulphur mustard is a lipophillic compound and rapidly penetrates the skin. Hence, upon contact the skin should be decontaminated immediately and completely. Decontamination, five minutes after SM contact may not be beneficial as sufficient quantity would have been absorbed by the skin (Vijayaraghavan et al, 2002). A number of proprietary formulations are available that can efficiently decontaminate SM. The personal decontamination kit (PDK) is a multipurpose physical and chemical decontamination of toxic chemicals and microbes (Chilcott et al, 2001; Vijayaraghavan et al, 2011). It consists of PDK-1 and PDK-2 that are Fuller's earth, PDK(CC2) suspension, which is chemically N,N'-dichloro-bis(2,4,6) trichlorophenyl urea, a chemical decontaminant and RDP wipe which is surfactant soaked napkin for removal of solid particles. The sequence of decontamination is PDK-1 (if contact is less) or PDK-2 (if contact is more), followed by PDK(CC2) and then RDP wipe (Figure 2). Fuller's earth will physically remove the chemical and biological particles due to its adsorptive effect and PDK(CC2) will oxidise them. The surfactant soaked napkin will wipe off all the liquid and solid matter. In humans the treatment of skin lesions are mostly symptomatic. Several studies showed that topical treatment with iodine or povidone-iodine ointment significantly reduced the skin lesions induced by SM and that the ointment should be applied immediately after SM exposure (Brodsky *et al,* 2006; Wormser *et al,* 1997). The combination of anti-inflammatory agents and iodine increased the counter-irritating activity. Povidone-iodine preparation combined with an anti-inflammatory agent is better for the skin lesions induced by SM at relatively long intervals between exposure and treatment (Wormser *et al.,* 2004). Pruritus, is a common problem among SM exposed veterans. A number of treatments like antihistamines, local anesthetics, and corticosteroids are prescribed in order to control pruritus in Iranian patients (Shohrati *et al.,* 2007). Once the blister breaks, topical antibiotics like framycetin or mupirocin skin ointment can be applied.

Fig. 2. Decontamination of chemicals with fuller's earth (PDK-1 or PDK-2) followed by PDK (CC-2) and RDP wipe.

Pharyngitis can be relieved by taking saline gargle. Intratracheal injection of SM in guinea pigs induced airway epithelial damage and corticosteroids like betamethasone showed

significant effect on airway epithelium (Calvet *et al.*, 1996). Chronic bronchitis is the most frequent late respiratory disease among Iranians exposed to SM during the Iran-Iraq war. Oral and intravenous corticosteroid therapy was investigated in improving the lung function in SM induced chronic bronchitis patients (Ghanei *et al.*, 2005). Doxycycline may be a promising therapeutic agent for SM (Guignabert *et al.*, 2005). For the lung lesions and systemic toxicity of SM, paracetamol-ibuprofen combination for controlling pain and fever, beclomethasone inhaler as an anti-inflammatory corticosteroid, codeine phosphate or sulphate as a cough suppressant, doxycycline as an antibacterial agent, and N-acetyl cysteine, as a mucolytic and glutathione sparing drug are recommended (Lindsay *et al.*, 2008, Bobb et al, 2005; Ghanei et al, 2007, Vijayaraghavan et al, 2011). If haemoconcentration and shock are present either whole blood transfusion or transfusion of plasma expanders should be considered.

5. Classification of flavonoids

The flavonoids are secondary metabolites of plants and once they were considered to be waste products of plant metabolism. Flavonoids are the derivatives of benzopyrone and are widespread in photosynthesising plants. More than 4000 different flavonoids are known. The flavonoids are polyphenolic compounds with the basic skeleton of a phenyl benzopyrone (Flavone) ring. Various substitutions, mainly phenolic OH groups, take place in 3, 5, 7, 3′ and 4′ positions of the flavone nucleus. Flavonoids are generally found in nature as glycosides with sugar moiety attached to the 3 or 7 position. Depending upon the position of OH groups and of the pyrone nucleus, flavonoids are classified into various categories, viz., isoflavones, flavonol, flavone, flavonones, flavans and chalcones (Bohm, 1998). The isoflavones have their phenyl group (B ring) attached to the 3 position of the benzopyrone nucleus. Isoflavones have been shown to possess potential estrogenic actions. Flavonols have a OH group attached to the 3 position of the ring. Quercetin, gossypin and rutin are important members of this group. Flavanones result from the saturation of the double bond in the 2-3 position of the benzopyrone nucleus. Reduction of the carbonyl group of the pyrone ring and subsequent saturation of this ring gives flavans. Catechin and epicatechin are typical examples of this group. At higher pH pyrone ring in 1-2 position of flavonone opens resulting in chalcones. Hesperidin methyl chalcone is the best example of this class.

