Non-alcoholic fatty liver disease (NAFLD) is the term used to describe the alcohol-like liver injury that occurs in the absence of alcohol abuse. It involves a range of histological abnormalities including simple steatosis or fatty liver, non-alcoholic steatohepatitis (NASH) and NAFLD induced cirrhosis. The predominant risk factor for NAFLD appears to be insulin resistance. Simple steatosis and NASH are generally asymptomatic and it is only the development of cirrhosis that gives rise to clinical consequences. At present, treatment for NAFLD concentrates on managing risk factors. However, in the future newer molecular targets like Molecular Chaperones and potential herbal therapies will play a key role in management of NAFLD. Molecular chaperones such as heat-shock proteins are a class of functionally related proteins whose expression is increased when cells are exposed to stress and are thought to play a critical role in protecting the liver against oxidative injury.

Key words: Non-alcoholic fatty liver disease, Alcoholic steatohepatitis, Molecular chaperones, Heat Shock Proteins and Oxidative Stress.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinicohistopathological entity with histological features which resemble alcohol-induced liver injury. It occurs in patients with little or no history of alcohol consumption. It encompasses a histological spectrum ranging from fat accumulation in hepatocytes (Angulo and Lindor, 2002) without concomitant inflammation or fibrosis (simple hepatic steatosis) to hepatic steatosis with a necroinflammatory component (steatohepatitis) that may or may not have associated fibrosis (Patel and Lee, 2001). The latter condition, referred to as nonalcoholic steatohepatitis (NASH) (Yu, 2002), may progress to cirrhosis of the liver (Clark, 2003).

The pathogenesis of nonalcoholic fatty liver disease has not been clearly defined. The most widely supported theory implicates insulin resistance as the key mechanism leading to hepatic steatosis, and perhaps also to steatohepatitis and alternatively oxidative injury, is required to manifest the necroinflammatory component of steatohepatitis. These two pathogenic factors consist of the accumulation of excessive hepatic fat primarily owing to insulin resistance, and oxidative stress owing to reactive oxygen species (Adams, 2006). Hepatic iron, leptin, antioxidant deficiencies, and intestinal bacteria all are reported to be the potential oxidative stressors.

NAFLD is associated with insulin resistance and the metabolic syndrome of Obesity (Clark, 2003), combined hyperlipidemia, Type II diabetes mellitus (Ben, 2001) and high blood pressure. Insulin resistance seems to be the major factor responsible for disease to steatohepatitis.

Multiple adiposkines have been identified which have significant effect on behavior and insulin sensitivity. The major adipokines are leptin, adiponectin and resistin, complement proteins and proteins of RAAS also act as adipokines.

Oxidation of free fatty acids can occur in mitochondria, peroxisomes or microsomes. This oxidation causes oxidative stress, owing to formation of reactive oxygen species, which can directly damage mitochondria. Reactive oxygen species can also increase their own production via positive feedback. They cause inflammation by inducing production of proinflammatory cytokines and chemokines. Reactive oxygen species and proinflammatory cytokines activate the NFκB pathway, which exacerbates inflammation and decreases insulin signaling.

Most patients with NAFLD have no or few symptoms. Patients may complain of fatigue, malaise, and dull right-upper-quadrant abdominal discomfort. Mild jaundice may, rarely be noticed.

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**Figure 1:** Micrograph of non-alcoholic fatty liver disease, demonstrating marked macrovesicular steatosis

