Review Paper:

Efficacy of Herbal Drugs in Treatment of Galactosamine-induced Hepatotoxicity in Rats

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Abstract

Liver plays an important role in drug metabolism. Presence of any toxic compound in drug or formation of toxic metabolites during hepatic metabolism of drug may cause hepatotoxicity. Galactosamine is the experimental drug which causes hepatotoxicity by two basic mechanisms i.e. inflammation and peroxidation. Natural compounds which are derived from trees or herbs have anti-inflammatory and antioxidant properties which are efficient to prevent or cure the drug- induced hepatotoxicity. These herbal compounds are easily available in nature and also their extraction procedures are less expensive.

Moreover, herbal drugs have negligible side -effects as compared to the synthetic drugs available in the market. This review mainly focuses on the different measures of Galactosamine-induced hepatotoxicity and also the herbal drugs which can be employed to cure Galactosamine-induced hepatotoxicity.

Keywords: Galactosamine, Hepatotoxicity, Inflammation, Peroxidation, Hepatoprotectivity, D-GalN.

Introduction

Hepatotoxicity is the liver damage caused by various chemicals. Drugs which are taken as medicine in our body, have some chemicals as their constituents which act as toxins. Liver plays very important role in drug metabolism and detoxification. Liver is the primary site for drug metabolism and also for the metabolism of xenobiotics. Hepatocytes possess many enzymes in its microsome and cytosole which are necessary for drug metabolism. Drug metabolism is the process in which drug is oxidised, hydrolysed and then attached covalently to the hydrophilic compound so that the drug can be easily excreted from body through kidney, bile, faeces after its action[1].

Liver also helps inenterohepatic recycling of drug. In this process, drug is reabsorbed from liver to the bile and then into small intestine. Drug is absorbed in the enterocytes and drug metabolites are present in the intestine, bile are converted back to parent drug by glucuronidase enzyme in the intestine and then transported back to liver.

Enterohepatic recycling improves the half life of drug and also the toxicity of drug which may affect the functionality of liver. Hence liver is the primary organ in the body which comes directly in contact with the parent drug or drug metabolites formed during drug metabolism. This is why the presence of toxicants, xenobiotics in the drug or the overdoses of drug can affect the liver and can reduce its functionality causing hepatitis[2].

WHO has reported that about 6 lakh persons in the world die every year because of acute or chronic hepatitis. Hepatitis also can be caused by viruses, alcohol and lipid oxidation products. About 900 drugs are now known which acts as hepatotoxicants including many therapeutic drugs and industrial chemicals. These hepatotoxins when taken into the body cause drug induced acute or chronic liver disease. Symptoms of drug-induced liver injury are mainly changes in morphology of liver by hepatic destruction, severe necrosis, sinusoidal dilation, central vein congestion, cellular boundary loss, elevation in the levels of liver enzymes in hepatocytes and blood serum, reduction in the activity of antioxidant enzymes in liver, loss of protein synthesis and DNA damage in liver cells, loss of hepatic functions, liver failure and hepatic encephalopathy[3].

Galactosamine: Galactosamine is one of the potent hepatotoxic drugs. It is the amino sugar derived from Galactose. Molecular formula of galactosamine is $C_6H_{13}NO_5$ and its molecular weight is 179.172g/mol. IUPAC name of Galactosamine is 2-Amino-2deoxy-D-galactose-hexopyranose which is also known as D-GalN. Galactosamine is one of the eight essential amino acids that function in cell to cell interaction. It helps in the treatment of joint inflammation and heart diseases. It is the constituent of glycoprotein hormones like FSH and LH.

Sources of Galactosamfine are mainly cattle bovine, shark meat and red algae. D-GalN is used as experimental hepatotoxin along with bacterial lipopolysaccharide (LPS) to induce hepatotoxicity in various animal models. Hepatic injury induced by D-GalN +LPS in these animal models is similar to the acute viral hepatitis in humans. Hence D-GalN is considered as ideal hepatotoxin for drug induced hepatotoxicity in animal model for its study[4].