6. Flavonoids in the treatment of diseases

The use of plants in many forms has been recorded in the history. Knowledge of the usefulness of certain plants for medicinal purposes has been passed from generation to generation by word of mouth. Flavonoids were identified in search for physiologically active natural products. They have been shown to possess remarkable physiological activity in mammalian system. The mechanism of action is not known in most cases, but with the growing sophistication of analytical tools particularly X-ray analysis of flavonoid enzyme interaction, continues to reveal more details. It is difficult to predict any underlying structural feature of flavonoids which lead to specific biological function. No structure function relationship has emerged that why certain flavonoids can function as cytotoxic in nature while others function as cytoprotective agent.

Reports suggest that flavonoids are known to have hepatoprotective effect from ancient time onwards. Wagner (1986) described studies of plants as a source of liver protecting drugs from Indian folk medicine, viz., *Butea monosperma*, *Eclipta alba* and *Wedelia calandulae*. Hydnocarpin has been shown to be active against several human tumors and Ehrlich ascites tumor in mouse, and also exhibit anti-inflammatory and hypolipidemic activity. Studies on human immune response and inflammatory reactions showed the involvement of nitric oxide as a major participant. It has now been demonstrated that natural and synthetic flavonoids have significant inhibitory effect on the production of nitric oxide (Lee & Kim, 2010). There are reports that kaempferol and quercetin can efficiently suppress oxygen induced cytotoxicity. Quercetin derivative, rutin has been found to be effective in reducing toxic effects of iron overloading in experimental animals (Gao et al, 2006). The flavonoids are thought to form complex with iron atoms thus preventing them from catalysing the conversion of superoxide ions to harmful hydroxyl radicals. Flavonoids can exert their antioxidant activity by various mechanisms, including scavenging free radicals, which initiate lipid peroxidation, by binding metal ions, and by inhibiting enzymes responsible for free radical generation (Haleagrahara, et al, 2011).

Among the various actions of flavonoids, the anti inflammatory action has been extensively studied by several workers (Gabor, 1986; Parmar & Ghosh, 1978). Flavonoids were found to exert a beneficial effect in rheumatoid arthritis and also in gingival inflammatory conditions (Carvel & Halperin, 1961). Flavonoids are effective in acute as well as chronic inflammatory conditions (Lee et al, 2004; Martinez et al, 1997; Agarwal, 1982). Flavonone glycosides like naringin and hesperidins when administered intraperitoneally showed good response in both acute and chronic inflammatory models (Perriera et al, 2007). 5,7-di hydroxy 7-methoxy flavone and Wogonin (5,7-dihydroxy 8-methoxy flavone) were reported to have moderate inhibitory activity of prostaglandins by inhibiting COX-2 enzyme (Daott et al, 2003). Several flavonoids were investigated on various *in vitro* assays for lipoxygenase inhibitory activity. A significant anti-inflammatory and anti-arthritic activity of silymarin, a mixture of flavano lignans, was reported in animal model of inflammation by inhibition of 5- Lipoxygenase (Gupta et al, 2000).

Morin and fisetin were shown to inhibit the oxidative modification of low density lipoproteins. Flavonoids could improve the capillary resistance in scorbutic animals more effectively than pure vitamin C (Rusznyak & Szent Gyorgi, 1936). Further studies conducted in rats revealed an unequivocal effect of flavonoids in maintaining capillary integrity in these species (Benko et al, 1970). Hesperidine methyl chalcone increased the capillary resistance of small intestine, large intestine and kidney of guinea pigs kept on a scorbutic diet (Gabor et al, 1968). Reports suggest that flavonoids also have vasodilatory activity. Hesperidine and catechin could develop collateral circulation after left coronary occlusion (Brkic & Laszt, 1972). Perflavone has been suggested to be useful in the treatment of angina, atherosclerosis and myocardial infarction (Wagner, 1977). The cataract observed in diabetic and galactosemic conditions can be treated by flavonoids as they inhibit the enzyme aldose reductase in the lens (Varma et al, 1977; Parmar and Ghosh, 1979). Gastric anti-ulcer effect of various flavonoids has been extensively studied by Parmar (1977). The antihistaminic effect, histidine decarboxylase inhibition and mast cell stabilising effect of flavonoids may play an important role in the anti ulcer and anti secretory property of these compounds (Reimann et al, 1977; Fewtrell & Gomperts, 1977; Ramaswamy et al, 1979).