**Figure:** Progression from steatosis to steatohepatitis
Mammalian heat shock proteins (hsps) induced in response to cellular oxidative stress forestall it by raising HSPs, reducing inflammation, and improving insulin signaling. Obesity and sedentary lifestyle perpetuate this chain, while dieting and exercise decline in anti-inflammatory HSPs allows inflammation to expand unhindered. Damage and allowing the accumulation of harmful proteins aggregates; and (c) signaling in turn reduces the expression of HSPs, leaving tissues vulnerable to (a) obesity-driven inflammation promotes insulin resistance; (b) impaired insulin signaling in turn reduces the expression of HSPs, leaving tissues vulnerable to damage and allowing the accumulation of harmful proteins aggregates; and (c) decline in anti-inflammatory HSPs allows inflammation to expand unhindered. Obesity and sedentary lifestyle perpetuate this chain, while dieting and exercise forestall it by raising HSPs, reducing inflammation, and improving insulin signaling. Mammalian heat shock proteins (hsps) induced in response to cellular oxidative stress serves as chaperones in refolding, disaggregation and degradation of damaged polypeptides. Amongst the family of heat shock proteins, Hsp70, Hsp60, Hsp90 and Hsp32 (also termed HO-1) have been implicated in protective mechanisms against increased oxidative stress in liver injuries. Upregulation of hsps in liver cells in culture has been shown to diminish the toxicity of a number of hepatotoxins. Immunohistochemical detection revealed elevated Hsp70 in livers of alcoholic patients as well as in non-alcoholic liver diseases. Oxidative stress due to depletion of glutathione (GSH) and induction of Hsp 70 has been closely linked. Thus, heat shock proteins confer a survival advantage to the cells preventing oxidative damage. In addition to its role in cytoprotection, hsp70 also inhibits inflammatory responses in immune cells. Hsp70 interacts with various components of the NFκB signaling pathway. Overexpression of Hsp70 leads to repression of NFκB mediated gene expression. Further, nuclear translocation of NFκB is also affected in cells transfected with the Hsp70 gene. Hsp70-induced NFκB inhibition is attributed to both increased IkBα expression and attenuated IkBα degradation. In addition, Hsp70 can directly interact with NFκB p65 and NFκB p50 to influence NFκB mediated responses.

Hsp90 also plays an important role in regulating the TLR signaling pathway and can thus influence inflammatory responses by chaperoning key signaling molecules. Inhibition of Hsp90 impairs function of its client signaling proteins and thus alters cell function. Treatment of cells with inhibitors of Hsp90 such as the benzoquinone ansamycin, geldanamycin activates a heat shock response without the stress. Geldanamycin also inhibits LPS-induced NFκB activity and TNFα production in macrophages. Hsp90 is crucial for biogenesis and activity of IKKz and IKKB, kinases responsible in IkBa phosphorylation and activation of NFκB, and inhibition of Hsp90 results in IKKα and IKKβ depletion. Thus Hsp90 can play a crucial role in maintaining the activity of various components of the NFκB signaling pathway (Pranoti M, 2007).

Release of HSPs in systemic circulation:

HSP is released into circulation by two mechanisms: a) Passive (left grey half of the cell): The passive release mechanism occurs when cells are infected (e.g., with lytic viruses, trauma or necrosis) resulting in the release of HSP’s into systemic circulation. b) Active (right half of the cell): The active release mechanism occurs in response to psychological stress (e.g., fear, immobilization, social isolation) or following stressful stimuli (e.g., heat, UV radiation, heavy metals) or in response to inflammatory mediators (e.g., IFN-γ, TNF-α, IL-1β, IL-10, IL-12) or receptor mediated events including ligation of HSP’s to its receptor (e.g., CD14, CD36, CD19, C-type lectin receptor LOX-1, CD 40). The active release mechanism is mediated by stimulation of the stress response which includes monomeric heat shock factor (HSF) which is in the cytosol to trimerize and translocate to the nucleus, where it binds to the heat shock element (HSE) and activates the synthesis of HSP. HSP are released into the circulation as free HSP and within exosomes to act as a danger signal.

Figure 4: Schematic representation of mechanisms by which HSP is released in circulation.

Heat shock proteins (HSP) are a class of functionally related proteins whose expression is increased when cells are exposed to stress (De Maio A, 1999). This increase in expression is transcriptionally regulated. The upregulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor (Wu C, 1995). Heat-shock proteins are named according to their molecular weight for e.g. Hsp60, Hsp70 and Hsp90 refer to families of heat shock proteins on the order of 60, 70 and 90 kilodaltons in size, respectively (Li Z and Srivastava P, 2004). The small 8 kilodalton protein ubiquitin, which marks proteins for degradation, also has features of a heat shock protein (Raboy, 1991).

Role of Heat shock proteins:

These are functionally related protein whose expression is increased when cells are exposed to increasing temperature & stress.

