Results and Discussion

4.0 RESULTS AND DISCUSSION

Medicinal plants have been used as sources of medicine in virtually all cultures. During the last decade, the use of traditional medicine has expanded globally and is gaining popularity (Devi *et al.,* 2008). Plants are known to contain various active principles of therapeutic value and possess biological activity against a number of diseases (Ayyanar, *et al.*, 2008). A number of plants with curative values have been identified in Ayurveda and other systems of Indian medicine.

India is well known for its herbal wealth. Management of Diabetes without any side effect is still a challenge to the medicinal community. There is continuous search for alternative drugs. Traditional antidiabetic plants might provide new oral hypoglycemic compounds which can counter the high cost and poor availability of the current medicines in developing countries (Prasad *et al.*, 2009). Plant extracts exhibit islet regeneration/protection properties and therefore has beneficial effects in diabetes mellitus that holds the hope of new generation of antidiabetic drugs (Bhat *et al.*, 2008).

Medicinal and aromatic plants, a gift of nature are being used against various infections and diseases. The importance of antioxidant constituents of plant material maintained the health and provides protection. They are the potential substances that protect the body from oxidative damage caused due to free radicals (Khalil *et al.*, 2007). Antioxidants are vital substances that possess the ability to protect the body from damages caused by free radical-induced oxidative stress. Plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Souri *et al.*, 2008).

In the present investigation, entitled "**Antidiabetic and Antioxidant effect of *Aristolochia bracteolata* in Streptozotocin induced Diabetic rats**", diabetes was induced by the administration of Streptozotocin (60mg/kg) dissolved in sodium citrate buffer (PH 4.5). The hypoglycemic effect of the selected plant was analyzed after 30 days of treatment in normal and Streptozotocin induced diabetic rats.

The antioxidant potential was evaluated in methanolic extract of *Aristolochia bracteolata* for the presence of enzymic (Catalase, Peroxidase, Superoxide dismutase, Glutathione-S-Transferase) and non-enzymic (Ascorbic acid, Reduced glutathione and α-Tocopherol) antioxidants. The free radical scavenging potential of the plant was determined interms of *invitro* Lipid peroxidation, Superoxide generation, Nitric oxide generation, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) radical formation. The possible improvement effects of *Aristolochia bracteolata* extracts on histopathological changes in liver tissue of normal and Streptozotocin induced diabetic animals were examined using haemotoxylin and eosin staining. The findings of the study are discussed under the following headings:

4.1. Antidiabetic effect of *Aristolochia bracteolata*

4.2. Antioxidant potential of *Aristolochia bracteolata*

4.2.1. Enzymic antioxidants

4.2.2. NonEnzymic antioxidants.

4.3. Free radical scavenging effect of *Aristolochia bracteolata*

4.3.1. Effect of plant extract on the inhibition of *invitro* lipid peroxidation, superoxide generation and nitric oxide generation.

4.3.2. Effect of plant extract on DPPH and ABTS radical formation.

4.4. Histopathological Examination

4.1 ANTIDIABETIC EFFECT OF *Aristolochia bracteolata*

The antihyperglycemic activity of the selected medicinal plant was analyzed and the blood glucose levels are presented in the table I and figure I.

TABLE I

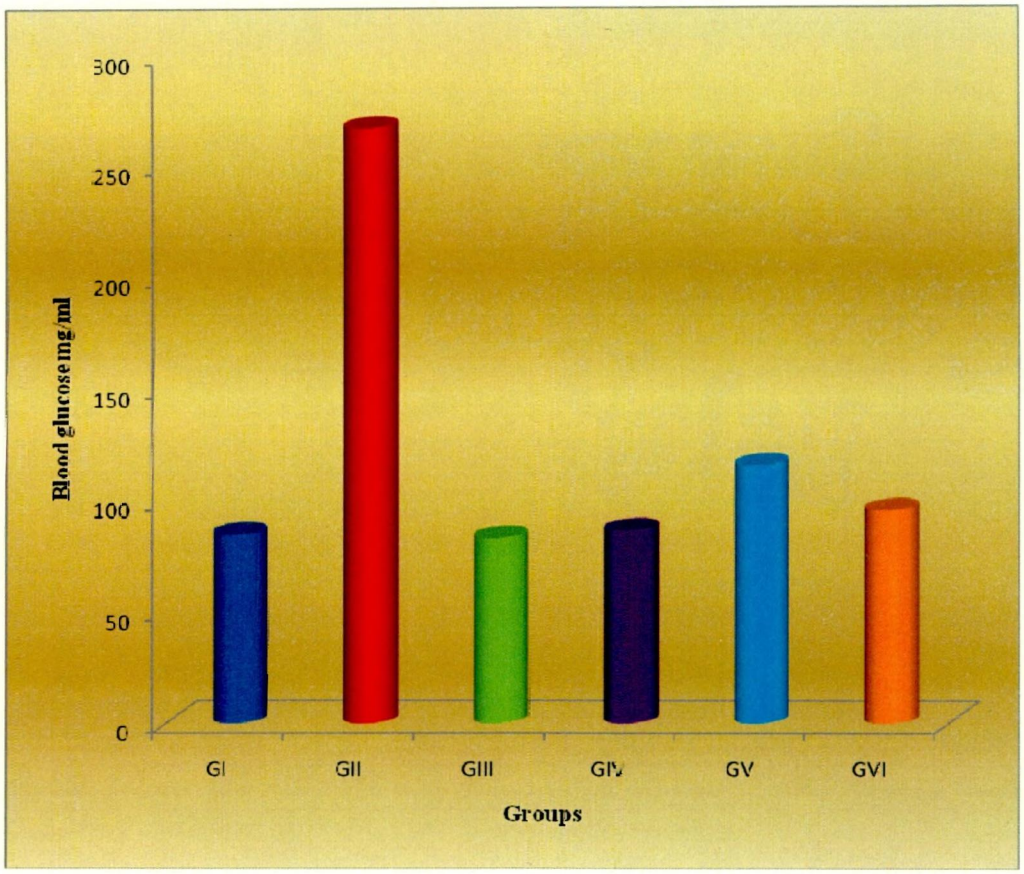
BLOOD GLUCOSE LEVEL IN NORMAL AND DIABETIC ANIMALS

S.NO	Groups	Blood glucose level (mg/dl)
1	Normal Control (GI)	84.69
2	Streptozotocin control (GII)	266.71
3	Dimethyl Sulphoxide (GIII)	83.09
4	Normal + *A.bracteolata* extract (500 mg/kg) (GIV)	86.61
5	Streptozotocin + *A.bracteolata* extract (250 mg/kg) (GV)	115.98
6	Streptozotocin + *A.bracteolata* extract (500 mg/kg) (GVI)	95.86
	CD(0.50)	**4.378**