Cisplatin, a widely used anticancer drug causes undesirable side effects such as nephrotoxicity and hepatotoxicity. The protective effect of silymarin against cisplatin induced hepatotoxicity was evaluated in rats. Cisplatin caused an increase in serum alanine aminotransferase and aspartate aminotransferase, elevation of malondialdehyde and nitric oxide in liver tissues as well as decrease in reduced glutathione and the activities of antioxidant enzymes, including superoxide dismutase and glutathione peroxidase in liver tissues. Silymarin significantly reduced the hepatotoxicity induced by cisplatin (Mansour et al, 2006). Doxorubicin is a widely used anthracycline anticancer agent. Doxorubicin causes cardiotoxicity which is the major limitation of its clinical use. 7-monohydroxyethyl-rutoside protected the negative inotropic action of doxorubicin *in vitro* in electrically paced mouse left atrium model and *in vivo* in the ST-interval lengthening in the ECG. This protection did not affect the antitumor effect (Bast et al, 2007). Natural antioxidants like catechin are now known to detoxify toxic metabolites of xenobiotics. The modulatory and protective effect of catechin on tamoxifen, an anticancer drug induced disruption of glutathione metabolism and antioxidant enzyme was evaluated. Tamoxifen treatment resulted in a significant increase in the lipid peroxidation, hydrogen peroxide generation and protein carbonyl content in liver and kidney, and catechin treatment significantly protected them. The decrease in reduced glutathione content was also corrected by catechin. Catechin pre-treatment showed restoration in the level of cytochrome P450 content and in the activities of glutathione metabolizing enzymes, viz., glutathione-S-transferase, glutathione reductase and glutathione peroxidase, and other antioxidant enzymes such as, glucose-6-phosphate dehydrogenase, catalase and superoxide dismutase in both liver and kidney. There is good evidence that catechin supplementation can reduce tamoxifen induced toxicity as prophylactic and post treatment (Parvez et al, 2006).

Interestingly, several flavonoids and flavonoid derivatives have also been found to possess anti-mitotic behaviour which suggests possible use as anti-cancer drugs. These flavonoids exhibited inhibition of the conversion of tubulin into microtubules (Panda & Srivastav, 2007). The isoflavone genistein and orobol have been shown to induce mammalian topoisomerase II dependent DNA cleavage with activities comparable to those of known antitumor agents (Bandele & Osheroff, 2008). Quercetin and fisetin had activities similar to that of drug adriamycin.

Flavonoids have been found to be active against a variety of viruses. Among the animal viruses are polio virus, herpes simplex, and pseudorabies virus (Ogra et al, 1985). One of the standard approaches for determining the potential medicinal use of flavonoids is to screen them for activity against a range of bacteria and pathogenic fungi (Sasaki et al, 2011). The range of organisms against which certain flavonoids are active is quite impressive and in many cases not trivial. Anti fungal activity of flavonoids has also been demonstrated clearly (Parvez et al, 2009). Many flavonoids both natural and synthetic have been tested as potential medicinal agents against human disease including malaria (Montip et al, 2011) and HIV (Zhao et al, 2010).

Though, the plants are source of abundant flavonoids their usefulness could not be established. The activity of the flavonoids will depend to the large extent on their pharmacokinetics. It is a widespread belief that some plant preparations have almost magical properties. It is almost certain that many do contain potentially useful compounds but, the issues of effective dose, duration of effect, transport to site of action, life time of the

effective agent and other factors must be considered before the claim can be said to have any pharmacological validity.

