5. DISCUSSION

Among the different groups of naturally occurring antioxidants from plants, carotenoids and polyphenolics are perhaps the two most important (Cadenas and Packer 2002). Carotenoids, including xanthophylls (oxygen-containing carotenoids) are naturally occurring coloured compounds that are abundant as pigment in plants. Carotenoids have the capacity to trap not only lipid peroxyl radicals, but also singlet oxygen species (Stahl and Sies 1997). The antioxidant capacity of carotenoids may also be related to the structure. Larger conjugated system such as astaxanthin is known to have a higher antioxidant activity (Miki 1991).

Polyphenolics is a highly inclusive term that covers many different subgroups of phenolic acids and flavonoids. More than 5000 polyphenolics, including over 2000 flavonoids have been identified, and the number is still growing (Harborne 1993). Polyphenolics vary in structures: hydroxybenzoic acids and hydroxycinnamic acids have a single-ring structure, while flavonoids can be further classified into anthocyanins, flavan-3-ols, flavones, flavonones and flavonols. Some of the flavonoids such as flavan-3-ols can be found in dimmers, trimers and polymers.

Miean and Mohamed (2001) indicated in his study that total flavonoid content in case of Colocasia esculenta is 133.5mg/kg of dry weight (found in myricetin). Lako et. al. (2007) showed that Colocasia esculenta var contained 130mg/100g TAC, 120mg/100g TPP and 1mg/100g Quercetin and Isorhamnetin each flavonols. In this study, the results revealed that the aqueous extract of Girardinia heterophylla possesses 8.66µg/gm fresh wt. of leaves of TPC and 113.5 µg/gm fresh wt. of leaves of TFC and Colocasia esculenta possesses 30µg/gm fresh wt. of leaves of TPC and 393.4µg/gm fresh wt. of leaves of TFC contents.

Phytochemicals in fruit and vegetables have been proven effective in the prevention of certain chronic diseases. Epidemiological studies have shown relationships between fruit and vegetable intake and chronic diseases such as coronary heart diseases, certain cancers and diabetes. Due to the prevalence of chronic degenerative diseases worldwide including the South Pacific and Australia, the availability of information on phytochemicals and
Antioxidant rich foods will help individuals make informed choices in the consumption of foods that could help protect them from such chronic diseases.

According to Jimoh (2010) the total polyphenol content of *Urtica urens* in case of aqueous extract is 4.58 mg tannic acid/g of dry plant material whereas flavonoids content is 0.36 mg quercetin/g of dry plant material. The value of our data shows the best results as compared to this study.

Polyphenols are especially important antioxidants, because of their high redox-potential, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen *et. al.* 1999). In addition, they have a metal chelating potential (Rice-Evans *et. al.* 1995). The antioxidant activity of the dietary polyphenolics is considered to be much greater than that of the essential vitamins, therefore contributing significantly to the health benefits of fruits (Wang *et. al.* 1996).

According to Pearson (1976), plant food that provides more than 12% of its calorific value is considered good source of protein. Therefore, the protein content of the leaves of these two plants will go a long way in meeting the protein requirement of the local people. Polyphenols are the major plant compounds with antioxidant activity. Some of the potential health benefits of polyphenolic substances have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation (Wichi 1988, Li *et. al.* 2005). As a group, phenolic compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species, the major cause of many chronic human diseases (Kyung *et. al.* 2005, Chen and Yen 2007). Phenolic compounds are plant substances which pass in common an aromatic ring bearing hydroxyl substituent. They may occur combined with sugar, as glycosides and they are usually located in the vacuole of the plant cells. The results strongly suggest that phenols are important components of these plants, and some of their pharmacological effects could be attributed to the presence of these valuable constituents.

Flavonoids and related polyphenols are ubiquitous in land plants, and have the general structure. Flavonoids generally consist of two benzene rings (e.g. A and B) linked by an oxygen-containing heterocycle ring (C). It should be noted that the chalcones are
considered by many authorities to be members of the flavonoids family, despite lacking the heterocyclic ring C. The fused A and C rings are often collectively termed the flavonoid nucleus.

The cellular sources of superoxide anion in mammalian cells include the microsomal electron transfer chain, entailing a slow electron transfer to O$_2$ via NADPH- cytochrome P-450 and NADPH- cytochrome b$_5$ reductase (Cadenas 1995a). Superoxide anions produced on incubation of liver microsomes with NADPH, can be detected by reduction of succinoylated ferricytochrome c (Kuthan and Ullrich 1982). e.g. Quercetin was an effective scavenger of superoxide anion in microsomes, however, other flavonoids had no effect on microsomal O$_2^-$ generation.

