DISCUSSION

Medicinal plants maintain the health and vitality of individuals and cure various diseases, including cancer without causing toxicity. In this view, the pharmacochemical characterization, GC-MS analysis and the pharmacological potential of the whole plant of *Polygala javana*, *P. chinensis* L. and *P. rosmarinifolia* 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 whole plant of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* are 8.36%, 9.54% and 9.28% 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, whole plant *P.chinensis* 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 whole plant of *Polygala javana* fluoresced light green under day light, yellowish green under short UV and dark green in long UV light. The powdered whole plant of *Polygala chinensis* emitted pale brown yellow under day light, light green, yellow under short UV light and dark blue in long UV light. The powdered whole plant of *Polygala rosmarinifolia* fluoresced pale brown under day light, light green under short UV and dark blue 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 whole plant of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 make 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 a 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 whole plant of *Polygala javana, P. chinensis* and *P. rosmarinifolia*. 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 whole plant of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* exhibited clear fluorescence behaviour at different radiations which can be taken as standard fluorescence pattern.

**GC – MS analysis**

Sixteen compounds were detected in ethanol extracts of *Polygala javana* whole plant. The results revealed that, Polygalitol (84.79%) was found as major compound followed by 1H- Perimidine, 2,3- dihydro – 2 (2,4,5- trimethoxyphenyl – (6.33%). 4H – 1 – Benzopyran – 4 – one, 5- hydroxy – 2- (4- hydroxyphenyl) - 3,7 – dimethoxy – (1.53%), Ledene oxide – (1) (1.43%) and phytol (1.28%). Fourteen compounds were reported in the ethanol extract of *polygala chinensis* whole plant. The major compounds include 1,5- Antydro – d- mannitol (92.30%), 9H- furo [2,3-H] chromene – 2,8 – dione, 4- methyl 1-9- (3,4,5- trimethoxybenzylidene)- (2.11%) and propane, 1,1,3-triethoxy (1.80%). Twelve compounds were reported in the ethanol extract of *Polygala rosmarinifolia* whole plant. The results revealed that 1,5 – Anhydro - d - mannitol (73.35%), was the major compound followed by Benzene 1,2 – dimethoxy – 4[(4- methylphenyl) sulfonyl methyl]- (9.80%) d- mannitol 1-Decysulfonyl (5.12%), 9- Octadecenoic acid (Z) – phenylmethyl ester (4.72%), Squalene (3.22%) and propane 1,1,3- triethoxy- (2.21%). Among the identified phytochemicals, 1,5- Anhydro – d- fructose is a metabolite of 1,5 – Anhydro - d- mannitol, is an useful anticarcinogenic agent, as it inhibits the growth of the oral pathogen *Streptococcus mutans*. It also shows antiinflammatory and anticancer effects. 1,5 Anhydro – d- mannitol is the major component found in the whole plant of *Polygala chinensis* and *Polygala rosmarinifolia* which is being used for pharmacological work.
Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in medicinal plants and this type of study will be helpful for further detailed study. Further investigations in the pharmacological importance of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medical systems.

**Pharmacological studies**

**Antioxidant activity**

**DPPH radical scavenging activity**

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva et al., 2002; Amarowicz et al., 2004; Raun et al., 2008; Wei et al., 2010). DPPH accepts an electron or hydrogen radical to become stable diamagnetic molecule (Siddaraju and Dharmesh, 2007). The use of DPPH scavenging assay among the assays involves in assessing the cell membrane integrity cell membrane stabilizing capacities of plant constituents and further it gives explanations as the possible ways by which phytomedicine could help to reduce diseases caused by infections, inflammation and oxygen radical generation that effects the cell membrane (Shahidi and Wanasundara, 1992).

DPPH radical scavenging system was used to evaluate the antioxidant property of ethanol extracts of *Polygala Javana*, *P. chinensis*, and *P. rosmarinifolia* whole plant. The experimental data of the plants extracts revealed that the extracts are likely to have the effects of scavenging free radicals. From the results, DPPH was observed a dose dependent relationship in the DPPH redial scavenging activity. The involvement of free radicals, especially their increased production, appears to be a feather of most of the human disease including cardiovascular disease and cancer. Flavonoids and phenols compounds of *Polygala Javana*, *P. chinensis*, *P. rosmarinifolia* are possibly involved in its antiradical activity.
Anticancer activity

The present investigation was carried out to evaluate the antitumour activity of ethanol extracts of whole plant of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* in DAL tumor bearing mice. The ethanol extracts of above said plants treated animals at the dose of 100 mg/kg significantly inhibited the tumour volume and tumour (viable) cell count and brought back the haematological parameter to more or less normal levels.

In DAL tumor bearing mice, a regular rapid increase in ascetic tumour volume was observed. Ascetic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumour growth would be a means to meet the nutritional requirement of tumour cells (Prasad and Giri, 1994). Treatment with ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals (Clarkson and Burchenal, 1965). It may be concluded that ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* by decreasing the nutritional fluid volume and arresting the tumour growth and increase the life span of DAL bearing mice. Thus, ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* has antitumour activity against DAL bearing mice.