Values are mean of six animals in each group

FIGURE I

BLOOD GLUCOSE LEVEL IN NORMAL AND TREATED ANIMAL GROUPS



GI – Normal control

GII – Streptozotocin control

GIII – Dimethyl sulphoxide

GIV – Normal – *A. bracteolata* extract (500mg/kg)

GV – Streptozotocin+ *A. bracteolata* extract (250mg/kg)

GVI – Streptozotocin + *A. bracteolata* extract (500mg/kg)

It is evident from the table that the oral administration of plant extract for 30 days produced a significant reduction in blood glucose level in the experimental groups GV and GVI when compared to diabetic control rats Thus, the above findings suggest that the methanolic extract of the selected plant might have reversed the increased blood glucose level to near normal values. Comparison between the two treatment groups GV and GVI revealed that GVI exerted a greater hypoglycemic effect than GV, indicating that the plant extract is more effective at higher concentration.

This is in agreement with the findings of Veeramani *et al.,* (2007) who have reported that the leaf extract of *Cardiospermum halicabum* at higher concentration (200 mg/kg) produced a better effect than the extract at lower concentration (50mg or 100mg/kg). Hossain *et al.,* (2007) have shown that the aqueous and ethanolic extracts of *Andrographis paniculata* exhibited a significant blood sugar lowering effects in diabetic rats.

Streptozotocin (STZ) is selectively toxic to cells in the pancreatic islets. It is well known that STZ causes specific death of B cells and induces diabetes mellitus (Bnouham *et al.,* 2009). Ghosh *et al.*, (2008) have reported that the ethanolic extract of *Bacopa monnieri* might have insulin like activity. The antihyperglycemic effect of the extract might be due to an increase in peripheral glucose consumption. Modak *et al.,* (2007) have reported that the aqueous extract of *Teucrium polium* had significantly decreased serum glucose level of the streptozotocin induced diabetic rats within 24 h and with the continual feeding of 2 ml aliquots of the extract per day, the blood glucose level of the diabetic rats decreased about 71% after 8 days and remained in the normal range.

4.2 ANTIOXIDANT POTENTIAL OF *Aristolochia bracteolata*

4.2.1 ENZYMIC ANTIOXIDANTS

Enzymic antioxidants in the selected medicinal plant

The level of Catalase, Peroxidase, Superoxide dismutase and Glutathione-S-Transferase in the selected medicinal plant is shown in Table II and Figure II.

TABLE II ACTIVITIES OF ENZYMIC ANTIOXIDANTS IN THE SELECTED MEDICINAL PLANT

S.NO	Concentration of plant extract (µg/ml)	Enzymic antioxidants (Units/g)			
		Catalase	Peroxidase	Superoxide Dismutase	Glutathione-S-Transferase
1	20	72.40	2.64	3.93	0.0417
2	40	131.60	7.92	6.03	0.0448
3	60	188.64	10.36	7.63	0.0583
4	80	196.53	12.23	8.83	0.0661
5	100	255.67	13.75	10.51	0.1024
CD (0.05)		1.976	0.311	0.427	0.00227

Values are mean of triplicates

Catalase - Amount of enzyme required to decrease the optical density by 0.05 units

Peroxidase - 1µm mole of pyrogallol oxidized per minute

Superoxide dismutase - Amount that causes 50% reduction in the extent of NBT oxidation

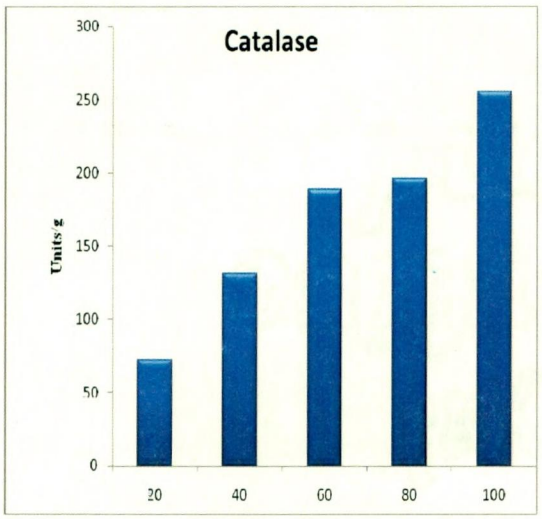
Glutathione-S-Transferase - µmoles of CDNB-GSH conjugate/min/g sample

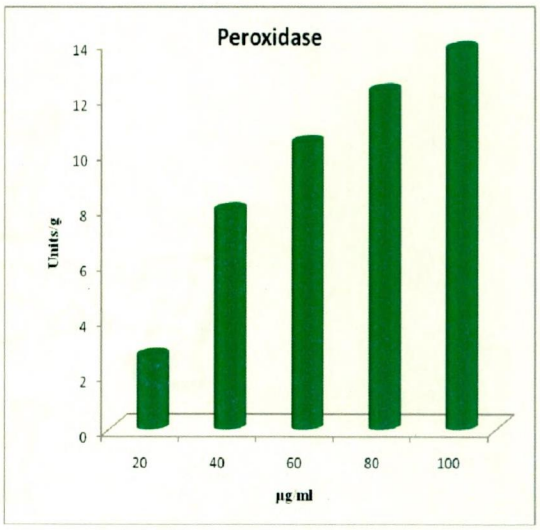
The enzymic activity was found to be maximum in the extract at higher concentration (100µg/ml) and minimum at lower concentration of (20 µg/ml). As the concentration of the plant extract increases the activity of all the enzymic antioxidants namely catalase, Peroxidase, superoxide dismutase and Glutathione-S-transferase increased.

