RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSION

Plant based medicaments had served as the most important therapeutic weapons available to man to fight various human and animal diseases. The exclusive use of herbal remedies to treat and manage ailments continued until the introduction of modern synthetic medicines. The advent of synthetic medicines in the health care system coupled with industrialization, urbanization in the most developed countries and embracing of western culture by the developing countries, made the use of herbal products to decline from about the beginning of the 20th century upto the 1970s (Ogbonnia *et al.*, 2008). However, in recent times, there is a renewal and growing interest in the use of plant-derived biologically active compounds as drugs or as leads in the manufacture of more potent medicaments. Plants, therefore, remain the main natural source of the active drugs and are still indispensable in the traditional medicine for treating a number of diseases. Traditional medicines are used by about 60 per cent of the world population both in the developing and developed countries (Mythilypriya *et al.*, 2007).

Diabetes mellitus, a disorder characterized by chronic hyperglycemia due to absolute or relative deficiency of insulin is estimated to afflict over 170 million people worldwide and this represents about 2 per cent of the world's population. The long-term effects of diabetes include progressive development of the specific complication of retinopathy, nephropathy and neuropathy with microvascular and macrovascular diseases (Edem *et al.*, 2008).

Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma and hepatorenal disturbances. Moreover they are not safe for use during pregnancy. Hence, the search for safer and

more effective hypoglycemic agents has continued. Following the WHO's recommendation for research on the beneficial uses of medicinal plants in the treatment of diabetes mellitus, investigations on hypoglycemic agents derived from medicinal plants have also gained momentum. Several investigations have been conducted and many plants have shown a positive activity (Barik *et al.*, 2008).

Hence, the present study has been designed to evaluate the antidiabetic effect of the alcoholic extracts of the fruit and the bark of *Helicteres isora* (Indian screw tree) on streptozotocin-induced diabetic rats. The observation made and the results obtained are presented and discussed under the following headings:

PHASE I

4.1 CHARACTERISATION OF THE FRUIT AND THE BARK OF *Helicteres isora*

4.1.1 Proximate principles

4.1.2 Antioxidants

Enzymic antioxidants

Non-enzymic antioxidants

4.1.3 Free radical scavenging activity of the fruit and the bark of *Helicteres isora*

4.1.4 Antimutagenic activity of the fruit and the bark of *Helicteres isora*

4.1.5 Toxicological evaluation of the fruit and the bark of *Helicteres isora*

Acute toxicity studies

Chronic toxicity studies

Body weight of the rats

Effect of *Helicteres isora* fruit and bark on hematological parameters

Effect of *Helicteres isora* fruit and bark on biochemical parameters

Effect of *Helicteres isora* fruit and bark on blood glucose level at intervals of 5 days for a period of 45 days

PHASE II

4.2 DETERMINATION OF ANTIDIABETIC EFFECT OF THE FRUIT AND THE BARK OF *Helicteres isora*

4.2.1 Effect of *Helicteres isora* fruit and bark on blood glucose and liver glycogen

4.2.2 Effect of *Helicteres isora* fruit and bark on plasma insulin concentration

4.2.3 Influence of *Helicteres isora* fruit and bark on serum fructosamine levels

4.2.4 Effect of *Helicteres isora* fruit and bark on hepatic key enzymes of carbohydrate metabolism

4.2.5 Effect of *Helicteres isora* fruit and bark on lipid profile

4.2.6 Effect of *Helicteres isora* fruit and bark on oxidative stress

4.2.7 Effect of *Helicteres isora* fruit and bark on serum protein

4.2.8 Histopathological study of rat liver, pancreas and kidney

PHASE III

4.3 IDENTIFICATION OF THE PHYTOCHEMICALS IN THE FRUIT AND THE BARK OF *Helicteres isora*

4.3.1 Qualitative detection of the phytochemicals

4.3.2 Screening of the phytochemicals by Thin Layer Chromatography

4.3.3 Isolation and quantification of the active constituents of *Helicteres isora* by High Performance Liquid Chromatography

PHASE I

4.1 CHARACTERISATION OF THE FRUIT AND THE BARK OF *Helicteres isora*

4.1.1 PROXIMATE PRINCIPLES

The medicinal value of plants lies in their chemical substrates that produce a definite physiological action on the human body. Therefore, there is a need to evaluate the herbs for their nutrient and mineral composition so as to assess the potential of indigenous sources of medicine (Edeoga *et al.,* 2003). Nutrients present in the fruit and the bark of *Helicteres isora* are depicted in Table 3.