Use of LPS in D-GalN induced hepatotoxicity in rat model: LPS i.e. Lipopolysaccharide is the major component of outer membrane of gram negative bacteria. When administered to body, LPS acts as endotoxin and stimulates the macrophages present in liver to produce large number of pro-inflammatory cytokines i.e. TNF-α, IL-6, IL1-β; thus creates inflammatory conditions in hepatocytes which helps to improve drug-induced hepatotoxicity in animal model.

Galactosamine is given intraperitonially to the rats along with LPS to induce hepatotoxicity in 24 hrs. Herbal drugs should have antioxidant, anti-inflammatory properties to act as hepatoprotective agents. Many research articles are available showing the results of successful hepatoprotectivity of herbal drug against D-GalN+LPS induced hepatotoxicity in rat model[5].

Galactosamine- induced Hepatotoxicity- Overview

Measures of D-GalN +LPS induced hepatotoxicity in rats: Figure 1 shows the different mechanisms by which galactosamine induces hepatotoxicity. It is found that galactosamine when given with lipopolysaccharide (LPS) acts as a potent hepatotoxic drug and causes hepatitis in rats. Galactosamine when injected intraperitonially in rats shows peculiar symptoms of hepatotoxicity including elevation of serum levels of AST, ALP, ALT enzymes in mice, increased bilirubin level, elevated levels of MDA, iNOS, MPO in liver leading to lipid peroxidation, decreased levels of antioxidant enzymes i.e. SOD, CAT, GPx, GR, GSH in liver cells leading to increased ROS formation, increased GST levels. Pro-inflammatory cytokine levels i.e. TNF-α, IL-6 get elevated due to activation of NFk-B pathway leading to liver cell inflammation.

Also, the levels of albumin and total protein present in liver cells are observed to be decreased. Other symptoms are disturbed: RNA and Protein synthesis in liver, changes in liver morphology including liver necrosis, apoptosis, infilteration of phagocytic cells at liver site, central vein congestion, sinusoidal dilation, cellular boundary loss. All these symptoms can be taken as a measure of hepatotoxicity induced by D-GalN+LPS in rats and reversion of these symptoms after injection of herbal drug can be considered as a efficiency of herbal drug to be used as a hepatoprotective agent against D-GalN +LPS induced hepatotoxicity.

Many herbal compounds we have found which are when injected in D-GalN +LPS induced hepatitic rat at their effective concentration, symptoms of hepatotoxicity get reversed revealing the hepatoprotective nature of these herbal drugs[6].

Clinical features of D-GalN +LPS induced hepatotoxicity: D-GalN changes the liver cell permeability and causes release of AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline phosphatase) enzymes and bilirubin in serum. Elevated serum levels of these enzymes are indication for hepatic tissue damage and abnormal liver functions. After injecting herbal drugs in hepatitic rat liver, serum levels of these enzymes get decreased to normal. D-GalN upregulates the expression of MDA, iNOS and MPO in liver cells.

These are the TBAR'S (Thiobarbuturic Acid Reactive Substances). MDA (Malondialdehyde) is a biomarker for oxidative stress[7]. Its level gets increased in D-GalN +LPS injected rats leading to ROS formation and lipid peroxidation. Increased iNOS (inducible Nitric Oxide Synthase) expression leads to the production of large amount of nitric oxide which induces hepatotoxicity[5]. MPO (Myeloperoxidase) is the indicator of neutrophil infilteration at liver site[7] which leads to liver necrosis. Levels of MDA, iNOS, MPO get reduced after injection of herbal drugs in hepatitic rat.

It is reported that D-GalN injection in rats leads to the decrease in the levels of SOD, CAT, GPx, GR, GSH in rat liver. All these are the antioxidant enzymes found in liver cells which control the production of ROS (Reactive Oxygen Species) in liver and prevent lipid peroxidation, liver tissue damage. D-GalN +LPS reduces the levels of SOD (Superoxide dismutase), CAT (Catalase) in liver. Also it causes the changes in glutathione metabolism[8] which results into decrease in the levels of GPx (Glutathione peroxidase), GR (Glutathione reductase), GSH (Reduced Glutathione) which ultimately results into ROS formation and oxidative stress in liver.