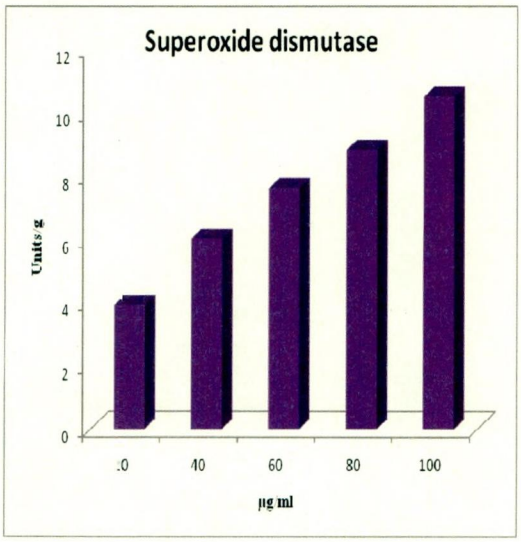
Superoxide dismutase (SOD) is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady-state level of O^{2-}.SOD is one of the most important enzymes and scavenges O_2 anion to form H_2O_2 and hence diminishes the

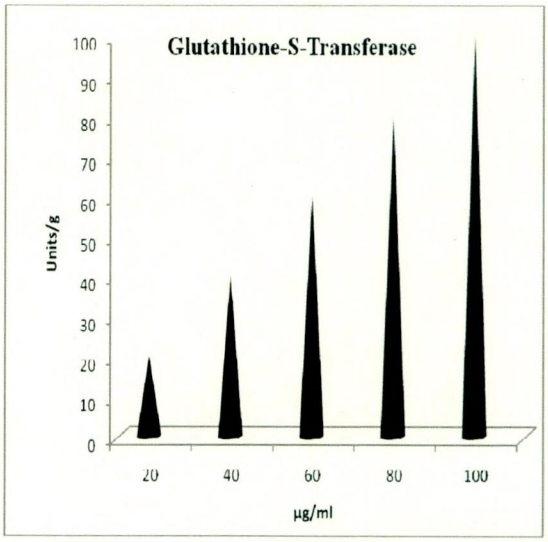
FIGURE II

LEVEL OF ENZYMIC ANTIOXIDANTS IN *Aristolochia bracteolata*









toxic effects. The O_2 anion is known to inactivate CAT and GPx (Kumar *et al.,* 2008). Kaviarasan *et al.,* (2008) have reported that the activity of SOD decreased significantly in high fat diet rats and the administration of flavonoid-rich seed fraction of *Spermacoce hispida* increased the enzymic activity.

Catalase (CAT) is a hemeprotein, localized in the peroxisomes or the microperoxisomes, which catalyses the decomposition of H_2O_2 to water and oxygen and thus protect the cell from oxidative damage produced by H_2O_2 (Sabu and Kuttan, 2004). Shetty *et al.,* (2007) have reported that rats treated with the aqueous extracts of *Ocimum sanctum* in the dose of 800 mg/kg body weight showed significant increase in the activity of catalase.

Peroxidases are referring to heme containing enzymes which are able to oxidize organic and inorganic compounds using hydrogen peroxide as co-substrate. The non-specificity of peroxidase makes the enzyme suitable to a broad range of electron donor substrates (Hakiman *et al.,* 2009). GST and GSH-Px are essential for maintaining a constant ratio of reduced glutathione in the cell. The enhancement in GST activity was observed in methanolic extract of *Solanum nigrum* berries treated animals (Jainu and Devi, 2004).

Enzymic antioxidants in normal and diabetic animal groups

Table (III) and Figure (III) represent the level of enzymic antioxidants in experimental groups. Normal animals supplemented with plant extracts at a concentration of 500mg/kg recorded maximum SOD activity and the group which received only Streptozotocin registered the lowest enzymic activity but when Streptozotocin induced diabetic rats administered with plant extracts exhibited a significant increase in SOD activity. The level of SOD was decreased in DMSO treated group (GIII) when compared with normal control group (GI). The maximum activity of Catalase, Peroxidase and Glutathione-S-Transferase was observed in extract treated experimental group (GVI) (500mg/kg) and the lowest activity was seen in the group II which received only Streptozotocin.

TABLE III LEVELS OF ENZYMIC ANTIOXIDANTS IN NORMAL AND DIABETIC ANIMAL GROUPS

S.NO	Groups	Enzymic antioxidants (Units/mg)			
		Catalase	Peroxidase	Superoxide Dismutase	Glutathione- S-Transferase
1	Normal Control (GI)	46.6	5.62	6.12	0.072
2	Streptozotocin control (GII)	19.09	2.02	2.44	0.028
3	Dimethyl Sulphoxide (GIII)	34.77	3.78	4.39	0.047
4	Normal + *A. bracteolata* extract (500mg/kg)(GIV)	45.22	4.43	6.23	0.071
5	Streptozotocin + *A.bracteolata* extract (250 mg/kg) (GV)	47.34	4.56	5.17	0.057
6	Streptozotocin + *A.bracteolata* extract (500 mg/kg) (GVI)	61.66	6.46	5.5	0.0858
CD (0.05)		**1.976**	**0.480**	**0.426**	**0.00560**

Values are mean of six animals in each group

Catalase - Amount of enzyme required to decrease the optical density by 0.05 units

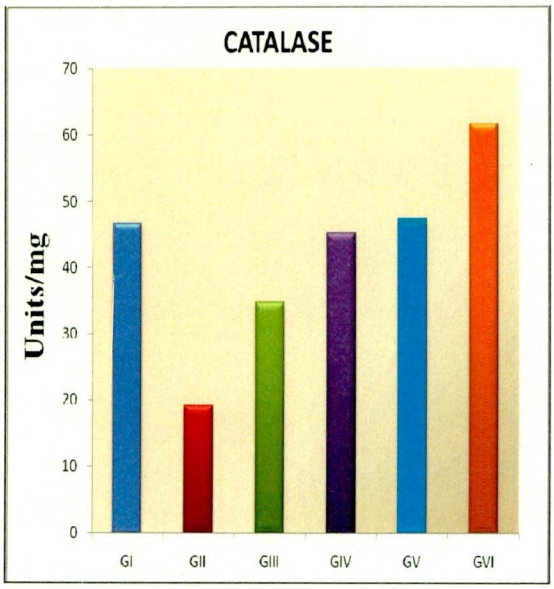
Peroxidase - 1μm mole of pyrogallol oxidized per minute

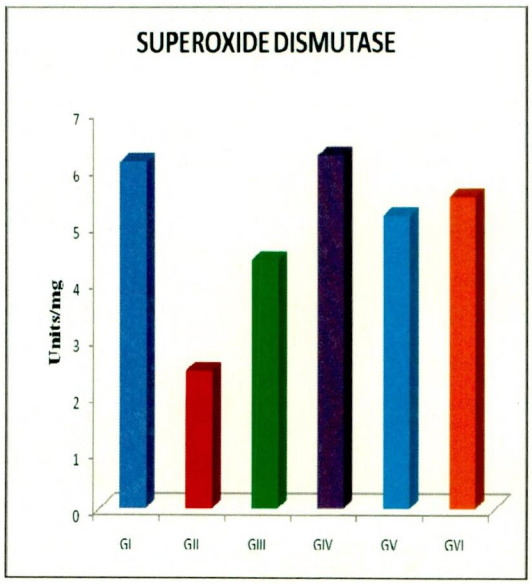
Superoxide dismutase - Amount that causes 50% reduction in the extent of NBT oxidation