TABLE 3

NUTRIENTS IN *Helicteres isora*

Nutrients (per 100g)	*Helicteres isora*	
	Fruit	**Bark**
Carbohydrate (g)	58.2 ± 2.40	62.0 ± 5.6
Protein (g)	4.8 ± 1.20	4.9 ± 1.2
Fibre (g)	1.0 ± 0.3	1.5 ± 0.5
Calcium (mg)	200 ± 43.3	100 ± 11.9
Phosphorus (mg)	36.8 ± 3.5	22.5 ± 4.2
Iron (mg)	45.1 ± 1.7	39.6 ± 2.1
Sodium (mg)	95.0 ± 1.6	58.0 ± 1.6
Potassium (mg)	84.0 ± 2.4	94.0 ± 1.4
Chromium (mg)	0.32 ± 0.1	0.16 ± 0.1

Values are mean ± SD of six replicates

It is clear from the above table, that the fruit and the bark of *Helicteres isora* contain appreciable quantities of carbohydrate, protein and moderate amount of fibre. The fibre content of the fruit is 1.0 g and that of the bark is 1.5 g. Fibre generally slows down the digestion and the absorption of carbohydrate, thereby reducing the rapid rise in blood sugar levels. It increases the sensitivity of tissues to insulin, thus preventing excessive secretion of insulin. It also improves the uptake of glucose by the liver and other tissues, thereby preventing a sustained elevation of blood sugar (Kochhar *et al.*, 2006).

Herbs or medicinal plants have high density of important nutrients such as minerals, vitamins and fibre which can prevent certain diseases as well as strengthen body tissue and improve the functioning of the nervous system (Fadzelly *et al.*, 2006)

Population studies as well as clinical and experimental research have shown that diabetes is one of the disorders most clearly related to inadequate dietary fibre intake. Frequent consumption of legumes and high fibre content has been shown to worsen the diabetic condition. Dietary fibres of *Ficus racemosa* (Indian Fig) when fed at 10 per cent dietary level to rats induced a greater resistance to hyperlipidemia than cellulose. They are found to influence total lipids, cholesterol, triglycerides and phospholipids of liver to varying extent and hence could be recommended for hypercholesterolemia and hyperglycemia (Mukherjee *et al.*, 2006). The fibre content of *Helicteres isora* might slow down the carbohydrate absorption and thereby prevent hyperglycemia.

Micronutrients regulate metabolism and gene expression and influence the displacement and the progression of many chronic diseases. These substances are the "magic wands" that enable the body to produce enzymes, hormones and other substances essential for the proper growth and development. These have been investigated as potential preventive and treatment agents for both type 1 and type 2 diabetes and for common complications of diabetes (WHO, 2008).

Herbs used in indigenous medicines in crude form for management of diabetes mellitus contain both the organic and the inorganic constituents. It is known that certain inorganic mineral elements (potassium, zinc, calcium and traces of chromium) play an important role in the maintenance of

normal glucose tolerance and in the release of insulin from β-cells of islets of Langerhans (Diwan *et al.*, 2006).

Chromium, an essential micronutrient, functions as a cofactor in all insulin regulating activities. Trivalent chromium (Cr^{3+}) is the only form of chromium that exhibits biological activity and is an integral component of the glucose tolerance factor (The Medica, 2008).