SOD, CAT, GPx, GR, GSH levels get increased to normal after injecting herbal drug into the D-GalN +LPS induced hepatitic rats showing reduced ROS formation. D-GalN brings about an increase in GST (Glutathione-S transferase) level leading to the oxidative stress, ROS formation and lipid peroxidation in liver. It is found that after injecting herbal drug in hepatiticrats, GST level get decreased.

Also, it is mentioned that in D-GalN +LPS induced hepatotoxic rats, level of HO-1 (Hemeoxygenase) get decreased. HO-1 brings about oxidative degradation of Heme into carbon monoxide (CO), free iron and biliverdin[9]. CO mediates the anti-inflammatory and anti-apoptotic effect. NO (Nitric oxide) level gets increased due to upregulation of iNOS expression by D-GalN which leads to endotoxemia and liver inflammation. These symptoms get reversed when rats are treated with herbal drug. It is evident that in D-GalN induced hepatiticrats, phosphorylated IKB-α and p65 levels get increased leading to the activation of NFk-B signalling pathway (Nuclear Factor Kappa Light-Chain- Enhancer of Activated B cell)[7] which regulates the cytokine production and cell survival.

NFk-B is present in the cytosole of almost all eukaryotic cells in its inactivated form. Inside the cytosole, NFk-B forms complex with IKB-α which is the inhibitor of NFk-B. Under stressed conditions, IkB Kinase (IKK) enzyme phosphorylates the IKB-α leading to its dissociation from NFk-B.

The activated, free NFk-B is then translocated to nucleus and transcribes the mRNA which codes for various pro-inflammatory cytokines i.e. TNFα (Tumor Necrosis Factor – alpha), IL-6 (Interleukin-6) promoting liver inflammation. NFk-B also plays important role in regulating immune response to infection. This tends to the infilteration of phagocytic cells in the liver leading to liver tissue necrosis.

Also TNF-α, IL-6 and IL1-β bind to TNFR-1 receptor and recruits and cleave caspase-8 through FADD, hence activate the extrinsic pathway of apoptosis in liver cells. These pro-inflammatory cytokines release some ROS and proteases which increase HMGB-1 serum level (High Mobility Group Protein B1) which induce liver inflammation and autophagy[10].

TNF-α, IL-6 and IL1-β levels are found to be decreased after treatment with herbal drugs. It is mentioned that in D-GalN +LPS induced hepatitic rats, levels of caspase 3, caspase 8, caspase 9 and cleaved caspase are found to be increased indicating the apoptosis of liver cells. It is evident from counting the number of TUNEL positive cells (Terminal deoxynucleotidyl transferase mediated dUTP nick labelling) in D-GalN +LPS induced hepatitic rats. TUNEL positive cells were found more in hepatitic rats than normal control rats[11].

After treatment with herbal drug, level of cleaved caspase is found to be decreased in hepatitic rats. It is reported that there is increased level of COX-2 (Cyclooxygenase-2) in DGalN-LPS induced hepatitic rats. COX-2 expression leads to the production of large amount of prostaglandins and thromboxanes.

Prostaglandins are responsible for mediating pain and inflammation. Treatment with herbal drug downregulates the expression of COX-2 and inhibits prostaglandin production; thus prevents inflammation at liver site. Albumin and total protein content of liver get decreased after injecting D-GalN +LPS in rats indicating the inability of liver to synthesize essential proteins. These levels get increased and reach to normal after treatment with herbal drug[5].

