5. DISCUSSION

Medicinal plants maintain the health and vitality of individuals and cure various diseases, including cancer without causing toxicity. In this view, the pharacochemical characterization, GC-MS analysis and the pharmacological potential of the leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* have been discussed.

Pharmacochemical Characterization

Physicochemical constituents

Ash values

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs (African Pharmacopoeia, 1986). Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica (Musa et al., 2006).

The ash values of leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* are 8.34%, 9.56% and 10.66% respectively. These ash values are indicative of the impurities present in the drug. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug. In the present study, aerial part of *Balanites aegyptiaca* has more ash value when compared with other investigated drug samples. Samples have more water soluble ash than acid insoluble ash. The ash values are generally the index of the purity as well as identity of the drug.
**Fluorescence analysis**

Many phytocompounds fluorescence when suitably illuminated. The fluorescent colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples (Pimenta et al., 2006).

The powder from the leaf of *Pleiospermium alatum* fluoresced green under day light, dark green under short UV and dark blue in long UV light. The powdered bark of *Pleiospermium alatum* emitted yellow under day light, greenish yellow under short UV light and dark blue in long UV light. The powdered aerial part of *Balanites aegyptiaca* fluoresced greenish yellow under day light, greenish yellow under short UV and violet in long UV light.

**Phytochemical studies**

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening; chemo profiling and marker compound analysis using modern analytical techniques. In the last two decades, HPTLC has emerged as an important tool for the qualitative, semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. A HPTLC method is fast, precise, sensitive and reproducible with good recoveries for standardization of herbal drugs.
In the present study, the preliminary phytochemical study on leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* have revealed the presence of alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, saponin, tannin, terpenoid, sugar, and glycosides from the methanol and ethanol extracts of the above said plants. HPTLC investigations also confirmed the presence of alkaloids, flavonoids, glycosides, saponins and steroids which could made the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because, the pharmacological activity of any plant is usually traced to a particular compound.

Therapeutically terpenoids exert wide spectrum of activities such as antiseptic, stimulant, diuretic, anthelmintic, analgesic and counter-irritant (Gokhale *et al.*, 2003). Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering (Handa and Kapoor, 1992). They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and antidote (Ali, 1994).

Saponins, a group of natural products occur in the leaf and bark extracts of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca*. In plants, the presence of steroidal saponins like, cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D etc., (Evans and Saunders, 2001). From plants, sapogenin, a synthetic steroid is prepared and used to treat a wide variety of diseases such as rheumatoid arthritis, collagen
disorders, allergic and asthmatic conditions (Claus, 1956). Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intra-luminal physicochemical interactions. Hence, it has been reported to have hypocholesterolemic effect and thus may aid lessening metabolic burden that would have been placed in the liver (Price et al., 1987).

Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles (Oliver-Bever, 1986; Rhemann and Zaman, 1989). Flavonoids are known to regenerate the damaged beta cells in the Alloxan induced diabetic rats (Chakravarthy et al., 1980). Flavonoids act as insulin secretagogues (Geetha et al., 1994). Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc, which are frequently implicated as having antidiabetic effects (Loew and Kaszhin, 2002).

To understand the nature of the fluorescence emission from these crude preparations under different conditions, the preliminary phytochemical analysis of these crude preparations were compared. The comparative analysis clearly showed a correlation between a compound present in it and their fluorescent behaviour under different conditions. The major bioactive compounds present in these crude preparations are the coumarins, flavonones, tannins, alkaloids and saponins. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition under short UV radiation. Flavonones which are light yellow in aqueous condition under UV light turns to bright yellow under alkaline conditions. Similarly the phytosterols when treated with 50% H₂SO₄ show green fluorescence under UV light. Terpenoids especially sapogenins exhibit yellow green
fluorescence under short UV light (Horborne, 1976). Quinine, aconitin, berberin and emetin show specific colour of fluorescence (Aconitin - light blue; berberin - light yellow; emetin - orange). Fixed oils and fats fluoresce least, waxes more strongly and mineral salts most of all (Evans, 1996). Haydon (1975) studied the photophysical characters of coumarins. Hydroxy methyl coumarin fluoresced in the 420 – 440 nm when observed in different solvents with increasing polarity (Chaltopudhyay et al., 2006). The fluorescence analysis of the crude drugs of *Pleiospermium alatum* and *Balanites aegyptiaca* exhibited clear fluorescence behaviour at different radiations which can be taken as standard fluorescence pattern.