It is evident from Table 1 that both the fruit and the bark of *Helicteres isora* might serve as very good sources of all the minerals. There are several reasons why people with diabetes should consume a high potassium diet. Potassium supplementation improves insulin sensitivity, responsiveness and secretion. Insulin administration induces a loss of potassium from body fluid. A high potassium intake reduces the risk of heart diseases, atherosclerosis and cancer. The estimated safe and adequate daily intake of potassium as set by the Committee on Recommended Daily Allowance is 1.9 g to 5.6 g. Hypokalemia or potassium depletion can result in reduced glucose tolerance. Calcium and iron content are found to be high in both the fruit and the bark of *Helicteres isora*. Calcium is the most abundant macro element in plants. The normal extracellular calcium concentrations are necessary for blood coagulation and for maintaining the integrity of the intracellular cement substances. Calcium is important for ossification and iron is necessary for normal hematopoiesis. Iron has several vital roles in the body. It is mainly involved in oxidation-reduction reactions of electron transport chain, hemoglobin-oxygen transport and also as a cofactor for numerous other enzymes (Rajendran *et al.*, 2007).

The chromium content of the fruit was found to be 0.32 mg/100 g and the bark 0.16 mg/100 g. In some clinical studies, supplementing the diet of the diabetics with chromium has shown to decrease fasting glucose levels,

improve glucose tolerance and decrease total cholesterol and triglyceride levels, while increasing HDL-cholesterol levels. Chromium seems to enhance the action of the insulin increasing the number of insulin receptors. Chromium has also been shown to improve various aspects of dyslipidemia in diabetic subjects (Zimmerman, 2000).

It is very significant to note that the elemental composition of *Murraya koenigii* (curry leaves), *Ocimum sanctum* (tulsi), *Aegle marmelos* (bilva) and *Mentha piperita* (pepper mint) was found to play an important role in the maintenance of normoglycemia by activating the β-cells of the pancreas (Narendhirakannan *et al.*, 2005).

4.1.2 ANTIOXIDANTS

Oxygen free radicals ($O_2^{\bullet}$, $^{\bullet}OH$, $HO^{\bullet}_2$) are highly reactive species with one or more unpaired electrons. H_2O_2 and activated oxygen species (AOS) are formed in almost every cell of the body at an astonishing rate during normal oxidative metabolism. Environmental factors such as UV light, ozone, tobacco smoke, different xenobiotics and herbicides facilitate their formation to a greater extent. They react very much with almost every cellular component and contribute to many types of pathology (Ruiz-Teran *et al.*, 2008).

Antioxidant defence mechanism counteracts free radicals formation and reactions. Antioxidants neutralize free radicals and increasing levels of antioxidants should decrease pathological disorders. Combinations of different natural antioxidants, which could be found in different medicinal plants, work better than the separate antioxidants alone. Many epidemiological studies also support the idea that antioxidants are interdependent (Bouhdid *et al.*, 2008).

Enzymic antioxidants

The activities of the antioxidant enzymes, catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase were determined and the results are given in Table 4.

TABLE 4

ACTIVITIES OF ENZYMIC ANTIOXIDANTS IN *Helicteres isora*

Enzymic antioxidants	Fruit	Bark
Catalase (U/mg)[#]	4.0 ± 1.08	5.4 ± 1.56
Superoxide dismutase (U/mg)[$]	8.1 ± 1.03	9.0 ± 0.72
Glutathione peroxidase(U/mg)[*]	154.0 ± 7.5	142.0 ± 9.8
Glutathione-S-transferase (U/mg)[◆]	0.2 ± 0.07	0.008 ± 0.003

Values are mean ± SD of six replicates

One unit of enzyme activity is defined as:

[#] Amount of enzyme that brings about a decrease in absorbance of 0.05 at 240 nm

[$] Amount of SOD that causes 50 per cent reduction in the extent of NBT oxidation

[*] μmoles of CDNB-GSH conjugate /min/mg protein

[◆] μ gram of GSH consumed/min/mg protein

The activities of the enzymic antioxidants, catalase, SOD, glutathione peroxidase and glutathione-S-transferase were found to be 4.0, 8.1, 154.0, 0.2 U/mg protein in the fruit and 5.4, 9.0, 142.0, 0.008 U/mg protein in the bark of *Helicteres isora*.