D-GalN brings about the depletion of UDP-glucose, UDP-galactose in galactose metabolic pathway and disturbs the sugar metabolism in liver cells. As a result, liver cells cannot consume sufficient amount of energy to synthesize the components (RNA and Proteins) essential for survival. This leads to changes in the structure of liver cell membrane, organelle, changes in the phospholipid composition of liver cell membrane. After treatment with herbal drug, all these changes get reversed to normal[4].

After injection with D-Gal+LPS in rats, there is observed a liver tissue necrosis and apoptosis, infilteration of phagocytic cells at liver site, sinusoidal dilation, central vein congestion and cellular boundary loss. All these symptoms get reversed and normal liver architecture is achieved after treatment with herbal drug.

It is mentioned that after D-GalN+LPS injection in rats, there is phosphorylation and activation of MAPK family proteins (Mitogen Activated Protein Kinases). MAPK is the serine threonine kinases which include number of signalling molecules like P38 MAPK, ERK (Extracellular Signal Regulated Kinase), JNK (c-Jun N Terminal Kinase) and ASK-1 (Apoptosis Signal Regulating Kinase -1). Activation of MAPK pathway signalling molecule results into the oxidative stress, inflammation and finally apoptosis. Ratio of phosphorylated JNK to total JNK was found to be increased in D-GalN injected hepatitic rats[11].

It is reported that in D-GalN +LPS injected rats, levels of autolysosomes in liver cells are found increased[10]. Rats which lack genes for IL-17, Leucocyte Cell derived chemotaxin-2, Complement C3 inhibitor of inflammosome, IL1R Associated kinase 4 (IRAK-4), TNF-α receptor are resistant to D-GalN +LPS induced hepatotoxicity. Galactosamine depletes the Uridine phosphate sugars, interfers with the Galactose metabolism and natural cycle of glycolysis in the liver cells.

As a result, liver cells cannot produce sufficient amount of energy to carry out their normal biological functions. Galactosamine activates the inflammatory system of the body at the liver site (Pro-inflammatory cytokines) resulting into liver cell necrosis. Galactosamine improves the ROS production in liver by different mechanisms and also activates the apoptotic machineries in the liver cells. These three processes finally tend to hepatotoxicity[11].

Treatments of Hepatotoxicity by using herbal drugs

By reviewing many research papers, we can conclude that inflammation and peroxidation (ROS formation) are two basic mechanisms by which galactosamine induces hepatotoxicity in rats. The result of the treatment of herbal drugs in D-GalN +LPS induced hepatotoxic rats reveals that the herbal drugs which are having antioxidant and anti-inflammatory properties can effectively reverse the symptoms of hepatotoxicity in D-GalN +LPS injected rats.

Significant results of hepatoprotectivity are found for the herbal drugs mainly - Maslinic acid, *Ricinuscommunis*, Diminazeneaceturate, Cordycepin, *Phyllanthusniruri*, Oeanolic acid, Forsythiaside A, Sea buckthorn, Linalool, Protocatechuic acid, Siilibinin, Vitamin E, *Pyropiayezoensis*, *Phyllanthusmaderaspatensis*, Epigallocatechingallate, Echinacosides, *Panax ginseng*, *Emblicaofficinalis*, Quercetin, Daburpolyherbal formulation, *Persicariachinensis*, *Piper puberlum*, *Acanthopanax Koreanum Nakai*, *Desmodiumadscendens* and Catechin[12].

Mechanism of hepatoprotectivity of herbal drugs

Herbal drugs deal with D-GalN +LPS induced hepatotoxicity mainly by upregulating the levels of antioxidant enzymes in liver (SOD, CAT, GPx, GR, GSH) which are decreased by D-GalN. Also they reduce the levels of GST and TBAR'S which are responsible for ROS formation and lipid peroxidation in liver and upregulate the expression of HO-1.