**GC – MS analysis**

Thirteen compounds were detected in ethanolic extracts of *Pleiospermium alatum* leaf. The results revealed that E-2-Hexenylbenzoate (13.84%) was found as major compound followed by 2,6,10,14,18,22- Tetracosahexane, 2,6,10,15,19,23-hexamethyl- (all-E) – [All-trans-squalene] (13.57%), 2H, 8H-Benzo [1,2-b:5,4-b’] dipyran-2-one, 8,8-dimethyl- (11.12%), Cyclohexanemethanol, 4-ethenyl-qá,á,4-trimethyl-3-(1- mithylethenyl)- [1R-91á,3á,4á] –[Elemol] (10.96%), 2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-Cyclohexen-1-one (10.35%), Lupeol (8.81%), 9,12-Octadecadienoic acid (Z,Z)- (8.36%), Acetyl turicine (8.15%), n-Hexadecanoic acid (6.11%), Phytol (5.13%). Lupeol is detected in *Pleiospermium alatum* leaf which exhibited a broad spectrum of biological activites and can be used as anticancer, antiprotozol, chemopreventive and antiinflammatory activities (Gallo and Sarachine, 2009). Eleven compounds were reported in the ethanolic extract of Pleiospermium alatum bark, the major compounds include 9, 12-Octadecadienoic acid (Z, Z)- (18.81%), All-trans-Squalene (17.55%), 1,2-Benzenedicarboxylic acid,
diisooctyl ester (13.38), n-Hexadecanoic acid (12.61%) and Diisooctyl adipate (11.80%). Among the identified phytochemicals, n-Hexadecanoic acid and Squalene have the property of antioxidant activity. 9-12, Octadecadienoic acid (Z, Z) have the property of antiinflammatory and antiarthritic as reported by the earlier worker (Lalitha Rani et al., 2009).

Five compounds were reported in the ethanol extract of aerial part of *Balanites aegyptiaca*. The result revealed that 3-O-Methyl-d-glucose (83.75%) was found as major compound followed by All -trans-Squalene (8.11%), 9,12-Octadecadienoic acid (Z,Z)- (3.92%), n-Hexadecanoic acid (3.17%), and Phytol (1.05%). Among the identified phytochemicals n-Hexadecanoic acid and squalene have the property of antioxidant activity. 9-12, Octadecadienoic acid (Z, Z) have the property of antiinflammatory and antiarthritic as reported by the earlier worker (Lalitha Rani et al., 2009). The results show that reactive oxygen species-promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi et al., 2009).

Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in these medicinal plants and this type of study will be helpful for further detailed study. Further investigations into the pharmacological importance of *Pleiospermium alatum* and *Balanites aegyptiaca* and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medical systems.
Pharmacological studies
Antidiabetic activity

Diabetes mellitus is one of the most common chronic diseases associated with carbohydrate metabolism. It is also an indication of co-morbidities such as obesity, hypertension, and hyperlipidaemia which are metabolic complications of both clinical and experimental diabetes. Alloxan, a beta cytotoxin induces chemical diabetes (Alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which paves the way for the decreased utilization of glucose by the tissues (Baynes, 1991; Saravanan and Pari, 2005; Bierman et al., 1975; Omamoto et al., 1981; Gurusamy et al., 2008). The prevention of diabetes is an urgent worldwide health concern. The period preceding the onset of type 2 diabetes is typically characterized by obesity and insulin resistance induced by over reacting and physical inactivity.

The ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part (Group IV & VI) were treated on Alloxan induced diabetic rats (Group II). The results were compared with control (Group I) and the positive control glibenclamide (Group VII) after fourteen days of treatment based on biochemical parameters. After the Alloxan induction, glucose, insulin, lipid profiles, protein and antioxidant were restored to control levels with the administration of the known drug glibenclamide and plant extracts *Pleiospermium alatum* and *Balanites aegyptiaca*. The result from the present study shows the significant changes in biochemical parameter during the experimentally inducted diabetes. Blood glucose, serum insulin, urea, creatinine levels were determined in control and ethanol extracts and glibenclamide treated rats. The administration of ethanol extracts of *Balanites*...
*Balanites aegyptiaca* aerial part decreases the blood glucose level where as serum insulin level was increased in glibenclamide treated rats compared to control rats. The hypoglycemic ethanol extracts of *Balanites aegyptiaca* aerial part were found to be inducing insulin release from pancreatic cells of diabetic rats (Sharma and Garg, 2009). Ahmed et al. (1991) have fed the ethyl acetate-soluble fraction of an absolute ethanol extract of *Pterocarpus marsupium* wood, which significantly lowered blood sugar level with corresponding increase in insulin level in Alloxan induced diabetic rats. It is evident from this study that there was an increase in insulin levels in diabetic rats treated with plant extracts. Many plants have been studied for their hypoglycemic and insulin release stimulatory effects (Al-Hader et al., 1994; Hikino et al., 1989; Ivorra et al., 1989 and Morrison et al., 1985 & 1987).

Extensive research has been conducted in the last few decades on plants mentioned in ancient literature and used traditionally for antidiabetic activity. Grover et al. (2002) have reported 45 medicinal plants and their products have been used in the Indian traditional system of medicine and shown experimental or clinical antidiabetic activity. The most effective and commonly studied antidiabetic plants are *Allium cepa*, *A. sativum*, *Aloe vera*, *Gymnema sylvestre*, *Syzygium cumini*, *Ficus benghalensis*, *Rubia cordifolia* and *Tinospora cordifolia* (Grover et al., 2002; Ziyyat et al., 1997 and Mohana Rao et al., 2005).

A significant elevation in serum constituents, urea and creatinine were observed in Alloxan induced diabetic rats (Group II), when compared to control rats. The ethanol extracts of *Balanites aegyptiaca* aerial part were administered orally to rats for fourteen days and this reversed the urea and creatinine level to near normal.
The administration of glibenclamide also decreased the levels of urea and creatinine to some extent.

Alloxan is taken as an indication of an abnormal glomerular function where a single injection of cisplatin at a dose of 5 mg/kg body weight in rabbits caused a marked reduction in the glomerular filtration rates, which was accompanied by an increase in the serum creatinine level, indicating the induction of acute renal failure. It is confirmed that there is a significant increase in serum creatinine in albino rats 14 days after Alloxan administration. The present result shows that, the treatment with ethanol extracts of *Balanites aegyptiaca* aerial part were effective in preventing Alloxan induced increase in serum creatinine level when compared with the control.

The levels of serum protein, albumin, globulin of control and Alloxan induced diabetic rats are presented in Table 16. A significant reduction in serum protein, albumin and globulin were observed in Alloxan induced diabetic rats (Group II), when compared to control (Group I) and glibenclamide treated rats (Group VII). On administration of ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part to the diabetic rats, protein, albumin and globulin levels were found to be restored in normal. These results were in accordance with the effect of *Artemisia herba-alba* and *Teucrium polium* in diabetic rats (Iriadam *et al.*, 2006). The increased level of serum protein, albumin and globulin in alloxan induced diabetic rats are presumed to be due to increased protein catabolism and gluconeogenesis during diabetes (Palanivel *et al.*, 2001).

Table - 16 summarizes the effect of Alloxan on the activity of the hepatic marker enzymes in serum. The animals treated with alloxan developed hepatic
damage which was evident from the increase in the enzyme activities. Pretreatment
with ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca*
aerial part and glibenclamide resulted in a decrease of transaminase activities in
Alloxan treated rats. The serum AST and ALT levels increases as a result of metabolic
changes in the liver such as administration of toxin, cirrhosis of the liver, hepatitis and
liver cancer including diabetes (Chalasani *et al.*, 2004). Similarly in the present study,
it was observed that the levels of SGPT and SGOT in Alloxan induced diabetic rats
were elevated. It may be due to leaking out of enzymes from the tissues and migrating
into the circulation by the adverse effect of Alloxan (Stanely *et al.*, 1999). AST and
ALT were used as markers to assess the extent of liver damage in streptozoctocin
induced diabetic rats (Hwang *et al.*, 2005).