The benefits of wild resources to inaccessible rural villages in Himalaya cannot be ignored. The number of wild leafy vegetables recorded in the present study area indicates its diversity is less as compared to other areas (Orech et. al. 2007b; Narayanan and Kumar 2007). According to several informants wild green leafy vegetables increase the amount of blood in the body which is likely to refer to the high iron content of many wild greens. However, chemical analyses were beyond the scope of this study, and therefore, the information on the nutrient contents is entirely based on literature. The majority of wild edible herbs eaten typically contain high levels of important nutrients especially for diets usually high in starch (Sundriyal and Sundriyal 2001; Grivetti and Ogle 2000; Naithani 1984; Orech et. al. 2007a).

There has been an increasing interest in the contribution of free radical reactions participating in reactive oxygen species to the overall metabolic perturbations that results in tissue injury and disease. Reactive oxygen species are generated in specific organelles of cells under normal physiological conditions.

Phagocytic cells ingest and kill invading pathogens with free radicals including superoxide anion, hydrogen peroxides, nitric oxide and hypochlorite (Moslen 1994). Peroxide generate hydrogen peroxide as a byproduct in the process of beta-oxidation of fatty acids, however, this molecule is locally decomposed by high concentration of catalase (Beckman and Ames 1997). The reduction of molecular oxygen (O$_2$) to water (H$_2$O) proceeds by a series of single electron transfers, therefore, highly reactive
intermediates such as superoxide anion (O$_2^-$), hydrogen peroxides (H$_2$O$_2$) and hydroxyradical (HO.) are generated in mitochondria (Cadenas 1989). Antioxidative constituents in plants have other various biological activities, especially in the case of flavonoids (Middleton and Kandaswami 1994). Oxidative damages and lipid peroxidation are also concerned with diabetes (Asayama et. al. 1989) and cataracts (Spector 1991). These additional activities of antioxidative phytochemicals would contribute to their pharmaceutical uses.

In living systems, dietary antioxidants such as alpha-tocopherol, ascorbic acid, carotenoids, flavonoids, and other phenolics may be effective in protection from oxidation damage (Namiki et. al. 1993; Stavric and Matula 1992). A number of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been developed, but their use has begun to be restricted due to their toxicity (Ito et. al. 1983; Namiki 1990). Vitamin E is an effective natural antioxidant but has limited usage (Fang and Wada 1993). As a result, there is considerable interest in food industry and in preventive medicine in the development of natural antioxidants from botanical sources (Schuler 1990; Okuda et. al. 1993; Andersson et. al. 1996). The presence of antimicrobial substances in the higher plants is well established (Srinivasan et. al. 2001). Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contribution towards human health (Conway 1973).

The present study investigated the antimicrobial activity of Colocasia esculenta extract against various bacteria and fungus. The Colocasia esculenta extract showed remarkable antimicrobial activity against all the tested microbial strains. The world has entered an era when health is increasingly managed with an eye to cost containment. Critical to developing a cost effective approach to the evaluation and management of clinical illness is the selective use of available diagnostic methods, therapeutics and preventive measures. The emergence of bacterial strains that are resistant to many commonly used anti-bacterial agents means that treatment failure may become more common. Appropriate herbal therapy with antimicrobial efficacy can shorten illness and reduce morbidity in some bacterial and parasitic infections and can be life saving in invasive
infection. The results support the use of *Colocasia esculenta* for antimicrobial treatment
disease or prevention of bacteria growth.

Tsuchiya *et al.* (1996) attributed the antimicrobial activities of flavonoids to their ability
to complex with extracellular and soluble proteins as well as their ability to complex with
bacterial cell walls. They suggested that more lipophylic flavonoids exert antimicrobial
activity by disrupting microbial cells membranes. Parekh *et. al.* (2006) reported *Bauhinia
variegate* L. an edible plant contained tannins, alkaloids and saponins. The plant extracts
possess antibacterial activity with more sensitivity for gram negative bacteria than gram
positive bacteria uses in the study. Although, herbal extracts needs to be assured for its
quality control and efficacy for a particular dose.

*Urtica urens* (dwarf nettle) is a member of the Urticaceae and native to Eurasia. *Urtica*
prefers wet, rich soil and tends to grow in large patches. The stems are covered with
stinging hairs but the leaves are smooth and more delicate (Wagner *et. al.* 1994, Hirano
*et. al.* 1994, Schottner *et. al.* 1997). The plant produces inconspicuous green-white
flowers in late spring or summer. The leaf, flower, seed, and root of nettle are used
differently and contain different chemical constituents. Like all green vegetables, nettle
leaf is a micronutrient dense, nutritious food; however, it should be steamed or cooked
before ingestion to destroy the stinging hairs, which contain histamine, formic acid,
acetylcholine, acetic acid, butyric acid, leukotrienes, 5-hydroxytryptamine, and other
irritants. Contact with the hairs leads to a mildly painful sting, development of an
erythematous macule, and itching or numbness for a period lasting from minutes to days.
Medicinal extracts of nettle do not cause this reaction as the hairs are destroyed in
1998).