Glutathione peroxidase is effective against H_2O_2 and fatty acid hydroperoxide. The role of superoxide dismutase is likely to prevent inactivation of nitrous oxide by superoxide, thereby protecting endothelium dependent arterial relaxation (Haan *et al.*, 2006).

The inactivation of nitrous oxide by advanced glycation end products formed in subendothelial space might also play a role in defective vasodilatory responses that occur in diabetes. Dietary or pharmacological treatments of animals with antioxidants have attenuated the injuries caused by oxidative stress in diabetes. The development of IDDM caused by the administration of streptozotocin / alloxan results in hydroxyl radical ($^{\bullet}OH$ or $NO^{\bullet}$) - abundance and beta-cell death. Antioxidants efficiently scavenge these free radicals and preserve beta-cells (Orzechowski, 2003).

Non-enzymic antioxidants

The non - enzymic antioxidants in *Helicteres isora* are presented in Table 5.

TABLE 5

LEVELS OF NON - ENZYMIC ANTIOXIDANTS IN *Helicteres isora*

Non-enzymic antioxidants	Quantity/100 g	
	Fruit	Bark
Vitamin E (mg)	4.5 ± 0.53	0.77 ± 0.07
Vitamin C (mg)	1.6 ± 0.78	0.63 ± 0.66
Flavonoids (mg)	33 ± 6.4	42 ± 6.5
Tannins (mg)	205 ± 2.3	180 ± 1.2
Total carotenoids (μg)	0.6 ± 0.15	0.2 ± 0.08
Reduced glutathione (mmoles)	9.96 ± 2.4	11.13 ± 2.2
Thiamine (mg)	ND	ND
Riboflavin (mg)	0.5 ± 0.2	ND

Values are mean ± SD of six replicates ND- Not detectable

It is evident from Table 5 that both the fruit and the bark of *Helicteres isora* are very good sources of non-enzymic antioxidants. Appreciable amount of flavonoids, tannins, glutathione and vitamin E are found to be present in the fruit as well as the bark of *Helicteres isora.*

Natural antioxidants strengthen the endogenous antioxidants defences from ROS and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention (Venukumar and Latha, 2002).

The reduced form of vitamin C represents a first line antioxidants defence against lipid peroxidation induced by free radicals. Among the lipid soluble antioxidants, α-tocopherol plays a central role as it controls radical-induced lipid peroxidation (Pari and Latha, 2004).

Flavonoids have shown a wide range of biological activity, including vasoprotective, antidiabetic, anti-inflammatory, antihepatotoxic and anticarcinogenic action. A large part of their pharmacological activity may be attributed to their ability to inhibit certain enzymes and to their antioxidant properties - iron chelating and oxygen free radical scavenging properties (Kessler *et al.*, 2003).

Sharma *et al.* (2008) has found that the flavonoids isolated from seeds of *Eugenia jambolana* (jamun) exert the maximum beneficial action by eliciting highly potent hypolipidemic and hypoglycemic activities.

Thus it could be concluded that *Helicteres isora* with all these enzymic and non-enzymic antioxidants, is an excellent medicinal plant, which can be used for treating various diseases like diabetes, cancer and neurodegenerative disorders, which are associated with free radical damage.

4.1.3. FREE RADICAL SCAVENGING ACTIVITY OF *Helicteres isora*

Considerable attention has recently been focused on the interrelationship of lipid peroxidation processes, free radical-related reactions and the development of a variety of pathological events. It is well established that lipid peroxidation is the deleterious result of free radical reactions, leading to disruption of biomembranes and dysfunction of cells and tissues. Therefore, lipid peroxidation is a crucial step in the pathogenesis of free radical related disease states, including inflammatory injury, gastrointestinal disease, cardiovascular disease, nervous system disorder, diabetes mellitus and cancer (Cho *et al.*, 2003).

Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated fatty acids. Their destruction can lead to cell death and also to the production of toxic and reactive aldehyde metabolites (Catala, 2008).

In recent years, there has been a global trend towards focusing on the area of natural phytochemicals present in herbs and functional foods as antioxidants. Since ancient times, humans have derived many benefits from natural plants and compounds. It has been recognized that traditional medicines and the polyphenols isolated from them have potential therapeutic roles in the prevention and the treatment of many human diseases related to excessive oxidative stress (Harnafi and Amrani, 2008).

The free radical scavenging activities (inhibition of *in vitro* LPO, SO, NO generation, DPPH radical scavenging and hydroxyl radical scavenging) of *Helicteres isora* were therefore assessed and the same are shown in Tables 6 and 7.

TABLE 6

PER CENT INHIBITION OF *in vitro* LIPID PEROXIDATION, SUPEROXIDE AND NITRIC OXIDE GENERATION OF *H.isora*

PER CENT INHIBITION	FRUIT	BARK
in vitro lipid peroxidation	80.6 ± 5.5	86.4 ± 3.9
Superoxide generation	66.0 ± 4.1	74.0 ± 5.4
Nitric oxide generation	46 .0 ± 4.7	22.0 ± 1.5

Values are mean ± SD of six replicates

Crude methanolic extracts of both the fruit and the bark of *Helicteres isora,* exhibited significant inhibition of *in vitro* lipid peroxidation. The extent of inhibition was found to be 80.6 in the case of the fruit and 86.4 in the bark. The per cent inhibition of superoxide radical generation and nitric oxide generation in the fruit was 66.0 and 46.0 and in the bark was 74.0 and 22.0 respectively. Inhibition of *in vitro* lipid peroxidation and superoxide generation by the bark was higher than the fruit, whereas the fruit had more pronounced effect in the inhibition of nitric oxide generation than the bark.

TABLE 7

DPPH FREE RADICALS AND HYDROXYL RADICALS SCAVENGING ACTIVITY OF *Helicteres isora*