7. Flavonoids as promising cytoprotectants for sulphur mustard and nitrogen mustard induced oxidative stress

Sulphur mustard is a bifunctional alkylating agent and readily reacts with a variety of macromolecules including nucleic acids, proteins and lipids, as well as small molecular weight metabolites such as glutathione. By alkylating subcellular components, SM disrupts metabolism and can lead to oxidative stress and multi-organ failure. Increased formation of reactive oxygen species, the presence of lipid peroxidation products and oxidised proteins, and increase in antioxidant enzymes such as superoxide dismutase, catalase, and glutathione-S-transferase are the effects of SM and other mustard agents. Antioxidants will be very useful as prophylactic as well as post treatment for SM and other mustard agent toxicity (Laskin et al, 2010). Antioxidants could enhance survival time, protect liver and lung from oxidative damage and reduce accumulation of purine metabolites in blood following SM intoxication. Oxidative stress and damage to macromolecules in the human body is considered as one of the major consequences of SM toxicity. Intradermal administration of SM in rats resulted in increased thiobarbituric-acid-reactive substances, plasma protein carbonyls and ferric-reducing antioxidant power. Subcutaneous administration of melatonin protected these effects (Pohanka et al, 2011a). Flavonoids inhibit lipid peroxidation and also act as free radical scavengers. Sulphur mustard is a potent cytotoxic compound and many studies suggest that flavonoids are strong cytoprotectants (Figure 3).

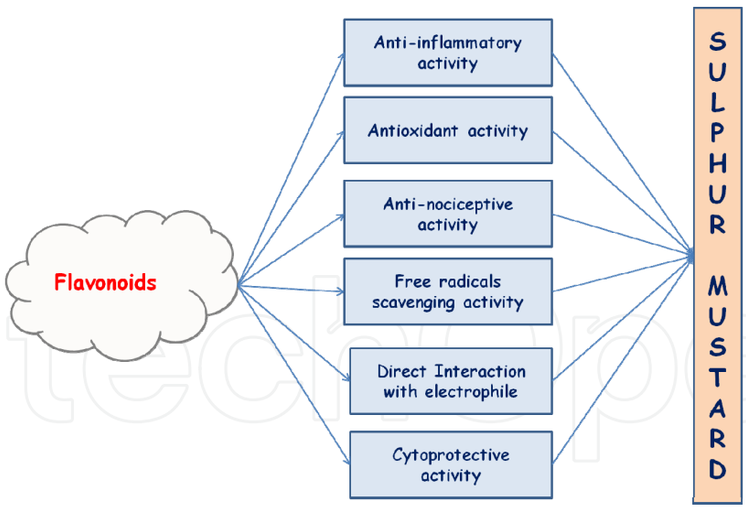

Fig. 3. Beneficial properties of flavonoids as a treatment for sulphur mustard toxicity.

When rats were exposed to SM at a dose of 20 or 80 mg/kg, depletion of reduced glutathione was observed. Epigallocatechin pretreatment did not show any appreciable protection, probably due to the use of high dose of SM (Pohanka et al, 2011b). The effect of dermal application of high dose of SM (155 mg/kg) on hepatic lipid peroxidation and the protective effect of flavonoids was investigated in mice. Sulphur mustard depleted reduced glutathione in blood and liver. Thiobarbituric acid reactive substances (TBARS) levels in the

liver showed an increase indicating lipid peroxidation. Administration of vitamin E or flavonoids, gossypin or hydroxyethyl rutoside after dermal application of SM did not alter depletion of reduced glutathione but did reduce the malondialdehyde level significantly. The survival time of mice was increased by gossypin and hydroxyethyl rutoside to a greater extent than by vitamin E or sodium thiosulphate (Vijayaraghavan et al, 1991).

Protective effect of intraperitoneal administration of various antioxidants, trolox (water soluble analogue of vitamin E), quercetin and glutathione, was studied against SM by percutaneous administration and inhalation in mice. Survival time increased significantly following trolox and quercetin treatments when SM was exposed either through inhalation or by percutaneous route. The protection was better than intraperitoneal administration of glutathione. Significant decrease in reduced glutathione and increase in the level of malondialdehyde indicated oxidative damage to liver and lung tissues following SM inhalation and percutaneous exposure. Trolox and quercetin protected the liver and lung tissues from oxidative damage caused by SM exposure through inhalation and percutaneous routes (Kumar et al, 2001). Quercetin was administered intraperitoneally to mice along with SM and oxidative stress parameters were evaluated after 7 days. Sulphur mustard decreased the body weight significantly and quercetin protected the mice significantly, in a dose dependent manner. The protection was better only when quercetin was administered as pretreatment or simultaneous treatment. The decrease in reduced and oxidised glutathione levels, and the increase in malondialdehyde level following dermal application of SM was protected by quercetin, when it was administered as pre-treatment or simultaneous treatment. The histological lesions induced by sulphur mustard on liver, spleen and skin were also significantly protected by quercetin as a pretreatment or as simultaneous treatment. This clearly proved that percutaneous administration of SM induces oxidative stress and quercetin can protect it only as a prophylactic agent (Gautam et al, 2007).