• Upregulation in stress (Santoro, 2000): Production of high levels of heat shock proteins can also be triggered by exposure to different kinds of environmental stress conditions, such as infection, inflammation, exercise, exposure of the cell to toxins (ethanol, arsenic, trace metals and ultraviolet light, among many others), starvation, hypoxia (oxygen deprivation). Consequently, the heat shock proteins are also referred to as stress proteins and their upregulation is sometimes described more generally as part of the stress response.

• Role as chaperone (Walter and Buchner, 2002): Heat shock proteins function as intra-cellular chaperones for other proteins. They play an important role in protein-protein interactions such as folding and assisting in the establishment of proper protein conformation (shape) and prevention of unwanted protein aggregation. By helping to stabilize partially unfolded proteins, HSPs aid in transporting proteins across membranes within the cell.

• Immunity (Nishikawa et al., 2008): Extracellular and membrane bound heat-shock proteins, especially Hsp70 are involved in binding antigens and presenting them to the immune system.

The mechanism by which heat-shock (or other environmental stressors) activates the heat shock factor has not been determined. However, some studies suggest that an increase in damaged or abnormal proteins brings HSPs into action. The central causal pathways include the following chain (Philip et al., 2009): (a) obesity-driven inflammation promotes insulin resistance; (b) impaired insulin signaling in turn reduces the expression of HSPs, leaving tissues vulnerable to damage and allowing the accumulation of harmful proteins aggregates; and (c) decline in anti-inflammatory HSPs allows inflammation to expand unhindered. Obesity and sedentary lifestyle perpetuate this chain, while dieting and exercise forestall it by raising HSPs, reducing inflammation, and improving insulin signaling.

Natural Herbal Remedies:

1) Natural sources of Omega 3 fatty acids:
This is a great supplement because of its wide range of benefits that include: Control of elevated triglyceride levels, Helps controlling Insulin Resistance. There are numerous studies that suggest diets containing high levels of omega-3 fatty acids (PUFA) can decrease the amount of liver fat. Omega-3 fatty acids are found in certain plant oils (such as canola, portulaca, cowberry and linseed oil) and marine fish (such as salmon). Nutritionally important n-3 fatty acids include ω-3-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), all of which are polyunsaturated. The human body cannot synthesize n-3 fatty acids de novo, but it can form “long chain” 20-carbon unsaturated n-3 fatty acids (like EPA) and 22-carbon unsaturated n-3 fatty acids (like DHA) from the “short chain” eighteen-carbon n-3 fatty acid ω-3-linolenic acid. The short chain n-3 fatty acids are converted to long chain forms (EPA, DHA) (Gerster, 1998). Recently it was demonstrated that the health benefit of omega-3 PUFA supplementation for people with liver steatosis proved to be extremely beneficial. It also reduced liver enzyme levels, led to a preferred blood fat ratio and improved the texture of the liver. Thus it is a compelling evidence to consider omega-3 PUFA for steatosis treatment (Capanpi & Calella, 2006).

2) Southern Ginseng:
Southern Ginseng, otherwise known as Gynostemma pentaphyllum or Jiaogulan, is an herb used in Chinese medicine to treat a wide range of ailments. Among the reasons for its use is southern ginseng’s ability to: Reduce cholesterol and blood coagulation, increase energy and stamina & Promote healthy cell development and liver protection due to antioxidant activity.

A 2006 study conducted at Chang Gung Memorial Hospital in Taiwan demonstrated a significant benefit to southern ginseng supplementation for patients with NAFLD. Researchers observed a marked reduction in liver enzyme levels, insulin resistance and body mass index when a daily dose of 80 mL of southern ginseng was added to dietary therapy (Chou et al., 2006).

3) Vaccinium ashei:
It is an herb mainly native North America to treat a wide range of ailments. Among the reasons for its use is its ability to: Reduce cholesterol, total blood lipid levels and liver protection due to antioxidant activity.

It contains a diverse range of micronutrients, with notably high levels (relative to respective Dietary Reference Intakes) of the essential dietary mineral manganese, vitamin B6, vitamin C, vitamin K and dietary fiber. It contains anthocyanins, other antioxidant pigments and various phytochemicals possibly having a role in reducing risks of some diseases, including inflammation and certain cancers.