In cancer chemotherapy the major problems that are being encountered are of myelosuppression and anaemia (Price and Greenfield, 1958; Hogland, 1982). The anaemia encountered in tumour bearing mice is mainly due to reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency order to haemolytic or myelopathic conditions (Fenninger and Mider, 1954). Treatment with ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* of brought
back the haemoglobin (Hb) content, RBC and WBC count more or less to normal levels. This clearly indicates that ethanol extracts of whole plant of *Polygala javana, P. chinensis* and *P. rosmarinifolia* posses protective action on the haemopoietic system.

The results of the present study demonstrates that the ethanol extracts of *Polygala javana, P. chinensis* and *P. rosmarinifolia* increased the life span of DAL tumor bearing mice, reduce tumour volume and improve the haematological parameters. The association between flavonoids and reduced cancer risk has been reported in previous studies that showed a decrease in cancer risk with consumption of vegetables and fruits rich with flavonoids (Ferguson *et al.*, 2004; Park *et al.*, 2008). The results of this study are accordance with this finding since the phytochemical screening showed the presence of flavonoids in ethanol extracts of *Polygala javana, P. chinensis* and *P. rosmarinifolia*. While the presence of alkaloids with flavonoids in *Polygala javana, P. chinensis* and *P. rosmarinifolia* extracts may explain its superior activity compared with other plants studied (Wamidh and Mahaaneh, 2010). The anticancer activity of total flavonoids and alkaloids isolated from different plants were reported earlier (Vijayan *et al.*, 2004; Park *et al.*, 2008). Plants derived compounds have played an important role in the development of several clinical useful anticancer agents (Cragg and Newmann, 2006). Since the phytochemical screening, *Polygala javana, P. chinensis* and *P. rosmarinifolia* whole plant showed the presence of alkaloids, flavonoids, terpenoids, steroids, saponins, glycosides and phenols which could make the plants useful for treating anticancer drug. Further, the isolation of the compounds responsible for the activity has to be taken up which may result in a modern drug from these plants.
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 preceeding 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* (GroupIII, IV & V) 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 of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia*. 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant 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 *Polygala javana, P. chinensis* and *P. rosmarinifolia* whole plant 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 *Polygala javana, P. chinensis* and *P. rosmarinifolia*, 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 *Polygala javana, P. chinensis* and *P. rosmarinifolia* 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 Polygala javana, P. chinensis and P. rosmarinifolia 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. 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 Polygala javana, P. chinensis and P. rosmarinifolia whole plant 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 - 20 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 Polygala javana, P. chinensis and P. rosmarinifolia whole plant 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 streptozocin induced diabetic rats (Hwang et al., 2005).

In this study, the ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 /HDL in control and experimental animals were investigated (Table - 21). Alloxan induced diabetic rats showed significantly increased serum lipid profiles except HDL-C, when compared with normal rats. The glibenclamide and ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normalcy. 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-mediator removal of LDL-cholesterol and decreased activity of insulin, during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglycemia 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 streptozocotcin diabetic rats. Administration of ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant and glibenclamide decreased the levels of phospholipids.

The results (Table – 22,23,24 ) showed increased lipid peroxidation (LPO) on serum, liver and kidney 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant and glibenclamide. These indicate that, plant extract inhibit oxidative damage due to the antiperooxidative effect of ingredients present in ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant. This could be correlated with previous study which reported that *Cassia auriculata* flower (Pari and Latha, 2002) *Syzigium cumini* (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 on serum, liver and kidney. 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant treated as well as glibenclamide treated rats.

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 whole plant of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity. Of the studied plants, *P. chinensis* whole plant showed more hypoglycemic activity.
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 ) 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 whole plant of Polygala javana, P. chinensis and P. rosmarinifolia 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 26). The administration of ethanol extracts of Polygala javana, P. chinensis and P. rosmarinifolia (Group IV, VI & VIII) 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 CCl₄ 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).

Hyoproteinemia 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 *Polygala javana*, and *P. chinensis* whole plant significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by ethanol extracts of *Polygala javana*, and *P. chinensis* is due to its antioxidant effect.

The recovery observed in various serum biochemical parameters after the treatment with ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* indicates that these plant extracts are effective in the treatment of CCl4 induced liver dysfunction in animal models.

**Antifertility activity**

The results revealed little change in the body weight of rats treated with whole plant *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia*. 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* can affect one or more aspects of spermatogenesis as well as spermiogenesis. Though a direct effect of ethanol extracts of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* toxicity. The hitherto unreported abnormal sperm morphology, coiled tail and malformed head could be attributed to both testicular and epididymal effects of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 Polygala javana, P. chinensis and P. rosmarinifolia 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 whole plant of Polygala javana, P. chinensis and P. rosmarinifolia 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 deeneared, 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* (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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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
In the present study, treatment of Polygala javana, P. chinensis and P. rosmarinifolia 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 whole plant extracts of Polygala javana, P. chinensis and P. rosmarinifolia 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 whole plant extracts of Polygala javana, P. chinensis and P. rosmarinifolia possessed varying degree of antiinflammatory activity when tested at two different doses. The whole plant extracts of Polygala javana, P. chinensis and P. rosmarinifolia at the dose of 200 mg / kg showed high significant antiinflammatory activity at 3rd hours, where it
caused 58.79%, 65.26% and 60.40% inhibition respectively, as compared to that of 10 mg/kg of Indomethacin (67.11%).

Since the plants, *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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.