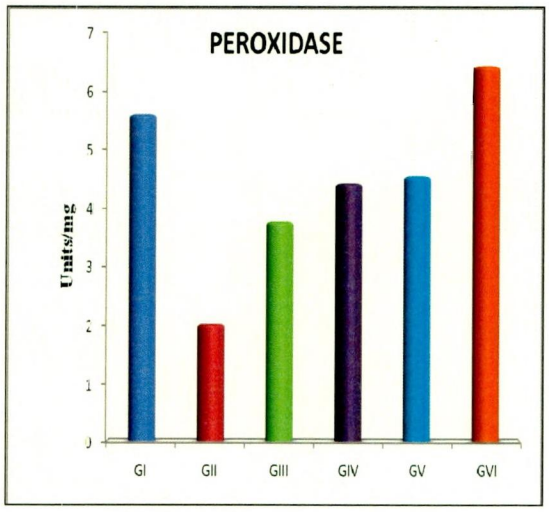
Glutathione-S-transferase - μmoles of CDNB-GSH conjugate/min/g sample

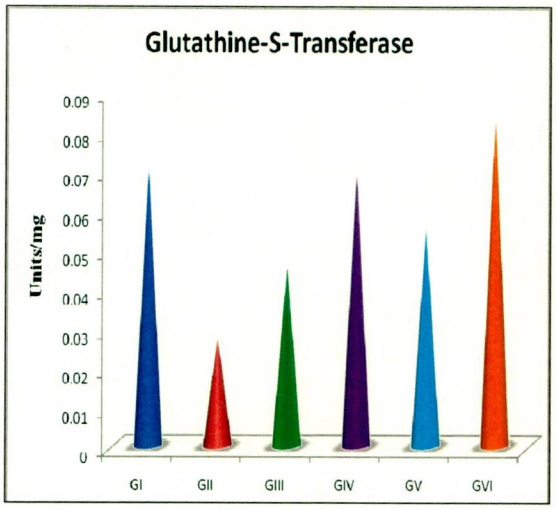
FIGURE III

LEVEL OF ENZYMIC ANTIOXIDANTS IN NORMAL AND TREATED ANIMAL GROUPS









GI – Normal control

GII – Streptozotocin control

GIII – Dimethyl sulphoxide

GIV – Normal + *A. bracteolata* extract (500mg/kg)

GV – Streptozotocin+ *A. bracteolata* extract (250mg/kg)

GVI – Streptozotocin + *A. bracteolata* extract (500mg/kg)

Catalase is heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. The decrease in Catalase activity is due to glycation of enzyme and accumulation of hydrogen peroxides. *Momordica charantia* seed extract treated groups showed a significant increase in Hepatic catalase activity of the diabetic rats (Sathishsekar and Subramanian 2005). Administration of ethanolic leaf extract of *Boerhaavia diffusa* increased the activity of catalase in diabetic rats, thus it can be concluded that the *Boerhaavia diffusa* extract has significant antioxidant activity (Sahu *et al.,* 2008)

Superoxide dismutase (SOD) catalyses the conversion of Superoxide anion to hydrogen peroxide and oxygen. In diabetic control group, liver and kidney SOD level was reduced and it was improved by treatment with methanolic extract of *Artanema sesamoides* (Selvam *et al.*, 2008). (Mazunder *et al.*, 2005) have also reported strong antihyperglycemic activity and antioxidant activity in methanolic extract of *Phyllanthus niruri.* Mandlik *et al.*, (2008) have reported that the reduced activities of superoxide dismutase and catalase in liver and kidney were observed in diabetic rats and these were reverted to near normal status on the administration of methanol extract of unripe matured fruits of *Diospyros peregrina* treatment.

Glutathione Peroxidase (GPx) and Glutathione-S- Transferase (GST) work together with glutathione in the decomposition of H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of Glutathione. The decreased activity of GST observed in diabetic state may be due to the inactivation caused by reactive oxygen species. Effect of ethanolic extract of Aloevera gel on tissue antioxidants is due to reduction in blood glucose level in diabetic rats, which prevent excessive formation of free radicals and also reduces glycation of the enzyme (Rajasekaran *et al.*, 2005).

4.2.2 NONENZYMIC ANTIOXIDANTS

NonEnzymic antioxidants in the selected medicinal plant

Table IV and figure IV show the level of ascorbic acid, α-Tocopherol and reduced glutathione in the selected medicinal plant. The level of nonenzymic antioxidants was found to be higher in the extract at the highest concentration (100µg/ml) when compared with lower concentration (20µg/ml). The content of Ascorbic acid, α-Tocopherol and reduced glutathione in the plant extract gradually increases with increase in concentration.

TABLE IV

LEVEL OF NONENZYMIC ANTIOXIDANTS IN THE PLANT SAMPLE

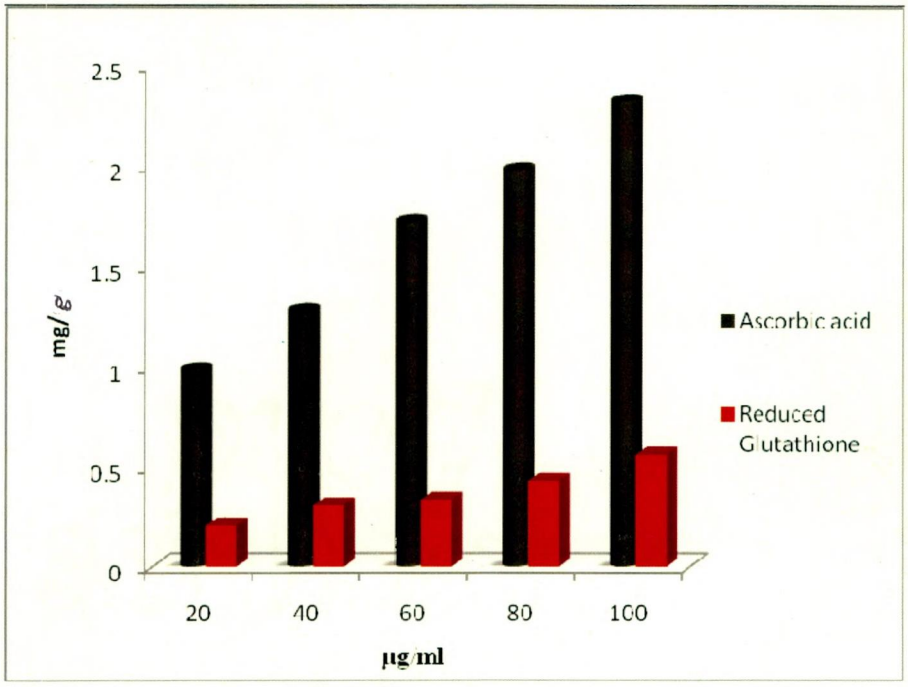
S.NO	Concentration of plant extract (µg /ml)	Non Enzymic antioxidants (mg/g)		
		Ascorbic acid	α -Tocopherol	Reduced Glutathione
1	20	0.986	0.0109	0.207
2	40	1.282	0.0167	0.308
3	60	1.721	0.0280	0.337
4	80	1.979	0.0325	0.432
5	100	2.321	0.0376	0.562
CD (0.05)		**0.0253**	**0.104**	**0.0015**