The water extract of *U.urens* showed activity against all Gram positive strains except
*Streptococcus pyrogens*. The aqueous extract was most active since it had activity against
all the organisms used in this study even at the lowest concentration (0.1 and 1.0 mg/ml)
(Jimoh *et.al.* 2010). In our aqueous extract of *G.heterophylla* the antimicrobial activity
were shown against all the selected bacterial strains. This study indicates that this value is
significantly different from the other at p< 0.0.5.
The high activity shown by the extracts of these two plants may have justified their use for medicinal purposes. Jimoh (2010) revealed in his study that the water extract of *U. urens* particularly showed high activity against all the selected bacterial strains as compared to fungal strains (Jimoh *et.al.* 2010). The German Commission E approves the use of nettle leaf as supportive therapy in patients with lower urinary tract infections (combined with immune and antimicrobial therapy) and to prevent and treat formation of urinary gravel (Blumenthal *et. al.* 1998). It has also been shown that the plant extracts were active against most of the Gram-positive strains and less of the Gram-negative strains. This observation may have supported the fact that, in general, the Gram-negative bacteria are less susceptible to antibacterial effect than the Gram positive ones (Grierson and Afolayan 1999, Afolayan 2003).

Ozyurt (2004) revealed that natural antioxidants such as dietary plant flavonoids are increasingly attracting attention. They are natural disease-preventing, health-promoting, and anti-ageing substances. Flavonoids are essentially ingested through food rather than being metabolically synthesized. There have been an increasing number of reports that directly contradict the putative role of flavonoids as antioxidant and anti-cancer agents (Hodnick *et. al.* 1998). Flavonoids are plant secondary metabolites widely distributed in the plant kingdom. More than 6000 flavonoids have been identified in plants (Harborne and Williams 2000).

The aqueous extract of the aerial parts of *Urtica dioica* L. have been occasionally used as a herbal medicine by cancer patients in Turkey (Akbay *et.al.* 2003). Ozyurt *et. al.* (2004) revealed in his study a sensitive and simple spectrophotometric method was developed for the determination of total flavonoid content of *Urtica dioica*. This method is based on the oxidation of flavonoids with Ce (IV) at room temperature, and the absorbance of unreacted Ce (IV) is measured at 320 nm. The reducing flavonoid content of the test sample may be expressed as quercetin equivalents.

Goel (2002) revealed in his study that the plant *Girardinia diversifolia* (Link) Fries possesses antibacterial activities against *Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa*. It also possesses antifungal activities against *Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes, Aspergillus fumigatus, Sporotrichum schenckii*. 
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Antiprotozoal activities against *Entamoeba histolytica* strain STA, *Plasmodium berghei*, *Giardia lamblia*. Anthelmintic activities against *Litomosoides carinii* and *Acanthocheilonema viteae*. Antiviral activities against *Encephalomyocarditis virus* and *Japanese B encephalitis virus*. Antifertility activities against *Anti-implantation in rats*. Effect on cardiovascular system against Effect on blood pressure, Effect on acetylcholine and Effect on isoprenaline. Effect on isolated tissue against Effect on guinea pig ileum. Effect on central nervous system and gross behaviour against Gross effects, Analgesia and Supramaximal electroshock seizure pattern test and Diuretic properties as well (Goel et.al. 2002).

Wahid (2009) revealed in his study that *Alocasia indica* Linn. are used in inflammation and in diseases of abdomen and spleen (Kirtikar and Basu 1975a). The juice of the leaves of the plant is used as digestive, laxative, diuretic, astringent and traditionally used for the treatment of rheumatic arthritis (Nadkarni 2000). *Alocasia indica* has antifungal properties (Bhatt and Saxena 1980). The plant contains flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, mallic acid, oxalic acid, aloesin, amino acids, succinic acid, and beta-lectines (Prajapati 2003).