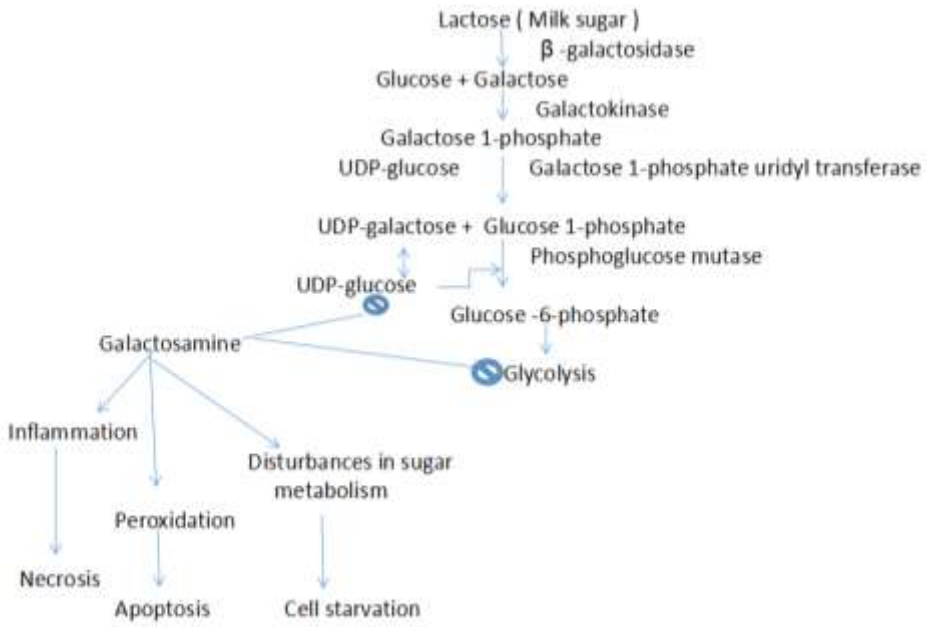

Fig. 1: Mechanism of Galactosamine induced hepatotoxicity

It ultimately results into reduction in oxidative stress in liver. Hepatoprotective herbal drugs deactivate the NFk-B signalling pathway in liver cells induced by galactosamine and responsible for phagocytic infilteration at liver site leading to necrosis.

Also, NFk-B causes overproduction of pro-inflammatory cytokines (TNF-α, IL-6, IL1-β). These pro-inflammatory cytokines bring about the inflammation and apoptosis in liver cells (by activating caspases). Reduction in the levels of pro-inflammatory cytokines by treatment with herbal drugs overcomes the symptoms of inflammation and apoptosis and also can recover the normal liver tissue structure. Inhibition of COX-2 expression is another mechanism of hepatoprotective drugs by which they mediate their anti-inflammatory action. These herbal drugs can help to regain the normal liver functioning as they recover normal AST, ALT, ALP, bilirubin levels in liver cells and also can improve the mRNA and protein production in liver cells which is essential for normal cellular metabolism in liver cells.

Maslinic acid can increase the level of HO-1 which acts as a shield against oxidative stress and activate the Nrf-2 signalling pathway. Nrf2 is the transcriptional factor which can stimulate the expression of many cytoprotective genes under the stressed conditions. Under normal conditions, Nrf2 is held into the cytosole by Keap-1. Under stressed conditions, Nrf2 gets free from Keap-1 and gets released into the nucleus. Inside the nucleus, Nrf2 binds to ARE in HO-1 promoter region and triggers the gene expression of cytoprotective genes.

During hepatotoxic conditions, Nrf2 induces the expression of antioxidant enzyme, NAD(P)H Quinone Oxidoreductase -1 (NQO- 1), Glutathione and some other enzymes which can reduce ROS level. It also interfers with the expression of TNFα, IL-6, IL1-β and shows anti-inflammatory effect[7].