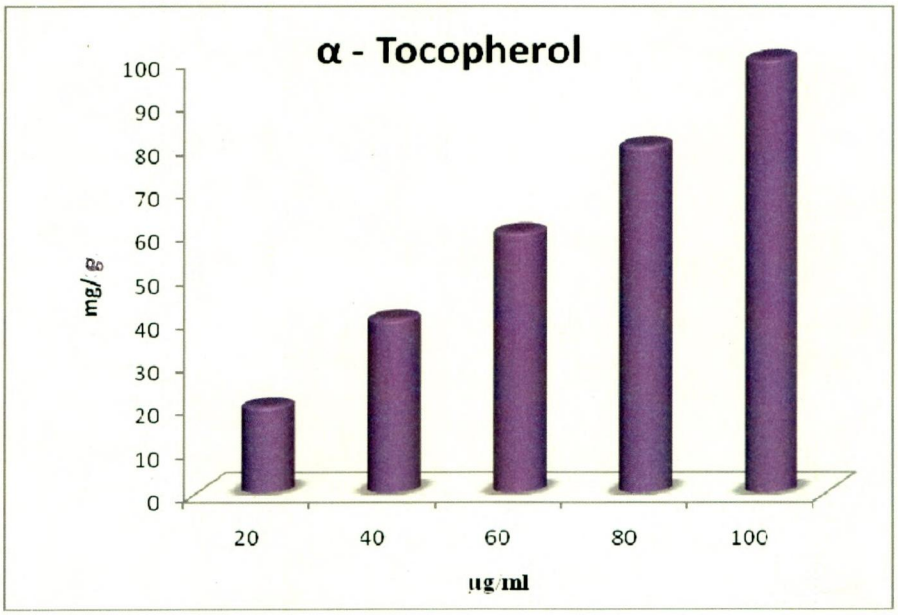
Values are mean of triplicates

FIGURE IV

LEVEL OF NON-ENZYMIC ANTIOXIDANTS IN

Aristolochia bracteolata





Vitamin E is an important lipid soluble, chain breaking free radical scavenger. Its unique location in cellular membrane enhances its efficiency to quench free radicals originating from the mitochondrial inner membrane (Banerjee *et al.*, 2003). The major aqueous-phase antioxidant, Vitamin C (ascorbate) traps peroxyl radicals in the aqueous phase before they can initiate lipid peroxidation. Vitamin C improves and normalizes endothelial vasodilator function in patients with heart failure by increasing the availability of the potential vasodilator nitric oxide (Kharb and Singh, 2004). Vitamin C is another important chain-breaking antioxidant, intracellularly and extracellularly. It neutralizes hydroxyl, superoxide, hydrogen peroxide radicals and prevents lipid peroxidation (Makker *et al.*,2009).

Vitamin C is regarded as the first line natural antioxidant defense in plasma and a powerful inhibitor of LPO non protein. Vitamin C is a water soluble antioxidant. It acts as a free radical scavenger. It scavenges peroxy redox system. In Oyster mushrooms, fresh samples have a higher level of Vitamin C when compared with powdered samples (Selvi *et al.*, 2007). Thus, the level of GSH was found to be higher in fresh compared with powdered samples. Glutathione constitutes a major reducing substance of the cytoplasm and is known to protect the cellular system against toxic effects of lipid peroxidation. GSH, Vitamin E and C exist in the interconvertible reduced and oxidized forms and thus participate in neutralizing free radicals when they are formed. There is a well established synergism between Vitamin E, Vitamin C and glutathione through the antioxidant network (Devi *et al.*, 2007).

NonEnzymic antioxidants in normal and diabetic animal groups

From Table V and figure V, it is evident that there was significant reduction in the level of ascorbic acid, α-Tocopherol and reduced glutathione in Streptozotocin induced diabetic control group (GII) animals.

Administration of methanolic extract of *A. bracteolata* orally for 30 days has significantly increased the nonenzymic antioxidant levels in the experimental groups (GV and GVI).The level of α-Tocopherol and reduced glutathione in DMSO treated group (GIII) and the group supplemented with only plant extract (GIV) was found to be lower when compared to normal control (GI) animals. Of the two different plant extract treated experimental groups, GVI has maximum content of nonenzymic antioxidants.

TABLE V LEVEL OF NON-ENZYMIC ANTIOXIDANTS IN NORMAL AND TREATED ANIMAL GROUPS

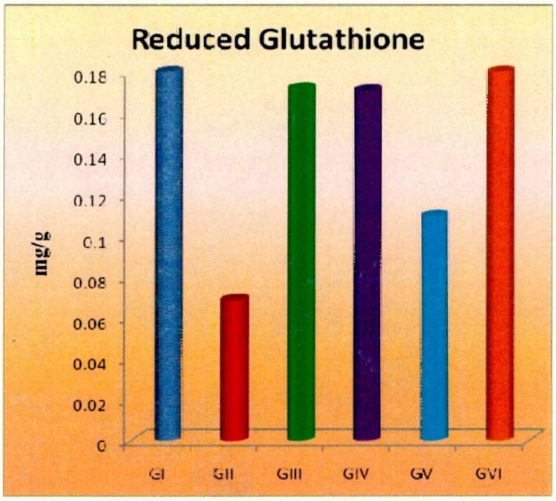
S.NO	Groups	Non Enzymic antioxidants (mg/g)		
		Ascorbic acid	α -Tocopherol	Reduced Glutathione
1	Normal Control (GI)	30.2	0.0284	0.186
2	Streptozotocin control (GII)	16.09	0.0171	0.069
3	Dimethyl Sulphoxide (GIII)	28.3	0.0191	0.172
4	Normal + *A.bracteolata* extract (500mg/kg)(GIV)	31.42	0.0243	0.177
5	Streptozotocin + *A.bracteolata* extract (250 mg/kg) (GV)	29.36	0.0208	0.115
6	Streptozotocin + *A.bracteolata* extract (500 mg/kg) (GVI)	40.7	0.0292	0.179
	CD (0.05)	**2.798**	**0.00532**	**0.0454**