Pari and Karthikesan (2007) who indicated that chronic alcohol intake leads to many cellular and tissue abnormalities such as alteration in liver enzymes (ALT, AST and ALP), which indicated the increased permeability, damage and necrosis of hepatocytes. Saravanan et. al. (2006) also showed that liver enzymes decreased significantly by extracts treatment. The observed decrease in these enzymes shows that aqueous extract of our plant extracts preserve the structural integrity of the liver from the toxic effect of ethanol. In our study, ethanol administration increased the value of AST, ALT, ALP, Bilirubin, Urea and Creatinine significantly. Concomitanly Deb (2002) reported during hepatic damage, cellular enzymes like SGOT, SGPT and ALP present in the liver cells leak into the serum, resulting in increased concentrations (Deb 2002). Ethanol administration for 90 days significantly increased all these serum enzymes. After treatment with aqueous extract of *Girardinia heterophylla* and *Colocasia esculenta*, these enzymes decreased significantly indicated hepatoprotective effect of extracts. Friedman et. al. (1980) revealed that albumin and globulin are two key components of serum proteins, because albumin is synthesized in the liver, one element is used to
monitor the liver function. In the present study, there was a concomitant decrease in serum protein levels. These results were in agreement with Ahmed et. al. (2002) who found significant decrease in serum protein in ethanol-administered mice. It demonstrates the decrease functional ability of ethanol-administered mice liver.

In the current study, significant increase in serum total protein was observed in aqueous extract of *Girardinia heterophylla* and *Colocasia esculenta* co-administered mice, that indicates the ability of these extracts to stimulate the regeneration of hepatic tissue which increase protein synthesis in damaged liver and improvement of the functional status of the liver cells.

Wahid (2009) stated that the phytochemical investigation of the hydroalcoholic extract of *A. indica* showed that it contains flavonoids, cynogenetic glycosides, citric acid, ascorbic acid, polyphenolic compounds. It is also stated that the toxicity created by the CCl$_4$ and paracetamol in normal rats elevated the levels of serum marker enzymes ALT, AST, ALP were observed significantly indicating acute hepatocellular damage and biliary obstruction. Pretreatment with AI produced a significant reduction in serum marker enzymes ALT, AST and ALP similar to silymarin (100mg/kg, bw.) treated group which seems to offer the protection and maintain the functional integrity of hepatic cells.

Histological examination of the liver tissue from CCl$_4$ and PCM had produced profound inflammation, severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus congestion especially in the sinusoids and occasional necrosis. Pre-treatment of animals with silymarin and AI reduced the inflammation, degenerative and steatosis.

The hepatotoxicity of CCl$_4$ has been reported to be due to the formation of the highly reactive trichloro (CCl$_3$) free radical, which alters functions of endoplasmic reticulum and causes peroxidative degradation of lipid membrane of the adipose tissue (Jain et. al.2008) leads to loss of metabolic enzymes located in the intracellular structures (Recnagal 1983). It is known that PCM induces liver injury through the action of its toxic metabolite, N-acetyl-p-benzoquinoneimine, produced by the action CytochromeP- 450. The metabolite causes depletion of glutathione (GSH) leading to cell death (Packer et. al. 1978, Shenoy et. al.2001). It is evident that the AI extract was able to reduce all the elevated levels of AST, ALT, ALP and bilirubin towards the normal value is as indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by hepatotoxins.
The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with AI extracts the normal cellular architecture was retained as similar to silymarin treated rats, thereby confirming the protective effect of the extracts. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume (Blancho *et. al.* 1990) as observed in the present study. Treatment with AI indicates the hepatoprotective effect due to presence of alocasin, flavonoids and other polyphenolic moieties present in it.

Patil and Ageely (2011) has been reported that *Colocasia antiquorum* is reported to possess hepatoprotective activity against experimentally induced liver injury in rats (Tuse *et. al.* 2009). *Colocasia esculenta* is reported to possess hypoglycaemic efficacy due to the presence of cyanoglucoside (Phillip *et.al.* 2002). Hypolipidemic and antihyperlipidemic activity has been reported due to the presence of arabinogalactan (Boban *et.al.* 2006) and mono and digalactocyl diacylglycerols (Tanaka *et.al.* 2005). Also it possesses antifungal activity due to presence of cystatin (Yang and Yeh 2005). Antibacterial activity of *Colocasia esculenta* has been mentioned by Ravikumar and co-workers (Ravikumar *et.al.* 2011).

In our study, elevations in the levels of MDA in kidney, liver of alcohol treated mice were observed. The increase in MDA indicates enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent excessive formation of free radicals. Treatment with AEGH and AECE significantly reversed these changes.

GSH is widely distributed in cells. It protects cells against free radical, peroxides and other toxic compounds. Deficiency of GSH within living organisms can lead to enhanced lipid peroxidation, tissue disorder and injury. Treatment with medicinal plant extracts was shown to be effective in restoring the GSH levels back to normal.