Ricinuscommunis has a potent bioactive compound Rutin which is having strong antioxidant activity. It decreases MDA levels and reduces lipid peroxidation in liver. Rutin also can protect bile duct from intoxication by D-GalN, it can stabilize hepatic cell membrane, can regenerate liver parenchyma[8]. Diminazeneaceturate (DIZE) works by decreasing the levels of caspase 3, caspase 8, caspase 9 and cleaved caspase 3 and hence it prevents the apoptosis of liver cells. On treatment with diminazeneaceturate, there is observed a significant reduction in count of TUNEL positive (apoptotic) cells in D-GalN +LPS induced hepatitic rats. Also mortality rate in rats was found to be reduced from 85% to 45% after DIZE treatment. DIZE can act as Angiotensin Converting Enzyme II Activator which plays important role in preventing liver inflammation[11].

Phyllanthusniruri has a bioactive compound Epicatechin which has antioxidant and anti-inflammatory properties[13]. Oeanolic acid is the triterpenoid present in many herbs like olive oil, garlic and syzygium species. It can inhibit the mRNA expression of TNF-α and ER responsive gene Gadd45 in D-GalN induced hepatitic rats. Also it inactivates NFk-B pathway and inhibits the expression of Caspase-3, Caspase-8 and COX-2; thus exhibits anti-apoptotic and anti-inflammatory action[14]. Forsythiaside A which is isolated from *Forsythia suspensa* can increase the expression of Nrf-2 and HO-1, thus mediates antioxidant, anti-inflammatory effect in D-GalN injected hepatotoxic rats[15].

Sea buckthorn shows hepatoprotectivity by inhibiting the expression of TLR-4, MAPK family proteins i.e. P-ERK

(Phosphorylated Extracellular Signal Regulated Kinase), P-JNK (Phosphorylated c-Jun N terminal Kinase), p-p38 MAPK (Phosphorylated Mitogen Activated Protein Kinase 38) and NFk-B (Nuclear Factor Kappa B) in Galactosamine injected hepatitic rats[16]. Linalool is the terpene alcohol found in mint, cinnamon, rosewood, birch and fungi. It mainly upregulates the expression of Bcl-2 and thus prevents the apoptosis of hepatocytes in hepatotoxic conditions in case of rats. Also it shows anti-inflammatory and antioxidant effects[10].

Protocatechuic acid (Dihydroxy-benzoic acid) is the polyphenolic compound found in Green tea, *Hibiscus sabdariffa*, Onion skin and Agaricus. In hepatotoxic conditions, it downregulates the AST, ALT, ALP, GGT (Gamma–glutamyltranspeptidase) serum level. Also it is reported that treatment of protocatechuic acid in D-GalN induced hepatotoxic rats brings about a decrease in the serum levels of kidney function markers i.e. Uric acid, Urea, Creatinine up to normal. Lipid profile levels in both plasma and tissues of liver and kidney were found to get normal[17].

It is reported that Silibinin which is an active component of Silymarin and vitamin E show good hepatoprotective activity against Galactosamine induced hepatotoxicity in rat model. Both silibinin and vitamin E downregulate the expression of ASK-1 (Apoptosis Signal Regulating Kinase - 1) and p38 MAPK which plays important role in TLR-4 signalling pathway that ultimately leads to cell death. Also silymarin and vitamin E can decrease the expression of NOX4 and increase the expression of Thioredoxin antioxidant system molecules i.e. Trx, TrxR (Thioredoxinereductase) and pp5 (Protein Phosphatase 5).

Trx forms a complex with ASK-1 on its N-terminal region and deactivates ASK-1. TrxR brings about the regeneration of reduced Trx. pp5 inhibits the kinase activity of ASK-1 by dephosphorylation of phosphothreonine residue of ASK-1. This ultimately results into prevention of apoptosis of liver cells in hepatitic rats by silymarin and vitamin E[18].

Pyropiayezoensis can downregulate the expression of COX-2 and iNOS and also it inhibits the phosphorylation of MAPK (Mitogen Activated Protein Kinase) to prevent apoptosis of liver cells in hepatitic rats.

Also it reduces the serum levels of GOT (Glutamic Oxaloacetic Transaminase), GPT (Glutamic Pyruvic Transaminase) which are the indicators of liver function. Pyropia can decrease the level of lipid peroxidation and hence proved hepatoprotective against D-GalN +LPS induced hepatotoxicity in rat (2).