Gossypin is a water soluble and naturally occurring bioflavonoid. The protection of varying doses of gossypin administered intraperitoneally was studied, prior to, simultaneous and 2 hr after percutaneous administration of SM in mice. The protection against systemic toxicity of SM was better when gossypin was given with lipophylic solvents (polyethylene glycol-300 or dimethyl sulphoxide) than with water. Good protection (8.0 folds) was observed when gossypin was administered (200 mg/kg in PEG-300; i.p.) at 30 min prior or simultaneous to SM exposure, but no protection was observed when gossypin was administered 2 hr post to SM exposure. A significant decrease in total antioxidant status of plasma, liver glutathione level (reduced and oxidised) and the activities of glutathione peroxidase, glutathione reductase and superoxide dismutase were observed after SM administration. Sulphur mustard treated mouse liver also showed necrosis. A significant protection was observed in these variables when gossypin was administered as pre-treatment or simultaneous treatment, and not as post treatment (Gautam and Vijayaraghavan 2007).

Hippophae rhamnoides (Linn) also known as sea-buckthorn is a high altitude (Indian Continent) and plain land (eg. Ukrain) growing shrub yielding small berries. The fruit is rich in vitamin C, antioxidants and flavonoids. Extracts of *H. rhamnoides* leaf and fruit, and *H. rhamnoides* flavone from fruit were evaluated against percutaneously administered SM in mice. Significant protection was observed with oral administration of ethanolic extract of *H.*

rhamnoides leaf and *H. rhamnoides* flavone. Reduced glutathione and oxidised glutalthione levels were decreased, and malondialdehyde was elevated after percutaneous administration of SM. Oral administration of ethanolic extract of *H. rhamnoides* and *H. rhamnoides* flavone significantly protected theses variables (Vijayaraghavan et al, 2006). Comparative evaluation of bioflavonoids viz., quercetin, gossypin and *H. rhamnoides* flavone, and tocopherol acetate (vitamin E) were carried out against the systemic toxicity of SM in mice (Figure 4). Quercetin, gossypin, *H. rhamnoides* flavone and tocopherol acetate were administered intraperitoneally prior to percutaneous administration of SM, and protection against the SM lethality and biochemical parameters were evaluated. The protection against the lethality of SM was very good with the flavonoids in the order gossypin showed 6.7 folds, *H. rhamnoides* flavone showed 5.6 folds and quercetin showed 4.7 folds protection. Tocopherol acetate did not give any protection (0.7 fold). Percutaneous administration of SM showed decrease in reduced and oxidised glutathione levels, and an increase in malondialdehyde level. Antioxidant enzymes like glutathione peroxidase, glutathione reductase and superoxide dismutase were significantly decreased. The total antioxidant status was also significantly decreased. Quercetin, gossypin and *H. rhamnoides* flavone significantly protected the reduced and oxidised glutathione level, and malondialdehyde level. Tocopherol acetate failed to offer any protection (Table 1). This study supported that SM induces oxidative stress and flavonoids are promising cytoprotectants against the toxic effects of SM better than vitamin E, but they all are effective only as a pretreatment (Vijayaraghavan et al., 2008).