Patil and Ageely (2011) revealed in their study that the AST, ALT and ALP activity in the medium were noted as 9.54±0.43, 10.85±0.47, and 12.93±0.93 after incubation of one hour in presence of CCl4, similarly after incubation of one hour in presence of paracetamol the activity recorded in AST, ALT, ALP were 9.64±0.34, 11.55±0.75 and 12.93±0.56 IU/ml of medium. Similarly significant findings were observed in our study.
In the assessment of Liver and Kidney damage induced by alcohol, the determination of enzyme levels such as AST & ALT is largely used. ALT is more specific to liver and thus is a better parameter for detecting liver injury (Williamson et al. 1996). High levels of ALT, AST indicate damage. Our results using alcohol induced toxicity in mice demonstrated that the medicinal plant extracts (AEGH and AECE) caused significant reduction in AST, ALT levels. The results also showed that the medicinal plant extracts showed significant reduction in the activity of ALP, Bilirubin, Urea and Creatinine levels.

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Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc). Inspite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations.

Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like AST, ALT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated.

Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost. There are numerous plants and traditional formulations available for the treatment of liver diseases. About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective
activity. In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations (Paranjape 2001).

However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy. Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds which are being evaluated (Kirtikar and Basu 1975b).

Cells have a number of mechanisms to protect themselves from the toxic effects of ROS. SOD removes superoxide by converting it to $H_2O_2$, which is then converted to $H_2O$ by CAT and GSH. Lipid peroxidation is an autocatalytic process which is a common consequence of cell death. M.D.A is one of the end products in lipid peroxidation (Kureta et. al. 1993). In order to elucidate protective mechanism of the plant extract, the levels of MDA and antioxidant enzymes were estimated.

Alcohol induced damage produces alteration in the antioxidant status of the tissues. The free radical scavenging enzymes such as SOD and CAT are key components of the antioxidant defense mechanism (Bandyopadhay et. al. 1999). They play an important role in the elimination of ROS derived from peroxidative damage in liver and kidney tissues. The observed increase in SOD and CAT activity suggests that AE GH and AECE have an efficient protective mechanism in response to ROS and prevents the accumulation of free radical and protects the kidney and liver from alcohol intoxication.

In addition, liver histology of ethanol administered animal showed pathomorphologic alterations in the form of obvious dilatation and congestion of blood vessels accompanied with marked fibrosis extending from the portal area in-between the hepatocytes. Such findings are supported and explained by those of (Charles et. al. 2003) who stated that alcohol increases hepatic collagen type I with a significant rise in mRNA for [alpha] 1 procollagen. This leads to cirrhosis, septal and perivenular fibrosis, this result was in agreement with (MacSween and Burt 1986) who observed a spectrum of histological abnormalities in the liver by alcohol administration.

Dixit (et. al. 2007) studies have shown that silymarin is effective in the treatment of both acute and chronic hepatitis. In acute viral hepatitis, administration of silymarin shortened treatment time and lowered the elevated serum bilirubin, AST, and ALT. In patients with
acute hepatitis who were given either silymarin (140 mg) or placebo three times daily for three weeks, the proportion of patients whose AST was normalized was much higher in the treated group (82%) than in controls (52%). In patients with chronic hepatitis, 420 mg silymarin per day for six months also yielded improved serum liver enzyme levels (Magliulo et.al. 1978).

Silymarin administration has demonstrated normalization of serum liver enzyme and total bilirubin levels in patients with alcoholic liver disease; there was also improvement in liver tissue histology (Fehu et.al. 1989). In patients with cirrhosis, long-term (41 months) administration of silymarin at 420 mg per day resulted in a significant increase in survival compared to a placebo group (Ferenci et.al. 1989).

Silymarin is a polyphenolic flavonoid, extracted using 95% ethanol, from the seeds of the milk thistle. The plant consists of approximately 70-80% of the silymarin flavonolignans and approximately 20-30% of a chemically undefined fraction, comprising mostly polymeric and oxidized polyphenolic compounds. The most prevalent component of the silymarin complex is silybin (50-60% of silymarin), which is the most active photochemical and is largely responsible for the claimed benefit of the silymarin. Besides silybin, which is a mixture of two diastereomers (A and B) in approximately 1:1 proportion, considerable amounts of other flavonolignans are present in the silymarin complex, namely silychristin (20%), silydianin (10%), isosilybin (5%), dehydrosilybin, and a few flavonoids, mainly taxifolin. The seeds also contain betaine, trimethylglycine, and essential fatty acids that may contribute to silymarin's hepatoprotective and anti-inflammatory effects (Luper 1998; Pepping 1999; Saller 2001; Bisset 1994; Gruenwald 1998).