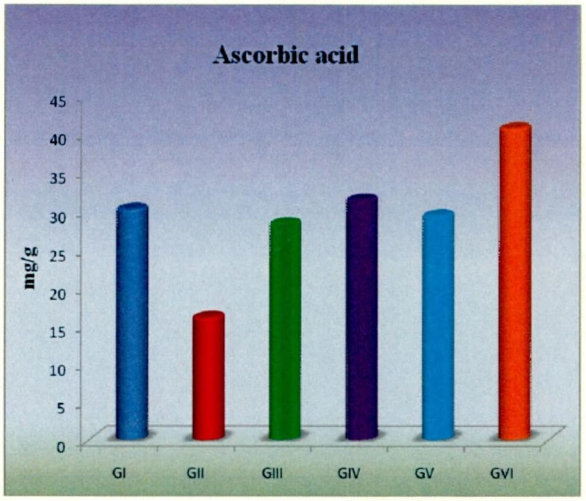
Values are mean of six animals in each group

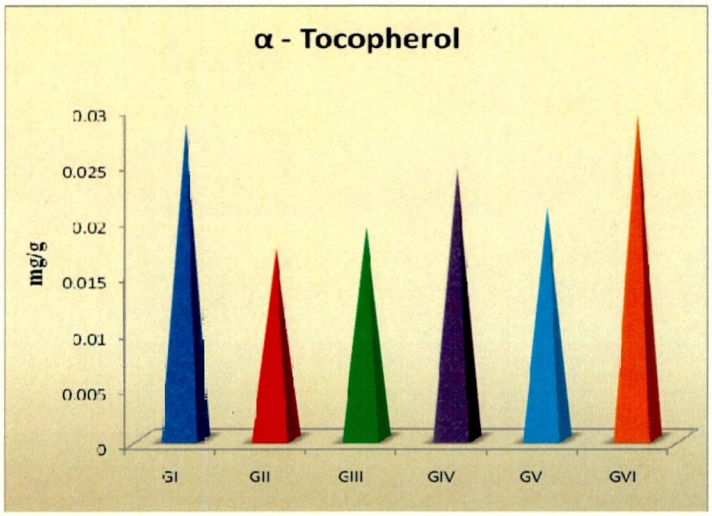
Rajadurai and prince (2005) have reported that α-Tocopherol protects against Low density lipoprotein oxidation. Administration of 200mg/kg leaf extract of *Aegle marmelos* was equally effective as α-Tocopherol (60mg/kg). Vitamin C is an electron donor and reducing agent widely acts on oxygen free radicals (Padayatty *et al.*, 2003). Ascorbic acid is known to act as an antioxidant in both *in vivo* and *in vitro*. It functions as a free-radical scavenger and successfully prevents detectable oxidative damage under all types of oxidative stress.

FIGURE V

LEVEL OF NON-ENZYMIC ANTIOXIDANTS IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC GROUPS







GI – Normal control

GII – Streptozotocin control

GIII – Dimethyl sulphoxide

GIV – Normal + *A. bracteolata* extract (500mg/kg)

GV – Streptozotocin+ *A. bracteolata* extract (250mg/kg)

GVI – Streptozotocin + *A. bracteolata* extract (500mg/kg)

Ascorbic acid plays an important role in detoxification of reactive intermediates produced by cytochrome P450, which detoxify xenobiotics (Prakasam *et al.*, 2005).Glutathione (GSH) protects the cell against oxidative stress by reacting with peroxides and hydroperoxides. The ethanolic extract of *Ocimum sanctum* leaves probably increased the levels of reduced glutathione in test group by facilitating reduction of oxidative free radicals by H donation (Sethi *et al.*, 2004). Sharma and Garg (2008) have also reported that GSH content also increased significantly, indicating that ethanolic extract *Butea monosperma* could either increase the biosynthesis of GSH or reduce the degradation of GSH.

4.3 FREE RADICAL SCAVENGING EFFECT OF *Aristolochia bracteolata*

4.3.1. Effect of plant extract on the inhibition of invitro lipid peroxidation, superoxide and nitric oxide generation

The high level of inhibitory effect of lipid peroxidation, superoxide and nitric oxide generation was observed in the highest concentration (100µg/ml) of methanolic extract of *A. bracteolata*. The minimum inhibitory level was found in the lowest concentration (20µg/ml) of plant extract. The percentage inhibition of invitro Lipid peroxidation, Superoxide generation and nitric oxide generation increases as the concentration of the plant extract increases. The maximum percentage inhibition was observed in nitric oxide generation was observed (84.87%) followed by lipid peroxidation (82.13%) and followed by superoxide generation (72.67%) as shown in (Table VI and figure VI).

Superoxide anion is very harmful to cellular compartments. Superoxide anion is oxygen-centered radical with selective reactivity. It has been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radicals (Ak and Gulcin, 2008). Jyothi *et al.*, (2008) have reported that *Euphorbia antiquorum* extract has reduced the super oxide anions and also scavenge off the hydroxyl radicals and hence, inhibit the cellulardamage. Superoxide has also been observed to directly initiate lipid peroxidation

