Andrographis paniculata

Andrographis paniculata has a broad range of pharmacological activities such as anti-inflammatory and hepatoprotective effects. Therapeutic efficacy of *A. paniculata* extract is mainly attributed to andrographolide. Andrographolide has anti-hepatotoxic activity against many liver toxins including acetaminophen- induced liver injury as an example.

For example, Acetaminophen is converted by drug metabolizing enzymes to a reactive metabolite, N-acetyl-*p*-benzoquinone imine (NAPQI that will covalently bind to proteins. At nontoxic doses, the metabolite was efficiently detoxified by glutathione forming an acetaminophen-glutathione conjugate. However, at toxic doses, the metabolite depleted hepatic glutathione by as much as 80–90% and subsequently covalently bound to protein. The amount of covalent binding correlated with the relative hepatotoxicity.

Reaction of NAPQI with glutathione occurs by conjugation to form 3-glutathion-*S*-yl-acetaminophen and by reduction to acetaminophen. Moreover, the reaction could be catalyzed by glutathione –S- transferase, and NAPQI is one of the best substrates ever described for this enzyme. Thus, detoxification of NAPQI is extremely rapid, and the rapid rate may explain why covalent binding to proteins was not observed in hepatocytes until glutathione was almost completed depleted.

An antioxidant is a molecule capable of terminating the chain reactions that damage cells by removing free radical intermediates, and inhibit other oxidation reactions by thereby reducing stress responsible for many degenerative disorders. The aqueous extract significantly increased the activities of catalase, superoxide dismutase and glutathione-S-transferase enzymes and reduced lactate dehydrogenase activity. The hepatoprotective effect of Andrographis paniculata against acetaminophen has been documented.

The hepatoprotective effect of Andrographis paniculata (AP) against Diclofenac (DIC) induced hepatotoxicity in rats has also been documented. Aqueous extract of AP significantly protected the hepatotoxicity. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) levels were significantly ($p < 0.01$) elevated in the DIC alone treated animals. Antioxidant status in liver tissue such as activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione s-transferase (GST), a phase II enzyme, and levels of reduced glutathione (GSH) were declined significantly ($p < 0.01$) in the DIC alone treated animals. Hepatic lipid peroxidation (MDA content) was enhanced significantly ($p < 0.01$) in the DIC treated group. AP also significantly decreased the lipid peroxidation in liver. Administration of AP (200 and 400 mg/kg) prior to Diclofenac significantly declines the activities of serum transaminases, GGT and ALP levels. Furthermore the hepatic antioxidant status i.e. SOD, CAT, GPx, GST and GSH were enhanced in the Andrographis paniculata plus Diclofenac treated group than the DIC alone treated group. The results of the present study concluded that the hepatoprotective effect of aqueous ethanol extract of AP against DIC -induced acute toxicity is mediated either by preventing the decline of hepatic antioxidant status or due to its direct radical scavenging capacity.

The mechanism of DIC hepatotoxicity involves covalent protein modification by reactive metabolites similar to acetaminophen, oxidative stress generation by peroxidase-

catalyzed reaction and mitochondrial injury propagation by reactive oxygen species. Antioxidants and also cytochrome P-450 inhibitors prevented this DIC-induced hepatic toxicity. It is logical to consider antioxidants as primary candidates to counteract such toxic effect. In recent years, accumulating evidence supported the protective effects of phenolic antioxidants from medicinal plants against oxidative stress–mediated disorders.

Administration of a single high dose of Diclofenac significantly ($p < 0.01$) elevated the serum transaminase, γ-glutamyl transpeptidase (GGT) and ALP activities compared to the normal animals. This indicated necrosis of hepatocytes that results in the leakage of transaminases, GGT and the elevation of serum ALP from a possible cholestasis. The significantly decreased serum transaminases, GGT and ALP activities in the Andrographis paniculata administered groups prior to DIC demonstrated its hepatoprotective effect. However, a single high dose of aqueous ethanol extract of Andrographis paniculata could produce only a partial protection. Hence, more prophylactic doses of extract of Andrographis paniculata are required to render a complete protection.

Cytochrome P-450 enzymes are the major catalysts involved in the metabolism of drugs. NSAIDs are mainly metabolized by cytochrome P-450 to form an electrophilic metabolite, N- acetyl-p-benzoquinonimine, which is primarily inactivated by conjugation with glutathione. A large number of the metabolites produced by NSAIDs are found to generate superoxide anion and other free radicals in the biological systems. However, at a higher dose of DIC (150 mg/kg), intermediate metabolites accumulate and cause liver damage. Depletion of glutathione beyond certain critical level can lead to oxidative stress and development of overt hepatotoxicity.

GSH is the most important endogenous antioxidant marker for chemical-induced toxicity to help eliminating the over produced ROS. The reduced hepatic antioxidant status is related to oxidative stress and elevation of lipid peroxidation that resulted in the leakage of hepatic enzymes to serum in the DIC alone treated animals. Treatment of Andrographis paniculata at 400 mg/kg plus DIC significantly enhanced the hepatic antioxidant activity including the hepatic GSH level compared to the DIC alone treated animals. The elevated hepatic reduced GSH level could partially explain the hepatoprotective mechanism of the Andrographis paniculata at 400 mg/kg dose. Reduced GSH can function as a reductant in the metabolism of hydrogen peroxide and various organic peroxides. The GPx present in the cells can catalyze this reaction. It is reported that depletion of GSH below a threshold value was associated with a significant conversion of xanthine dehydrogenase to reversible xanthine oxidase, a superoxide radical generation reaction-catalyzing enzyme. Therefore the enhanced hepatic GPx and SOD activities in the high dose Andrographis paniculata plus DIC treated group further support its hepatoprotective effect. The elevated antioxidant status in the liver of Andrographis paniculata (400 mg/kg) plus DIC treated group is related to the decreased MDA level could maintain the membrane integrity and prevented the leakage of hepatic enzymes to serum. The histopathological analysis of liver section indicates a moderate centrilobular necrosis, fatty infiltration and lymphocytic infiltration in the high dose Andrographis paniculata plus DIC treated animals with respect to the DIC alone treated animals. In the present study decreased hepatic GST activity of the DIC alone treated animals in the present study could support the enhanced lipid peroxidation. Administration of Andrographis paniculata plus DIC significantly and dose dependently elevated the hepatic GST activity and protected the liver toxicity. The enhanced GST

activity, a phase II enzyme, can also explain the increased detoxification of the reactive metabolites generated from the DIC metabolism in the liver of Andrographis paniculata treated animals.

Treatment with the extract of *Andrographis paniculata* was able to rescue the gentamicin-induced toxicity, probably via augmenting the antioxidative defense mechanisms explained above. Thus, taken together results from these studies indicate that this protective activity of the plant extract helps in maintaining the integrity of plasma membrane and also enhances the regenerative and reparative capacity of the liver. The examples highlighted above have similar pathological mechanisms to common liver toxicants a pet could encounter in a normal environment and thus the administration of AP extracts could provide prophylactic protection to pets. Andrographolide was quickly and almost completely absorbed into the blood following the oral administration of AP extract. Since a large part (55 %) of AND is bound to plasma proteins and only a limited amount can enter the cells. The absorbed compound is widely distributed among the organs of the viscera and daily administration could provide therapeutic protection against many live toxins.

The hepatoprotective activity of AP is higher than silymarin, known for its hepatic activity.