Dixit (et. al. 2007) study revealed that the chemopreventive action of silymarin helps in inhibit the carcinogenic action of many chemicals. The incidence of urinary bladder neoplasms and preneoplastic lesions induced by N-butyl-N-(4-hydroxybutyl) nitrosamine were significantly reduced (Vinh et.al.2002). Silymarin also significantly inhibited azoxymethane-induced colon carcinogenesis in rats (Kohno et.al. 2002). Skin carcinogenesis induced by benzoyl peroxide or 12-O-tetradecanoylphorbol-13- acetate was also inhibited by silymarin (Lahiri et.al. 1999; Agarwal et.al. 1994; Zhao et.al.1999).
Due to its antioxidative activity, silymarin has been found to be useful in treatment and prevention of many neurodegenerative and neurotoxic processes. Wang et. al demonstrated that silymarin could effectively protect dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting the activation of microglia which represents macrophage-like population of brain cells and which act in host defense and tissue repair in the CNS (Wang et. al. 2002). Many gastrointestinal problems can be treated and/or prevented by silybin/silymarin preparations. In the pancreas, silybin acts mainly as chemoprotectant and can stimulate recovery after intoxication. Alloxane, which causes necrosis of b-pancreatic cells and lack of insulin secretion, causes production of $\text{H}_2\text{O}_2$, which produces cellular damage followed by cell death. Silymarin, due to its antioxidant action, has been found to prevent a rise in both plasma glucose and pancreatic lipid peroxidation in the hyperglycemic rats (Soto et. al. 1998; Soto et. al. 2003)

During cancer therapy, the use of cardioprotective drugs, e.g. doxorubicin, is limited by the cardiotoxicity that is known to be mediated by oxidative stress and induction of apoptosis. Silybin, due to its antioxidant effect, can be very effective in such cardioprotective applications. In the study conducted by Chlopovkova et al., the cell membrane stabilizing and radical scavenging potency of silymarin and its isolated components helped to protect cardiomyocytes (rat) against doxorubicin-induced oxidative stress (Chlopovkova et. al. 2004).

Silymarin has been shown to exhibit preventive effects against photocarcinogenesis in various animal tumor models. Topical application of silymarin to mouse skin reduced UVB-induced tumor incidence, multiplicity, and size compared to that in nontreated animals (Katiyar et.al.1997). Silybin inhibited photocarcinogenesis in mice whether applied topically or administered in the diet (Mallikarjuna et.al.2004).

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Hussein et. al. (2007) proved in his study that histopathological examination of liver tissue reveals that ethanol abuse for 1 month had a damaging effect on the liver tissue. Dilatation with or without congestion of blood vessels, necrosis of cells and the presence of fibrosis were used as parameters for damage. Using garlic and onion oils treatment, study revealed the hepatoprotective effect of edible plants against ethanol toxicity. In the present study, sections taken control mice showed the normal structure of liver tissue composing of hepatocytes arranged in cords radiating from a central vein in an astomosing manner to form a sponge work or labyrinth. These cords are separated from each other by blood sinusoids, which are nearly equal in size (A). The hepatocytes appear polyhedral in shape. Their acidophilic cytoplasm takes a lace-like or granular appearance with clumps of basophilic material. The nuclei are vesicular, large and rounded or ovoid in shape with well-defined one or two nucleoli. (C). Ethanol treatment for 90 days had an obvious damaging effect on liver tissue in the form of dilatation with congestion of the portal vein, with thickening of its wall and marked fibrosis in the portal area extending from it in between the hepatocytes in many directions. The fibrous tissue in the portal area is formed of irregular collagenous fibers with fibrocytes. (B). Using aqueous extracts of our plants showed marked improvement in liver tissue structure in spite of the presence of slight thickening and fibrosis in blood vessels’ walls especially central veins. Hypertrophy of Kupffer cells in blood sinusoids can be explained by increased phagocytic activity to remove debris of dead cells in the stage of regeneration (D, E) Using aqueous extracts of our plants showed slightly little improvement as fibrosis remained surrounding the components of the tract and extending between the hepatocytes towards neighboring areas. Slight dilatation of blood sinusoids and hypertrophy of Kupffer cells denote the presence of edema, while the hepatocytes retain their normal structure.