In this study, the ethanol extracts of *Pleiospermium alatum* leaf and bark and
*Balanites aegyptiaca* aerial part regulated the activity of SGPT, SGOT and ALP in
liver of rats intoxicated with Alloxan. The effect of glibenclamide on the recovery of
hepatic enzyme activity in serum was very similar to that of the earlier study (Preethi
and Kuttan, 2009).

The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG),
HDL – C, LDL-C, VLDL-C, PL and LDL /HD in control and experimental animals
were investigated (Table - 17). Alloxan induced diabetic rats showed significantly
increased serum lipid profiles except HDL-C, when compared with normal rats. The
glibenclamide and ethanol extracts of *Pleiospermium alatum* leaf and bark and
*Balanites aegyptiaca* aerial part treated rats showed a significant decrease in the
content of lipid profiles, when compared with diabetic induced rats. Similarly HDL-C
level decreased in Alloxan induced diabetic rats when compared with normal rats. On administration of ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents a risk factor for coronary heart diseases (Mironova *et al.*, 2000). Lowering the serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease (Scott and Grundy, 1999).

During diabetes, there is an enhanced activity of the enzyme, increased lipolysis and releases more fatty acids into the circulation (Agarth *et al.*, 1999). The increased fatty acid concentration also increases the β-oxidation of fatty acids, producing more acetyl Co-A and cholesterol during diabetes. In normal condition, insulin increases receptor-mediated removal of LDL-cholesterol and decreased activity of insulin, during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceremia have been reported to occur in diabetic rats (Mironova *et al.*, 2000). The increased concentration of free fatty acid may be due to lipid break-down and this may cause increased generation of NADPH-dependent microsomal lipid peroxidation. Phospholipids were increased in Alloxan induced diabetic rats. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core (Cohn and Roth, 1996). Increased phospholipids levels in tissues were reported by Venkateswaran *et al.* (2002) and Pari and Satheesh (2004) in streptozocnin diabetic rats. Administration of ethanol extracts of *Pleiospermium alatum* leaf and bark and
*Balanites aegyptiaca* aerial part and glibenclamide decreased the levels of phospholipids.

The results (Table - 18) showed increased lipid peroxidation (LPO) of Alloxan induced diabetic rats. Earlier studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats (Latha and Pari, 2003a and Ananthan et al., 2004). This may be because the tissues contain relatively high concentration of early peroxidizable fatty acids. In the present study, an increase in the levels of LPO was found and these levels were significantly reduced after the supplementation of the ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part and glibenclamide (Table - 18). These indicate that, plant extract inhibit oxidative damage due to the antiperoxidative effect of ingredients present in ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part. This could be correlated with previous study which reported that *Cassia auriculata* flower (Pari and Latha, 2002) *Syzigium cuminii* (Prince and Menon, 1998 and Prince et al., 2004); *Tinospora cordifolia* (Prince et al., 1999) and *Scoparia dulcis* (Latha and Pari, 2003b) has antiperoxidative and antihyperlipidaemic effect of diabetic animals. Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the lipid metabolism. Insulin is a potent inhibitor of lipolysis, since it inhibits the activity of hormone sensitive lipase in adipose tissue and suppresses the release of free fatty acids (Loci et al., 1994).

The level of serum superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx) and reduced glutathione (GSH) in control and experimental rats were studied. A highly significant reduction in the activity of scavenging mitochondrial
enzymes is observed in Alloxan induced rats. These adverse changes were reversed to near normal values in ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part treated group III as well as glibenclamide treated rats group VII (Table -18).

Mitochondria are the energy reservoir of the cell and the damage inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leading to cell death (Sohal and Dubey, 1994). Subcellular membrane, associated with thiol bearing enzymes, represents sensitive sites for detoxification causing perpetuation of cellular function (Kyu and Byung, 1997). Reactive oxygen species can themselves reduce the activities of anti-oxidant defence mechanism. In the present study, ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part have enhanced mitochondrial enzymatic antioxidant activity and suppressed lipid peroxidation.