4) Camellia sinensis:
Green tea (Camellia sinensis), a popular tea in the Orient, contains a high dose of catechin, a chemical plant. By comparison, black tea, which is popular in the United States, has undergone the process of fermentation, resulting in a lower concentration of catechin. Catechin is a flavonoid with antioxidant properties that has the ability to stabilize cell membranes. Therefore, the proclaimed liver-protective properties of catechin are similar to those that are claimed for milk thistle.

Experimentally induced liver damage in rats and in liver cell cultures has demonstrated the protective effects on the liver afforded by catechin. It has been suggested that higher dosages should be used on humans in order to reap the herb’s benefit of liver protection.

5) Vitis coignetiae:
Vitis coignetiae is a plant belonging to the genus Vitis that is native to the temperate climes of Asia, where it can be found in Russia; Korea and Japan. It was described botanically in 1883.

Little is known about the plant uses but the potential phytoconstituents can possibly play a challenging role in the treatment of non-alcoholic liver diseases and NAFLD.

Recently the true facts based on the research work carried out showed that anthocyanins from fruits of Vitis coignetiae Pulliat (known as meoru in Korea) on human hepatoma Hep3B cells inhibited cell invasion in a dose-dependent manner in invasion assays. They also inhibited expression of matrix metalloproteinase (MMP)-2 and MMP-9 and activation of nuclear factor kappaB (NF-kappaB) stimulated by tumor necrosis factor alpha. Taken together, the results of this study indicate that the anthocyanins isolated from fruits of V. coignetiae Pulliat have anti-inflammatory effects on human hepatoma Hep3B cells and inhibit MMP-2 and MMP-9 expression at least in part through the inhibition of NF-kappaB activation. The research indicates the possible use of the herb in the management of non-alcoholic liver disease (Shin, 2009).

6) Tephrosia purpurea
Tephrosia purpurea also known as ahulu, ahuola, or oho commonly called as Fish poison, Wild Indigo, is a species of flowering plant in the pea family, Fabaceae, that has a pantropical distribution. Native Hawaiians, It is found throughout India. The roots, leaves and seeds contain tephrosin, deguelin and quercetin, the roots contain isothiocyanate and rotenone. In the roots and leaves 2.5% rutin is found. Purpurin, a flavone has been isolated from the seeds, as also 8- substituted flavonoid and 3- substituted oxygenated chalcones. Octacosanol, sitosterol- C- glucopyranoside and a flavone glycoside have been isolated from the whole plant. The plant contains tephrosin which is a flavonoid and eclip sine as well as coumarin. The herb contains wedelolactone and dimethyl wedelolactone which possess potent anti-hepatotoxic activity. The herb is a rich source of ascorbic acid. The plant juice, mixed with an aromatic essential oil, is used in the treatment of jaundice. The herb is used as a tonic and deobstructive in hepatic enlargements and is used in combination with aromatic oils for catarrhal jaundice. The plant possess antihypertotic.

7) Eclipta prostrata
Eclipta prostrata commonly known as False Daisy, bhringraj is a plant belonging to the family Asteraceae. It is widely distributed throughout India, China, Thailand, and Brazil. This species is widely used in traditional Chinese herbal medicine, and in Ayurveda. It is considered to be the best remedy as liver tonic. The whole plant contains the alkaloids nicotine and eclip sine as well as coumarin. The herb contains wedelolactone and dimethyl wedelolactone which possess potent anti-hepatotoxic activity. The herb is a rich source of ascorbic acid. The plant juice, mixed with an aromatic essential oil, is used in the treatment of jaundice. The herb is used as a tonic and deobstructive in hepatic enlargements and is used in combination with aromatic oils for catarrhal jaundice. The plant possess antihypertotic.

8) Hydrastis canadensis L.
Goldenseal (Orange-root, Orange-root; Hydrastis canadensis) is a perennial herb belonging to family Ranunculaceae, native to southeastern Canada and the northeastern United States.