The excessive consumption of ethanol by humans may be associated with a variety of haematological changes. These include macrocytosis, megaloblastic and sideroblastic erythropoiesis, stomatocytosis, thrombocytopenia, abnormal platelet function and, occasionally, granulocytopenia (Lindenbaum 1977). Malik and Wickramasinghe (1986), study suggested that neither the mice exposed to high concentrations of ethanol vapour for 24 h (acute ethanol intoxication) nor those exposed to lower concentrations for 20-43
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days (chronic ethanol intoxication) showed a reduction in the haemoglobin level. Acute ethanol intoxication caused a slight increase in the absolute blood reticulocyte count; this may have been caused by an increased release of reticulocytes from the marrow, an ethanol-induced prolongation of the maturation time of reticulocytes in the blood, or both. Macrocytosis is a very frequent abnormality in chronic alcoholics and in most cases appears to be independent of folate deficiency (Wu et al. 1974; Wickramasinghe and Saunders 1977). It was hoped that alcohol-treated mice may also develop macrocytosis, thereby providing a model with which to investigate the mechanisms underlying this abnormality. However, neither acute nor chronic ethanol intoxication caused a significant degree of macrocytosis. This finding may either reflect some species-dependent difference between mice and men in the response of the erythron to ethanol-induced damage. Alternatively it may indicate that some factor other than the intake of ethanol itself is necessary for the development of macrocytosis in humans and that this factor was absent in the mouse model under investigation.

Liver damage is associated with cellular necrosis, increases in tissue lipid peroxidation as MDA and level caused by oxidative stress and depletion in the tissue GSH levels. Moreover, serum levels of liver function parameters like ALT, AST, bilirubin, and alkaline phosphatase are elevated. The mechanism of liver fibrosis is not understood, but no doubt that oxidative stress and reactive oxygen species (ROS) play an important role in pathological changes in the liver. In this study, ethanol administration for 90 days led to induced liver fibrosis, which has been proven by the significantly difference of biochemical markers between the ethanol control and normal control groups. At the same time, the hepatoprotective effect exhibited by AEGH and AECE at dose 16mg/100 gm body wt was comparable to Silymarin at dose 10 mg/100 gm body wt in alcohol-induced liver injury mice. Treatment with the AEGH and AECE at dose 16mg/100 gm body wt has accelerated the return of the altered levels of liver function enzyme and to the near normal profile. The abnormal reconstruction of the lobular architecture, the appearance of widespread fibrosis in addition, nodular lesions of the hepatic parenchyma are the main characteristics of liver cirrhosis (Li and Crawford 2004). Our histological findings prove that the alcohol AEGH and AECE affected the recovery of liver structure in alcohol-induced liver cirrhosis mice. Indeed, there was remarkable reduction in fibrosis extent
and a decrease of stellate infiltration in mice treated with plant extract compared to control alcohol group. Histological studies confirmed the hepatoprotective effect of AEGH and AECE alcohol treated mice liver sections showed fatty degeneration of hepatocytes and necrosis of cells. The extract treatment (16mg/100 gm body wt) almost normalized these effects in the histoarchitecture of liver. Therefore, from this study the AEGH and AECE could be a hepatoprotective against alcohol induced liver damage in mice.

It has been reported that *Girardinia heterophylla* and *Colocasia esculenta* contains flavonoids, steroids, and lactones. Presence of these compounds in the extracts may be responsible for the protective effect on alcohol induced damage in mice. The antioxidant capabilities of the phenolic compounds are important for the human body to destroy the free radicals that exist in our body. Many of the polyphenols such as flavonoids have been identified as powerful antioxidants; moreover, play a significant role in the treatment of many diseases, including liver cirrhosis (Hollman and Arts 2000). On the other hand, there was a study on the effect of Silybum marianum and Cichorium intybus extracts on liver cells suggested that hepatoprotective action due to the presence of flavonoids and their antioxidant effects (Madani et.al. 2008). *G. heterophylla* and *C. esculenta* has been reported to possess antioxidant activity; furthermore, the extracts exhibited significant radical-scavenging activity probably due to the higher concentration of phenolic and flavanoids. In this study, reduced lipid peroxidation was revealed by a significant decrease in MDA level in groups treated with ethanol extracts. The results of the hepatoprotective effects of this extracts can be due to the presence of the great amount of phenolic and flavonoids compounds and their antioxidant effects besides the free radical scavenging property of these plants. Likewise, the hepatoprotective activity of the extracts could be due to neutralization of the toxic compounds produced by converting alcohol to a highly toxic metabolite during cytochrome p-450 pathway as mentioned above. On account of these *G. heterophylla* and *C. esculenta* extracts, it has been reported recently to affect cytochrome p450 enzyme system through its inhibition. Consequently, the toxic metabolite of alcohol is affected by the *G. heterophylla* and *C. esculenta* extracts that might lead to reduce the progress of liver necrosis.
6. CONCLUSION

The Himalaya is the reservoir of a variety of important plant species that produces secondary metabolites under environmental stress. As a result, the plants have both medicinal and aromatic properties. Medicinal and aromatic plants (MAPs) found in the Himalayan region include species of high ecological and economic potential and were known as high-value low-volume crops (Maikhuri et. al. 2005). Therefore, herbal medicine has been shown to have genuine utility and about 80% rural population depends on its efficacy for their primary health care. Over the years WHO advocated that countries should interact with traditional medicine with a view to identify and exploit the aspects that provide safe and effective remedies for ailments of both microbial and non-microbial agents (WHO 1978). In recent years, although technology and medicine have developed extensively, due to decreases in natural richness and drawbacks some countries have made it obligatory to use natural products for many goals. Thus, like in other countries in the world, plants known by people are picked and used for the treatment of various diseases (Basile et. al. 2000). The presence of antimicrobial substances in the higher plants is well established (Srinivasan et. al. 2001). Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contribution towards human health.