Hydroalcoholic extract of *Phyllanthusmaderaspatensis* (Madras leaf flower) has many bioactive components like Quercetin, Catechin, Rutin, Ellagic acid, Kaempferol which are hepatoprotective in nature and can be used against galactosamine induced hepatitis. Also it is mentioned that n-hexane extract of *P.maderaspatensis* shows hepatoprotectivity against CCl_4, Acetaminophen, Thioacetamide induced hepatotoxicity[19].

It is reported that Epigallocatechingallate (EGCG) which is a component of Green tea, can act as hepatoprotective against D-GalN induced hepatotoxicity in primary culture of rat liver cells. EGCG decreases the percentage of lactose dehydrogenase leakage from hepatocytes which is induced by Galactosamine. LDH leakage is the marker of cytotoxicity. Also it is mentioned that high dose of EGCG can induce lipid peroxidation and hepatotoxicity[20].

Echinacoside which is derived from the stem of Chinese herb Cistanche salsa reduces the levels of inflammatory cytokines, TBAR'S, HMGB-1 and prevents liver apoptosis under hepatotoxic conditions[21]. Cambial meristematic cells of *Panax ginseng Meyer* are rich in Ginsenosides having antioxidant, anti-inflammatory and immunomodulatory properties and can mediate hepatoprotectivity in Galactosamine induced hepatitic rats[9].

One of the reports explains the efficient hepatoprotective nature of *Emblicaofficinalis Gaertn*, also called as Indian gooseberry or Amla. It has many phytochemical compounds including Quercetin, Gallic acid, Corilagin, Ellagic acid having antioxidant and anti-inflammatory properties. Amla can protect against the hepatotoxicity of many drugs like Galactosamine, Ethanol, Carbon tetrachloride, Paracetamol, Hexachlorocyclohexane, ochratoxins and also heavy metals, anti-tubercular drugs[22].

D-GalN downregulates SIRT-1 gene expression in rat liver cells and increases AST, ALT, ALP and bilirubin levels in liver which results in hepatotoxicity. SIRT-1 gene codes for the protein Sirtuinin-1 which is a NAD+ dependent Class III histone deacetylase and regulates stress responses, genomic stability and cell survival. Quercetin, a phenolic compound which is found in many of the drugs, can act as an activator of SIRT-1 and thus mediates hepatoprotective response. When compared for the activity of Quercetin with the SRT 1720 (Synthetic allosteric SIRT-1 activator) against D-GalN +LPS induced hepatotoxic rats, it was found that quercetin can more efficiently stimulate the expression of SIRT-1 in hepatitic rats than SRT 1720[23].

Dabur India limited has designed one herbal formulation (DRDC/AY/8060) which can reverse the symptoms of hepatotoxicity induced by D-GalN and paracetamol also. Ingredients present in this herbal formulation are mainly Amlakibhumi (*Phyllanthusniruri*), Guduchi (*Tinosporacordifolia*), Neem (*Azardiractaindica*), Kalmegh (*Andrographispaniculata*), Harataki (*Terminaliachebula*), Amla (*Emblicaofficinalis*), Bahera (*Terminaliabellerica*), Kutki (*Picrorhizakurroa*)[24]. *Piper puberulum* is a Chinese herb which upregulates the expression of Nrf2, metallothionin and also HO-1, Quinone oxidoreductase-1, Glutamate Cysteine ligases (GCLC) and thus can mediate

hepatoprotective action against various hepatotoxins in rat model like Galactosamine, Carbon tetrachloride, Acetaminophen[25].