Goldenseal contains the isoorotlines alkaloids: hydastine, berberine, berberastine, hydastinine, tetrahydroberberastine, canadine, canadilide and 8-oxotetrahydrohalifeldine were identified.

Berberine is a major constituent of Hydrastis Canadensis. Berberine exerts upregulating activity on both low-density lipoprotein receptor (LDLR) and insulin receptor (InsR). This one-drug-multiple-target characteristic might be suitable for the treatment of metabolic syndrome, it acts possibly by preventing insulin resistance through increasing insulin receptor expression. Berberine lowers elevated blood total cholesterol, LDL cholesterol, triglycerides and aterogenic apolipoproteins (apo B), but the mechanism of action is distinct from statins. Berberine reduces LDL cholesterol by upregulating LDLR mRNA expression post transcriptionally while downregulating the transcription of propionate convertase subtilisin/kexin type 9 (PCSK9), a natural inhibitor of LDL receptor (LDLR), and increasing in the liver the expression of LDL receptors through extracellular signal-regulated kinase (ERK) signaling pathway. Berberine reduces hepatic fat content in the rats of non-alcoholic fatty liver disease (NAFLD). Berberine also prevents proliferation of hepatic stellate cells (HSCs), which are central for the development of fibrosis during liver injury. Thus, Hydrastis Canadensis which is a good source of Berberine can be utilized by its multi-functional mechanism like reducing hepatic fat content, preventing insulin resistance for the treatment of Non-alcoholic fatty Liver disease (Lee et al., 2006).

9) Cordyceps sinensis:
Cordyceps sinensis is a member of the ascomycetes class of fungi and has been used as an herbal medicine for centuries in Asian Societies. According to many ancient descriptions of Chinese herbs, Cordyceps sinensis possesses important pharmacological activities. It has been proven to enhance hepatic function, decrease plasma cholesterol levels and ameliorate liver diseases in case of liver cirrhosis. It has been reported that Uncoupling protein 2, a newly identified mitochondrial inner membrane carrier protein, uncouples fuel oxidation from Adenosine triphosphate synthesis and regulates lipid metabolism to prevent liver from steatosis and subsequently it was been reported that Cordyceps sinensis might be a valuable therapeu-
Silymarin, has a longstanding history of supporting liver health. Studies prove milk thistle may:

- Quicken the rate of liver cell regeneration
- Inhibit liver fibrosis
- Protect the liver from damage
- Reduce cholesterol absorption

According to a study, milk thistles extract significantly reduced cholesterol absorption in rats fed a high cholesterol diet, as well as caused significant decreases in LDL cholesterol and triglycerides (Sobolova et al., 2006). Researchers concluded that the inhibition of cholesterol absorption caused by milk thistle could extract contribute to positive changes in cholesterol profiles and lipid content in the liver and thus can possibly be used as a potential remedy for the treatment of NAFLD.

11) Rizoma Polygoni Cuspidati:
*Rizoma Polygoni Cuspidati* is traditional to China belonging to the family Polygonaceae. Resveratrol contained in Rhizoma polygoni cuspidati is an active ingredient that has good antioxidant character. The real-time qPCR method had been used to detect and analyze the non-alcohol fatty liver disease (NAFLD) model in medical intervention in this research. The level of TNF-alpha mRNA in the liver and thus can possibly be used as a potential herb for the treatment of NAFLD.

CONCLUSION:
Non alcoholic fatty liver disease is the hepatic manifestation of the metabolic syndrome. It is common, under-diagnosed and increasing in prevalence, generally asymptomatic and may progress through steatosis to NASH to cirrhosis undiagnosed and present with such complications as variceal haemorrhage, liver failure or hepatocellular carcinoma. It is usually, although not invariably, associated with features of the metabolic syndrome, and more severe NAFLD is seen in patients as they show increasing components of the metabolic syndrome. The pathogenesis remains to be fully understood but insulin resistance appears to be the driving force. Reducing insulin resistance and treating the components of the metabolic syndrome appears to improve NAFLD but there is a clear need for more newer molecular targets and therapeutically potential herbal remedies. A multidisciplinary approach to treat NAFLD and its related co-morbidity is essential.

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