TABLE VI

EFFECT OF *A. bracteolata* ON INHIBITION OF INVITRO LIPID PEROXIDATION, SUPEROXIDE AND NITRIC OXIDE GENERATION

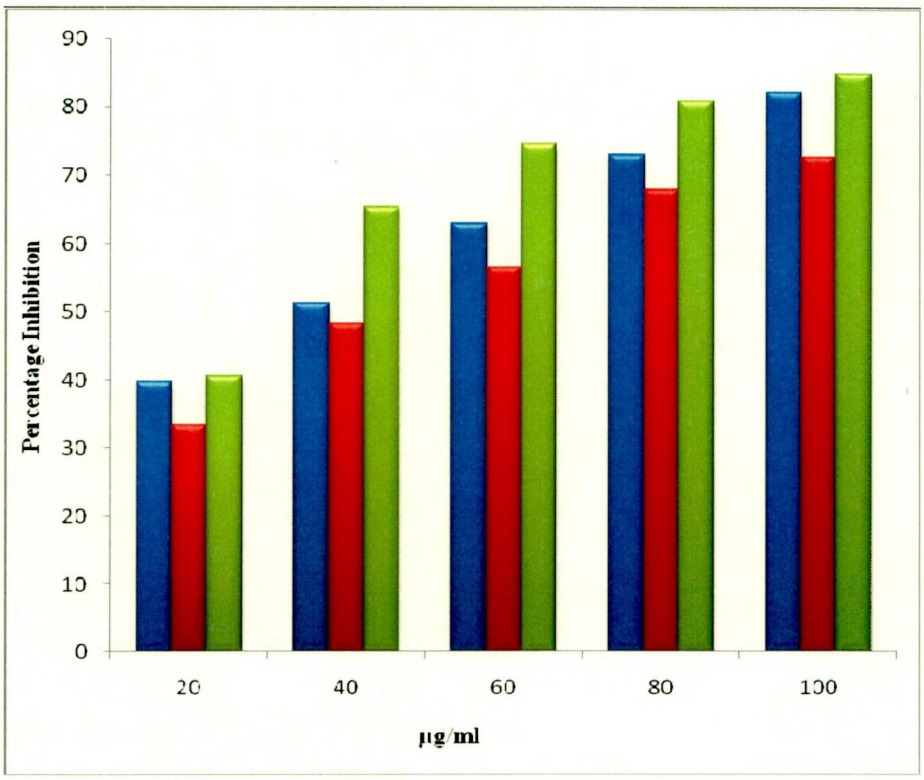
S.NO	Concentration of plant extract (µg /ml)	Inhibition of		
		Invitro lipid peroxidation (%)	Invitro superoxide generation (%)	Invitro Nitric oxide generation (%)
1	20.0	39.75	33.31	40.61
2	40.0	51.23	48.24	65.41
3	60.0	62.89	56.51	74.65
4	80.0	73.04	67.83	80.83
5	100.0	82.13	72.67	84.87
CD (0.05)		**0.392**	**1.479**	**0.923**

Values are mean of triplicates

. The superoxide scavenging activity of *Cyperus rotundus* rhizomes was increased markedly with the increase in concentrations. Thus, higher inhibitory effects of the rhizomes extracts on superoxide anion formation possibly render them as promising antioxidants.

FIGURE VI

PERCENTAGE INHIBITION OF LIPID PEROXIDATION, SUPEROXIDE AND NITRIC OXIDE GENERATION BY *Aristolochia bracteolata*



Gupta *et al.*, (2006) suggested that Nitric oxide reacts with superoxide anion to form peroxynitrite results in protein tyrosine nitration that is widely recognized as hallmark of nitrosative stress. This leads to impairment of proteins and nucleotide damage and furthermore mitochondria may lack DNA repair mechanisms. Hazra *et al.*, (2008) have reported that the methanolic extract of *Spondias pinnata* has more potent nitric oxide scavenging activity than standard Curcumin.

Lipid peroxidation is a free radical mediated process leading to oxidative deterioration of polyunsaturated lipids. Lipid peroxidation serves as a marker of oxidative stress. Administration of Methanolic Extracts of *Acorus calamus* (AC) effectively decreased LPO levels indicating that AC extracts effectively reduced the oxidative stress (Li *et al.*, 2007). Methanolic extract of *Garcinia mangostana* exhibited excellent activity for various radical scavenging activities and suppressed lipid peroxidation. The extract prevented lipid peroxidation, which may explain its cytoprotective property on cell membrane damage caused by radicals or toxic substances (Kosem *et al.*, 2007).

4.3.2 Effect of plant extract on DPPH and ABTS radicals

Table VII and Figure VII reveal the free radical scavenging activity of methanolic extract of *Aristolochia bracteolata*. The potential decrease in concentration of DPPH radical was observed due to scavenging property of the selected plant. There was maximum percentage inhibition of DPPH (75.92%) and ABTS (62.88%) radical formation at the highest concentration (100µg/ml) and the minimum inhibition was found in the lowest concentration (20µg/ml) of the plant extract. The plant has greater scavenging effect on DPPH radicals compared to ABTS radical formation. The above findings suggest that *Aristolochia bracteolata* could serve as a potent free radical inhibitors or scavengers, acting possibly as primary antioxidants.

TABLE VII

DPPH AND ABTS RADICAL SCAVENGING EFFECT OF *Aristolochia bracteolata*

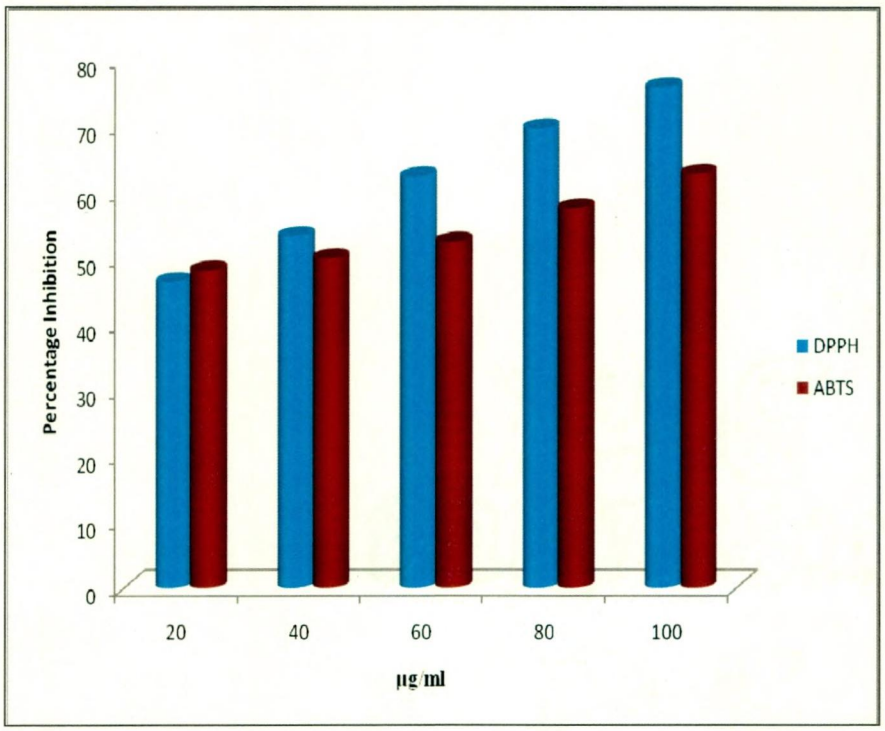
S. No	Concentration of plant extract (µg/ml)	DPPH Scavenging activity (%)	ABTS Scavenging activity (%)
1	20	46.44	48.22
2	40	53.42	50.08
3	60	62.43	52.53
4	80	69.69	57.63
5	100	75.92	62.88
	CD(0.05)	**0.557**	**0.681**