In living systems, various reactive oxygen species are generated and can cause cell damage. A major form of cellular oxidative damage is lipid peroxidation, which is initiated by reactive oxygen species through the extraction of a hydrogen atom from unsaturated fatty acids of membrane phospholipids (Farber et. al. 1990). Membrane lipids are particularly susceptible to oxidation, not only because of their high polyunsaturated fatty acid content, but also because of their association in the cell membrane with enzymic and non-enzymic systems capable of generating free radical species (Halliwell and Guttridge 1990a). Peroxidation of membrane lipid is a cardinal feature of oxy radical toxicity (Cross et. al. 1987). The chain reaction of lipid peroxidation yields several types of secondary free radicals and a large number of reactive compounds, resulting in the destruction of cellular membranes and other cytotoxic responses (Bus and Gibson 1979).
Oxidative degradation of polyunsaturated fatty acids occurs in two sequential steps (Svingen et al. 1979). The initiation reaction involves reactive oxygen species such as hydroxyl radical as initiators, forming a conjugatively stabilized carbon centered radical (L'). This reacts rapidly with oxygen to form peroxy radical (LOO•), which abstracts a hydrogen atom from another fatty acid to form lipid hydroperoxides (LOOH) and a new carbon centered radical (L') until the chain reaction is terminated (propagation). Therefore, antioxidative materials acting in living systems are classified as preventive antioxidants and chain-breaking ones (Halliwell and Gutteridge 1990b).

Antioxidative constituents in plants have other various biological activities, especially in the case of flavonoids (Middleton and Kandaswami 1994). Flavonoids in G. heterophylla and C. esculenta show potent activities in the pathogenesis of cirrhosis and in kidney diseases. These additional activities of antioxidative phytochemicals would contribute to their pharmaceutical uses. Both these plants have antimicrobial activities also. Both antioxidative and antimicrobial actions of plant materials are required in the food industry. Many kinds of plant have been used for prevention and treatment of various diseases and food preservation. Explication of their active constituents and mechanisms of their action would lead to further utilization of plant materials.

To conclude the results of this study demonstrate that the plant extracts AEGH and AECE have potent antioxidant and free radical scavenging properties and a very good hepatoprotective properties attenuates the toxic effects of alcohol by acting as an in vivo antioxidant as well as the hepatoprotective thereby inhibiting the initiation and promotion of lipid peroxidation or by an accelerated scavenging of free radicals and their products by conjugation with GSH.
7. RECOMMENDATIONS

We have selected two plants for the evaluation of nutraceutical properties. Broadly this term comprises antimicrobial, antioxidant and hepatoprotective properties. After this study, the significant observations are the aqueous extract of *Colocasia esculenta* shows best antimicrobial activity among the two plants. Antioxidative constituents in plants have other various biological activities, especially in the case of flavonoids. Flavonoids in *G. heterophylla* and *C. esculenta* show potent activities in the pathogenesis of cirrhosis and in kidney diseases. These additional activities of antioxidative phytochemicals would contribute to their pharmaceutical uses. This study demonstrates that the plant extracts AEGH and AECE have potent antioxidant and free radical scavenging properties and a very good hepatoprotective properties attenuates the toxic effects of alcohol by acting as an *in vivo* antioxidant as well as the hepatoprotective thereby inhibiting the initiation and promotion of lipid peroxidation or by an accelerated scavenging of free radicals and their products by conjugation with GSH.

Studied edible plants like *Girardinia heterophylla* and *Colocasia esculenta* are used for prevention and treatment of various diseases and food preservation. These plants are required in food industry. They are also used in clinical aspects. If they are consumed in our daily life, so we will far away form the risk of liver and kidney disorders. They are nutritious as well as free from reactions i.e. supposed to be ayurvedic in nature. This study will give the platform to highlight the submerged wild edible plants. It will also help to cultivate the wild edibles and to protect them from extinction. The proposed work will help in control, treatment and prevention of different kinds of human diseases and to understand the basic mechanism of action.

Through this study, we will give the satisfactory documentary proof regarding our work.