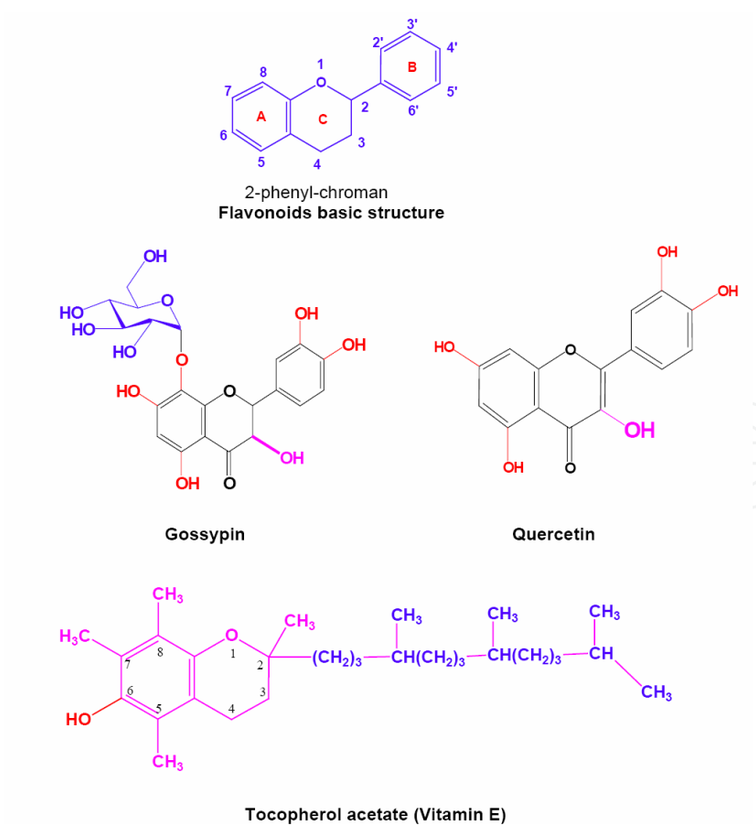

Fig. 4. Chemical structure of flavonoids and tocopherol acetate.

Groups	Dose (mg/kg)	Route	PI
Flavonoids:			
Quercetin	200	i.p.	4.7
Gossypin	200	i.p.	6.7
H. rhamnoides -flavone	200	i.p.	5.6
Herbal Extract:			
Aloe vera Leaf Extract	1000	p.o.	1.9
Ginkgo Biloba Extract	1000	p.o.	4.0
H. rhamnoides Leaf Extract	1000	p.o.	2.4
H. rhamnoides Fruit Extract	1000	p.o.	1.7
Other antioxidants:			
Tocopherol Acetate	200	i.p.	0.7
N-acetyl cysteine	125	i.p.	2.8
Melatonin	125	i.p.	5.6

Table 1. Protective efficacy of flavonoids and herbal extracts against sulphur mustard induced toxicity compared with other antioxidants in mice. LD50 of sulphur mustard by percutaneous route in mice is 8.1 mg/kg.

Nitrogen mustards are also alkylating agents and damage cellular nuclear DNA after penetrating the tissue. Rats were exposed to nitrogen mustard and treated with proanthocyanidin. A segment of the cortical tissue was prepared and evaluated by electron microscopy. Degeneration of the cortical neural cell nuclei with edema and axonal degeneration in the subcortical neural tissue were observed. Proanthocyanidin treatment showed less edema and degeneration (Tekiner et al, 2009). Hesperidin was investigated in mouse bone marrow cells against the genotoxicity induced by cyclophosphamide. Mice were orally pretreated with hesperidin and injected intraperitoneally with cyclophosphamide. Hesperidine could significantly protect the toxicity induced by cyclophosphamide on occurrence of the micronucleated polychromatic erythrocytes. Hesperidin due to its antioxidant effect reduced the oxidative stress and genotoxicity induced by cyclophosphamide in mouse bone marrow cells (Ahmadi et al, 2008). The preventive effect of hawthorn (*Crataegus microphylla*) fruit extract which is a rich source of flavonoids was investigated in mouse bone marrow cells against genotoxicity induced by cyclophosphamide. Hawthorn contains high amounts of phenolic compounds, chlorogenic acid, epicatechin and hyperoside. Mice were given hawthorn extract orally and cyclophosphamide intraperitoneally. Hawthorn extract could significantly protect the toxicity induced by cyclophosphamide on the occurrence of micronucleated polychromatic erythrocytes (Hosseinimehr et al, 2008).

8. Conclusion

Flavonoids are promising as antidotes, due to their plethora of beneficial effects that can mitigate the varied symptoms of sulphur mustard and nitrogen mustard. As a chemical they are very safe and the only limitation is its poor absorption by oral route. If their structure is modified to improve the bioavailability through oral route, and also with a stronger nucleophilic property by simplifying the structure and adding electron donating functional groups, they can be recommended as treatment and also as supportive therapy for a variety of diseases. They also will be highly useful for scavenging variety of toxic chemicals.

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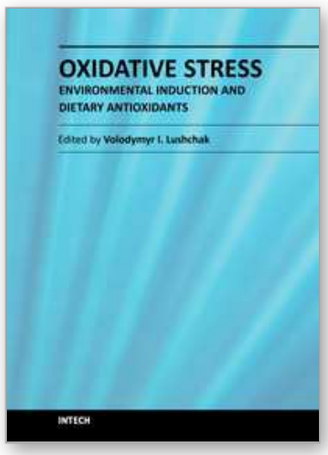

Oxidative Stress - Environmental Induction and Dietary Antioxidants

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This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

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