Values are mean of triplicates

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen from a stable diamagnetic molecule (Molyneux *et al.,* 2004). Ara and Nur, (2009) reported that the DPPH antioxidant assay is based on the ability of a stable free radical (DPPH) to decolorize in the presence of antioxidants. Methanolic extract of leaves and flowers of *Lippia alba* exhibited a significant dose dependent inhibition of DPPH activity hence the plant extract has got profound antioxidant activity. Similar reports have been shown by Thirugnanasampandan *et al.*, (2008) in the ether and ethyl acetate extracts of *Aristolochia tagala.*

FIGURE VII

DPPH AND ABTS RADICAL SCAVENGING EFFECT OF *Aristolochia bracteolata*



Adedapo *et al.*, (2008) suggested that proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance at 734 nm which decreases with the scavenging of the proton radicals. Higher concentration of methanolic extracts of *Buddleja saligna* was more effective in quenching free radicals in the system.

4.4 Histopathological examination

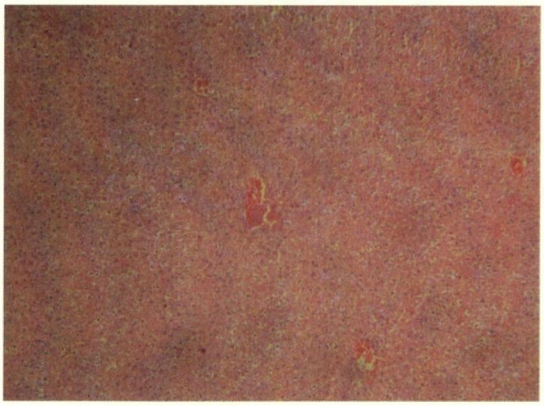
In the present study, the histopathological and histochemical examinations of the liver of control rats showed mild dilation of the central vein and appearance of wound. In the liver of diabetic rats (GII) showed congested vein, periportal inflammation and marked glycogenation. These results are in agreement with Gayathri *et al.*, (2008) who reported that the liver of diabetic rats was characterized by periportal necrosis of the hepatocytes and inflammatory cell infiltration. The liver of treated group (250 mg/kg) showed appearance of hepatocytes like control group and appearance of wound is observed. The liver of extract treated group (500mg/kg) had no appearance of wound and the hepatocytes were like control group having no inflammation (Plate 4).

The damages that occur in the liver of diabetic's rat may be due to Oxygen Free Radicals (OFR). Cellular defense mechanisms such as antioxidants and antioxidant enzymes offer protection to cells and tissues from oxidative injury. The imbalance between OFR production and cellular defense mechanisms could be critical in influencing vascular injury. The increase in the level of OFRs in diabetes could be due to their increased production and/or decreased destruction by non-enzymic and enzymic [Catalase (CAT), glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD)] antioxidants (Oraby *et al.*, 2008).

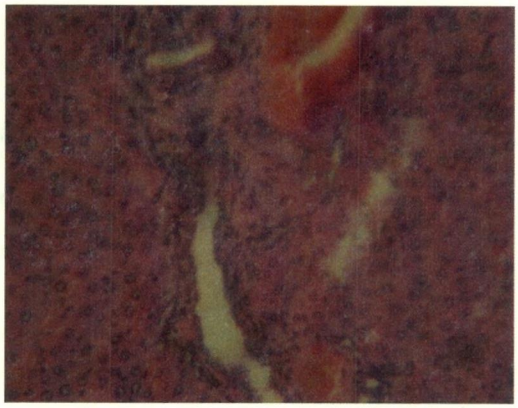
Water extract is more potent than hexane and ethanol extracts of clover flowers (CF) of *Trifolium alexandrinum* in the improvement of histological alterations in the diabetic rat's liver. These effects may be due to the presence of a high content of flavonoids which acts synergistically as antioxidants. Thus, the result of the present study provides a scientific rationale for the use of *Trifolium alexandrinum* as an antidiabetic agent, but further investigation to determine the chemical structure of the active compounds and their mechanisms of action is necessary (Rawi 2007).

PLATE 4

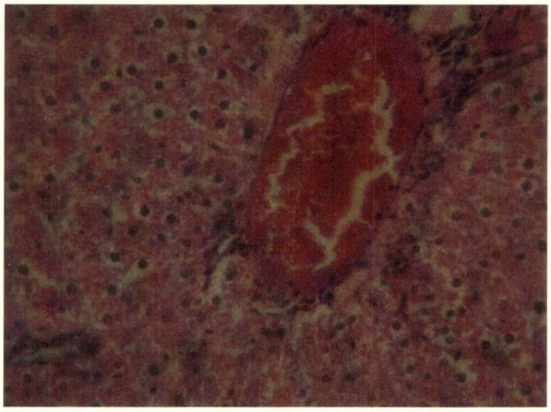
HISTOLOGICAL OBSERVATIONS OF CONTROL AND EXPERIMENTAL RAT LIVER



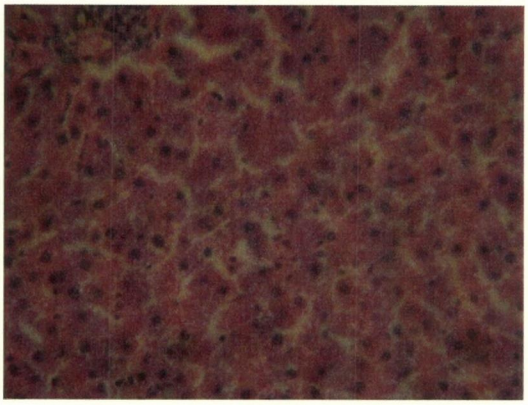
A) Normal structure of hepatocytes in control rat



B) Diabetic rats show periportal necrosis with inflammatory inflammation



c) Diabetic rats treated with extract shows the hepatic lobule that appears like control



D) Diabetic rats treated with extract shows normal hepatic lobule that appears like control