Free radical reacts with lipids causing peroxidation, resulting in the release of products such as malondialdehyde, hydroperoxide and hydroxyl radicals. These extracts have the capacity to scavenge free radicals directly or interfering with generation of free radicals (Reddy and Lokesh, 1992 and Dhuley *et al.*, 1993). Thus, the inhibitory effects of these extracts on oxidative damage may be attributed to the suppression induced peroxidation (Selvendiran *et al.*, 2004). It is well known that CAT, SOD and GPx play an important role as protective enzymes against free radical formation in tissues (Oberly and Buettner, 1974). Several investigators have reported that, the reduced activities of CAT and SOD genes are induced by free radicals and also by certain humoral factors (Anderson *et al.*, 1994 and Slaga, 1995). The present
study indicates the reduction in the activity of SOD, CAT, GPx and GSH in Alloxan induced rats (Group II). These results reveal the protective role of plant extract in decreasing lipid peroxidation and by normalizing antioxidant system.

It is concluded that, medicinal plants have been reported to possess antihyperglycemic activity. The preliminary investigation on the antidiabetic efficacy of ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity. Of the studied plants, *Balanites aegyptiaca* aerial part showed more hypoglycemic activity.

**Antihyperlipidaemic activity**

Lipid is an important part of a healthy body because it is used to form cell membranes, sexual hormones and is necessary for other cellular functions. The various forms of lipids cannot dissolve in the blood and must be transported to and from the cells by low density and high density lipoprotein. High density lipoprotein cholesterol (HDL-C) tends to carry cholesterol away from arteries back to the liver. Therefore, high serum cholesterol level can be due to hepatic dysfunction (Gupta et al., 2008). Triton X-100 induced rise in serum TC, TG, LDL-C, VLDL-C and PL and fall in serum HDL-C in Group II rats. The rise in serum triglycerides is possibly due to hypoactivity of lipoprotein lipase in blood vessels which breaks up triglycerides. The high TG level along with decreased absorption of fatty acids by adipose is associated with a low level of HDL-C, insulin resistance and increased risk of antherosclerosis (Terasawa et al., 2000). In the present study, high cholesterol level in Triton X-100 intoxication may also be due to decreased activity of cytochrome P450
enzymes (Witmer et al., 1994). The rise in serum lipid profiles may also be attributed to increased lipolysis, medicated by increased nonepinopterine release which act through interface with the intracellular fraction of Ca^{2+} in the cytoplasm (Liu and Lin, 1997).

Administration of Pleiospermium alatum leaf and bark and aerial part Balanites aegyptiaca extracts caused a significant decrease in serum TC, LDL-C, VLDL-C and PL suggesting beneficial modulatory influence on cholesterol metabolism and turn over. Elevated serum triglycerides is considered as independent risk factor for cardiovascular disease (Asia Pacific Cohort Studies Collaboration, 2004). A significant decline in the serum triglycerides level observed in Pleiospermium alatum leaf and bark extracts treated rats supports the cardiovascular protective influence.

Triton X-100 treated rats caused a considerable accumulation of TL, TC and TG in liver. These results are supported by many earlier studies (Mehta et al., 2003 and Purohit and Vyas, 2006). Simultaneous administration of Pleiospermium alatum leaf and bark and aerial part of Balanites aegyptiaca extracts caused a significant decline in the TL, TC and TG contents in liver indicating hypolipidaemic effects.

The cholesterol lowering effects of the Pleiospermium alatum leaf and bark and aerial part of Balanites aegyptiaca extracts is possibly associated with a decrease in intestinal absorption of cholesterol resulting in an increase in fecal excretion of neutral lipids (Purohit and Vyas, 2006).
From these results it can be concluded that, ethanol extracts of leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegytiaca* contains active compounds which decreases serum lipid profiles and lowers the risk of atherosclerosis in antihyperlipidaemic rats.

**Hepatoprotective activity**

Liver diseases remain as one of the most serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play an important role in the management of various liver disorders. A number of plants show hepatoprotective activity (Malhotra *et al.*, 2001). Based on the promising results shown by the plant extracts in the *in vitro* studies, *in vivo* hepatoprotective studies were carried out in experimental rats using CCl$_4$ induced heptotoxicity.