Metallothionin provides protection against metal toxicity and oxidative stress in liver and kidney. Quinone oxidoreductase-1 helps in superoxide scavanging and GCLC helps to regenerate GSH, thus contributes in antioxidant activity of drug. *Acanthopanaxkoreanum Nakai* down regulates the TLR-4 expression in rat model of hepatotoxicity and thus inhibits the TLR-4 dependent cytokine production and mediates hepatoprotective response[26]**.**

A comparison is made between pure D-pinitol extracted from *Desmodiumadscendens* and *Desmodiumadscendens* decoction as both curative and preventive hepatoprotective agent against Galactosamine induced hepatotoxicity. Decoction is found to be preventive hepatoprotective agent but not curative[27]. It is reported that Catechin which is a natural phenolic antioxidant found in many plants like tea plant, black grapes, apricot, strawberries, Mimosa plant, upregulates the expression of Bcl2 and downregulates the expression of p53 and mediates hepatoprotective effect in D-GalN induced hepatitic rat.

Bcl2 is the apoptosis suppressor and p53 can mediate apoptosis by modulating the expression of Bcl2, by activating Bax and caspases and by increasing the expression of death receptors in apoptosis pathway. Galactosamine upregulates the expression of p53 and downregulates Bcl2 expression, thus brings about apoptosis of liver cells. Catechin can reverse these conditions and can recover normal liver physiology[28]**.**

Another report on the hepatoprotective nature of methanolic extract of *Persicariachinensis* reveals that this herbal drug inhibits the expression of AP-1 (Activator Protein – 1) in D-GalN induced hepatitic rats. D-GalN + LPS binds to TLR-4 receptor and activates MAPK signalling pathway (Mitogen Activated Protein Kinase). MAPK family proteins i.e. ERK (Extracellular signal regulated kinase), c-Jun N-terminal Kinase (JNK) and p38 also get activated. MAPK family proteins bring about the activation of AP-1 which is a heterodimeric transcription factor made up of c-Fos, c-Jun, ATF and JDP proteins. Activation of AP-1 leads to the increased inflammatory response.

Downregulation of AP-1 expression by *Persicariachinensis* proved helpful against Galactosamine induced hepatotoxicity in rats[29]. Cordycepin can inhibit TLR-4 expression and NFk-B pathway and prevent cytokine production, inflammation. It also decreases NADPH oxidase activity and prevents ROS formation. It decreases the expression of vascular adhesion molecule -1 (VCAM -1) and prevents neutrophillinfilteration. Cordycepin also can increase LC3-II, p62 levels and decreases the levels of autolysosomes in liver[30].

Some other herbal compounds which are found effective in inhibiting the inflammation and peroxidation in case of D-GalN +LPS induced hepatotoxicity are mainly - Vitamin D3[4], Betaine[31], Oxyresveratrol[32], *Xineaamericana* Var. Caffa root extract[33], Liquiritigenin from the roots of *Glycyrrhizaglabra*, Swertiamarin from *Enicostemmaaxillare*[34], Chrysin (Flavenoid)[35,36], carvacrol[37–39], Leucasaspersa[40], Genistein, Tenuigenin[41], Mountan cortex radices[42], Cassia abbreviate[43], phyllanthusrheedi, Cichoriumglandulosum[44], spirulinaplatensis[45], Bicalin[46], syringing[47].

Conclusion

Galactosamine is a potent hepatotoxic agent which can induce hepatotoxicity by stimulating liver cell inflammation, peroxide formation and by interfering with the process of glycolysis in liver cells. The symptoms which reflect the hepatotoxicity are mainly elevation in the serum levels of AST, ALT, ALP enzymes, elevation in the MDA, iNOS, MPO levels leading to lipid peroxidation, downregulation of antioxidant enzyme levels (SOD, CAT, GPx, GR, GSH) leading to high ROS formation, increased GSH and pro-inflammatory cytokine levels.

Also changes in the liver anatomy disturbed RNA and protein synthesis in liver cells which ultimately lead to liver cell death. Many herbal compounds possess anti-inflammatory and antioxidant potential which can reverse these clinical conditions responsible for hepatotoxicity and can help to regain normal liver structure and metabolism. Use of these herbal drugs for medical purposes to cure drug induced hepatotoxicity can give efficient therapeutic outputs with negligible side-effects.

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