Any increase in the level of serum AST, ALT, ALP, ACP and LDH activity is an indication of hepatic disease. Defect in protein metabolism, evidenced by changes in total protein and / or albumin level, are used to indicate the severity of the hepatic disease (Henry, 1984). In the present investigation, the rats treated with hepatotoxicant CCl$_4$, transaminases (AST and ALP), ALP were increased (Table 22) remarkably in plasma by the release of these enzymes from hepatic parenchyma cells, which were indicating a considerable hepatocellular injury (Bishayee *et al.*, 1995). Oral treatment with drug silymarin, ethanol extracts of *Pleiospermium alatum* *Balanites aegytiaca* leaves attenuated these increased enzyme activities produced by CCl$_4$. In the present investigation, results coincide with the reports of Shah *et al.* (2002) who showed an elevation in the levels of AST, ALT and ALP in hepatotoxic rat models and its restoration by *Phyllanthus debilis* plant extract. An elevation of LDH
levels in hepatotoxic rats and its restoration by curcumin in in vitro liver slice cultures was reported by Naik and Ghaskadbi (2004).

The CCl$_4$ treated group showed an elevation in the levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin when compared to control (Table 23). The administration of ethanol extracts of *Pleiospermium alatum* and *Balanites aegyptiaca* (Group III & IV) showed significant restoration of levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin. The present results coincide with that of the results of Rajkapoor *et al.* (2002), who showed an elevation in AST, ALT, ALP and total bilirubin, in hepatotoxic rats and their restoration to near normal levels by *Nigella sativa* administration. Sethuraman *et al.* (2003) have also shown a similar curative effect of *Sarcostemma brevistigma* against CCl$_4$ induced hepatic damage in rats.

The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effect or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin (Hukkeri *et al.*, 2003).

Aspartate and alanine transaminases are present in high concentrations in liver, due to hepatic necrosis or normal membrane permeability. These enzymes are released from the cells and their levels increase in the blood. It is a sensitive indicator of acute liver damage.
Alkaline phosphatase is a membrane bound enzyme and its elevation in the plasma indicates membrane disruption in the organ. The level of this enzyme increases in cholestasis (Shah et al., 2002).

Hypoproteinemia is most frequent in the presence of advanced chronic liver diseases (Venukumar and Latha, 2002). Hence, the decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. The lowered level of total proteins recorded in the serum of CCl₄ treated rats reveals the severity of hepatopathy. The attainment of near normalcy in total protein content of serum of the treated rats confirms its hepatoprotective nature.

The increase in malondialdehyde (MDA) levels in plasma suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with ethanol extracts of *Balanites aegyptiaca* whole plant significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by ethanol extracts of *Balanites aegyptiaca* is due to its antioxidant effect.

The recovery observed in various serum biochemical parameters after the treatment with ethanol extracts of *Pleiospermium alatum* leaf and bark and *Balanites aegyptiaca* aerial part indicates that these plant extracts are effective in the treatment of CCl₄ induced liver dysfunction in animal models.

**Antifertility activity**

The results revealed no change in the body weight of rats treated with leaf and bark extracts of *Pleiospermium alatum* and *Balanites aegyptiaca* aerial part extracts
(200 mg / Kg body weight) for fourteen days. The testis and other accessory sex organs were decreased significantly during the experiment. Among the accessory sex organs, a significant weight reduction was seen in the testis, caput and caudal epididymal segments and the weight reduction was dose dependent. Reduction in the weight of testis and other accessory sex organs might be due to low level of androgen, which was not enough to maintain the weight of gonads and accessories (Sharma and Jacob, 2001). It is known that the accessory sex organs viz., epididymis and vas deferens are androgen dependent target organs and manifest differential sensibility to androgens for maintenance of their structure and function. It is also known that any change in circulating androgens would affect the internal micro environment of epididymis and thereby lead to alternation in sperm motility and metabolism (Khan and Awasthy, 2003).

In the present study, ethanol extracts of *Pleiospermium alatum* and *Balanites aegyptiaca* treated rats decreased the sperm motility and sperm density in cauda and caput epididymal segments. Drastic effect on the nature of the normal sperms in the caput and caudal region was observed in ethanol extracts of *Pleiospermium alatum* and *Balanites aegyptiaca* treated rats. Further tail region of the sperm in all the treated groups (Groups II, III and IV) were much affected than the head regions. The development of normal and mature sperm is the key to optimum male fertility. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary (Steinberger, 1971). FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in the leydig cells of the testis (Kerr and Klester, 1975). Many studies on
the testis of rat treated with plant extracts has also demonstrated that the inhibitory activity on the proliferation of spermatogonia in mammals (Steinberger et al., 1964; Mancini et al., 1967 and Krueger et al., 1974). Spermatogenesis is therefore, a complicated process, covering proliferation of the spermatogonia, long-lasting process of the tissue meiosis and numerous changes in the spermatids during their pre-formation (Steinberger, 1971 and Kerr and Klester, 1975). The result of the present study suggests that, ethanol extracts of Pleiospermium alatum and Balanites aegyptiaca may affect the normal function of the sertoli and leydig cells on continuous oral administration for twenty one days.

Sexual cells can occur during the reproductive phase, mitotic division of the spermatogonia or during the maturation of the spermatozoa, thereby affecting the number and quality of the sperm cells produced in the testis. Among the ethanol extracts treated groups III and IV (200 mg / kg body weight) produced a significant reduction in total sperm count and viable sperms. This may be as a result of the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis (Bowman and Rand, 1985 and William, 2000). The presence of immature sperms was also observed in the experimental rats treated with 200 mg / kg body weight of ethanol extracts of Pleiospermium alatum and Balanites aegyptiaca. This suggests that the 200 mg / kg body weight dose level could affect the maturation of the spermatozoan in the male rats, which might also be a contributory factor to the decrease in the mean total sperm count. The data generated in the present study, by and large, confirm to those already reported and studied with
various plant extracts (Njar et al., 1995; Raji and Bolarinwa, 1997 and Parveen et al., 2002). The decrease in the caudal epididymal sperm counts are clear indications that ethanol leaf extracts of Pleiospermium alatum and Balanites aegyptiaca can affect one or more aspects of spermatogenesis as well as spermiogenesis. Though a direct effect of ethanol extracts of Pleiospermium alatum and Balanites aegyptiaca on the cellular mechanisms of spermatogenesis cannot be concluded, it is likely that the impairment of the hormonal mechanisms concerned with the regulation of spermatogenesis may be the underlying cause.

The various other sperm abnormalities, like sluggish motility, coiled tail and sperm maturation are also due to ethanol extracts of Pleiospermium alatum and Balanites aegyptiaca toxicity. The hitherto unreported abnormal sperm morphology, coiled tail and malformed head could be attributed to both testicular and epididymal effects of Pleiospermium alatum and Balanites aegyptiaca extracts. Coiling of the sperm tail is usually the product of abnormal axoneme and / or the outer dense fibril. The outcome of the present study affirms the male reproductive toxic effects of Pleiospermium alatum and Balanites aegyptiaca extracts when applied as a therapeutic agent. Since male reproductive toxicology and male contraception are two sides of the same coin, the negative consequence of Pleiospermium alatum and Balanites aegyptiaca extracts on the sperm may be taken as an advantage for further study. By the treatment employed, in the study, no toxic effect was produced in the liver and kidney, neither was it directly involved on the development and functioning of the male reproductive system nor in the reproductive organs.
In the present study, a significant decrease in the sperm density and motility was observed in the cauda epididymis in all the treatment group, which leads to proven in the impairment of fertility in all the treated groups. The results presented in this study also indicated that the treatment with the ethanol extract of leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* by adult male rats reduces the number of female’s impregnation. In addition, the number of implantations and the number of viable features were also decrease, this decreased could be a reflect and may be due to the decrease in sperm motility and sperm density observed in this study. Hence, this may be due to the effects of the given plant extracts on the enzymes involved in the oxidative phosphorylation process.

The present study revealed a decrease in the serum level of testosterone. This observation was similar to the earlier findings of Udoh and Kehinde, (1999); Udoh and Ekpenyong (2001) and Udoh *et al*., (2005a). The reduction in the serum level of testosterone could be probably due to the decrease of serum levels of LH / ICSH observed in this investigation. Leydig cells secrete testosterone by the stimulatory effect of LH (Udoh and Udoh 2005 and Udoh *et al*., 2005 b). In males reduction of testosterone level may impair spermatogenesis and cause male infertility. This study further observed a dose dependent increase in the serum estrogen level. This increase might probably be due to the conversion of testosterone to estrogen (Carr and Blackwell, 1993 and Chinoy and Padman, 1996).

Treatment with the ethanol extracts of *Pleiospermium alatum* and *Balanites aegyptiaca* (200mg / kg body weight) was highly effective in producing reversible functional sterility. The drug treated male rats clearly indicated structural and
functional alteration in testis, epididymis and seminal vesicle. Depletion of sperm count and sperm motility in the drug treated rats suggests alteration in sperm production in the testis and maturation in the epididymis. Changes in both sperm count and motility resulted in partial infertility within twenty one days. This resulted in abnormal sperm functions which ultimately gave rise to complete male sterility. Among the plant based contraceptives, inhibition of male fertility after administration of natural substances has been related to decreased spermatozoa density (Watcho et al., 2001). For male contraception, it is not necessary to stop spermatogenesis, but it is enough to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm (Dwivedi et al., 1990).

The activity of *Pleiospermium alatum* and *Balanites aegyptiaca* species has been attributed to the action of various steroidal saponin. Saponins are important mainly because of their steroid structure. They are precursors for the hemisynthesis of birth control pills (with progesterone and estrogens) as well as similar hormones and corticosteroids (Crabbe, 1979). Recently many laboratories are engaged in developing male contraceptives from plants (US National Academy of Sciences, 1992). Plant products as contraceptives will be more acceptable for economic reasons in terms of self reliance and the possible practicability for a male pill approach in countries where population pressure is high. Recently, extensive efforts have been made to study the antifertility drugs from plants (Handelsman, 1994; Khan and Awasthy, 2003 and Upadhyay et al., 1993). In the present study, treatment of *Pleiospermium alatum* and *Balanites aegyptiaca* extracts show marked alterations in the male reproductive organs. Further studies are needed to prove whether the
alterations are reversible or permanent after cessation of treatment and for understanding the exact mechanism.

**Antiinflammatory activity**

In the present study, the antiinflammatory activity of the leaf and bark extracts of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* have been established. The extracts were found to be significantly inhibiting the carrageenan-induced rat paw oedema, a test which has significant predictive value for antiinflammatory agents acting by inhibiting the mediators of acute inflammation. Carrageenan-induced inflammation is useful in detecting orally active antiinflammatory agents (Di Rosa *et al*., 1971 and Ismail *et al*., 1997). The development of carrageenan-induced edema is believed to be biphasic (Vinegar *et al*., 1969). The initial phase is attributed to the release of histamine and serotonin. The edema produced at the peak 3 hours is thought to be due to the release of Kinin-like substances, especially bradykinin (Crunkhon and Meacock, 1971). The second phase of edema is due to the release of prostaglandins, protease and lysosomes and it is sensitive to most antiinflammatory drugs (Vinegar *et al*., 1969 and Di Rosa *et al*., 1971).

Results of the present study are suggesting that, the drugs under investigation predominantly inhibit the release of prostaglandin like substances. The leaf extracts of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* possessed varying degree of antiinflammatory activity when tested at two different doses. The leaf extracts of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* at the dose of 200 mg / kg showed high significant antiinflammatory activity at 3rd hours, where it caused 52.88% and 64.46% inhibition respectively, as compared to that of 10 mg/kg
of Indomethacin (56.86%). n-Hexadecanoic acid, 9,12-Octadecenoic acid (Z,Z), squalene and lupeol were reported in the ethanol extracts of *Pleiospermium alatum* whereas n-Hexadecanoic acid, squalene, phytol and 9,12-Octadecenoic acid (Z,Z) in *Balanites aegyptiaca* aerial part extracts were reported by GC –MS analysis. These compounds may have the role in antioxidant and antiinflammatory effects.

Since the plants, *Pleiospermium alatum* and *Balanites aegyptiaca* extracts are useful in traditional medicine for the treatment of various ailments, it is important to standardize its use as a drug. Further studies can be made on this investigation to correlate the pharmacological and the phytochemical principles to elucidate the exact mechanism in their activity.