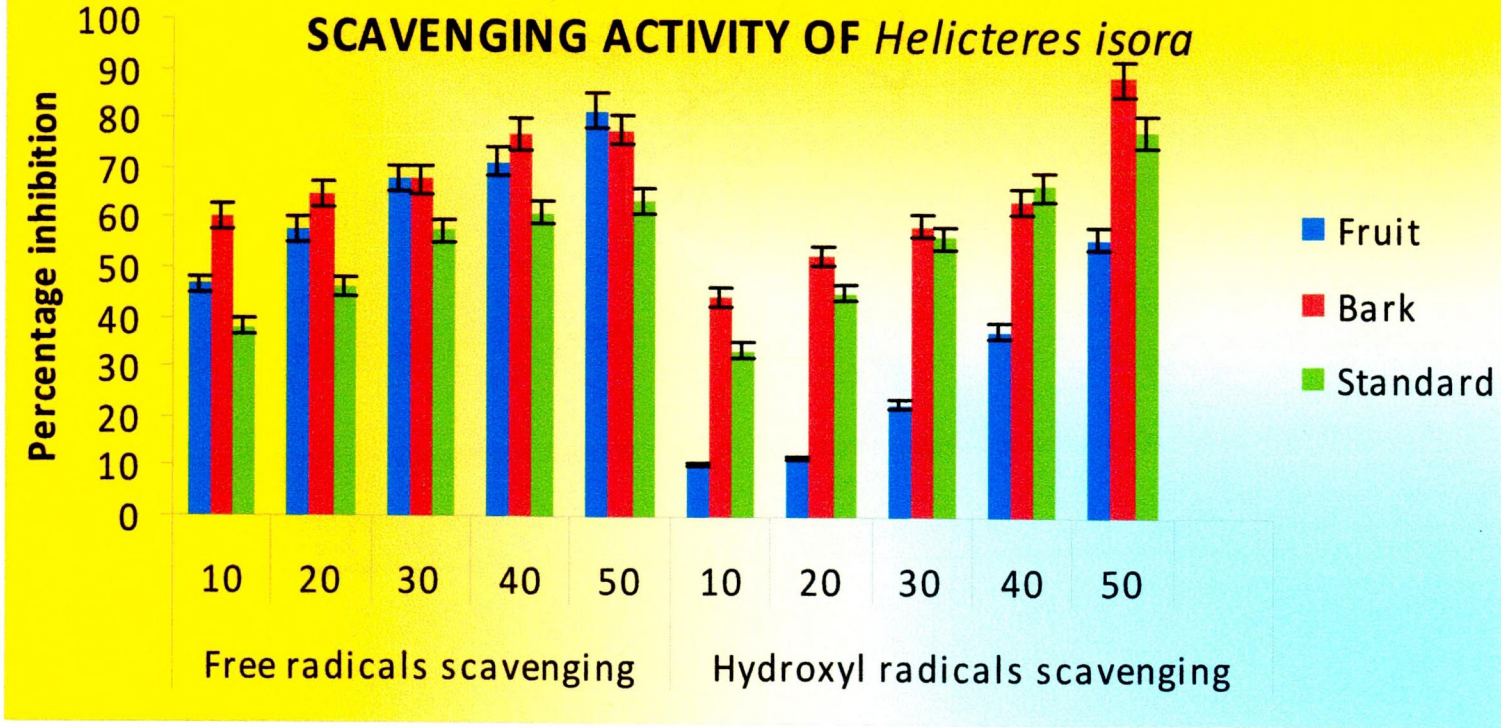
Extract	DPPH Free radicals scavenging activity (mg/ml)						Hydroxyl radicals scavenging activity (mg/ml)					
	10	20	30	40	50	IC_{50}	10	20	30	40	50	IC_{50}
Fruit	46.5	57.7	68.0	71.3	81.7	13.0	10.4	12.0	22.7	37.4	56.0	45.0
Bark	60.4	64.6	67.7	76.8	77.8	6.5	44.3	52.6	58.5	63.4	88.3	15.5
Standard	38.0	46.3	57.6	61.2	63.4	23.5	33.6	45.1	56.1	66.4	77.4	24.0

Values are mean of six replicates

Standard - Free radical scavenging activity - Ascorbic acid
Hydroxyl radical scavenging activity – Copper sulphate

FIGURE 2 a

DPPH FREE RADICALS AND HYDROXYL RADICALS SCAVENGING ACTIVITY OF *Helicteres isora*



The IC_{50} values for free radicals and hydroxyl radicals scavenging activities were found to be 13.0 and 45.0 mg/ml in the fruit and 6.5, 15.5 mg/ml in the bark respectively. When compared with the standard, bark extracts showed better activities for both the radicals. However, the fruit extract is effective only against free radicals alone.

The fruit and the bark extracts of *H.isora* are rich in the antioxidants, vitamins E and C, polyphenols, flavonoids and glutathione as indicated in Table 5. These antioxidants might have contributed to the effective inhibition of *in vitro* lipid peroxidation, superoxide generation, nitric oxide generation, free radicals and hydroxyl radicals scavenging activity. These results were similar to that of Khalaf *et al.* (2008) who have reported the antioxidant activity of some common plants.

The results of the present study indicate a strong *in vitro* antioxidant activity of the methanolic extract of both the fruit and the bark of *H.isora*. Therefore, it can protect cells from oxidative damage and toxic effects of ROS and control several diseases. Further studies are required to reveal *in vivo* antioxidant activity of *Helicteres isora* and its potential for therapeutic use.

4.1.4 ANTIMUTAGENIC ACTIVITY OF THE FRUIT AND THE BARK OF *Helicteres isora*

The purpose of the bacterial reverse mutation assay is to evaluate a chemical's genotoxicity by measuring its ability to induce reverse mutations at selected loci in several bacterial strains (Mc Cann *et al.*, 1975). The results of the evaluation of the antimutagenicity of *H.isora* in Ames *Salmonella* microsome assay are given in Table 8 and Plate 2.

TABLE 8

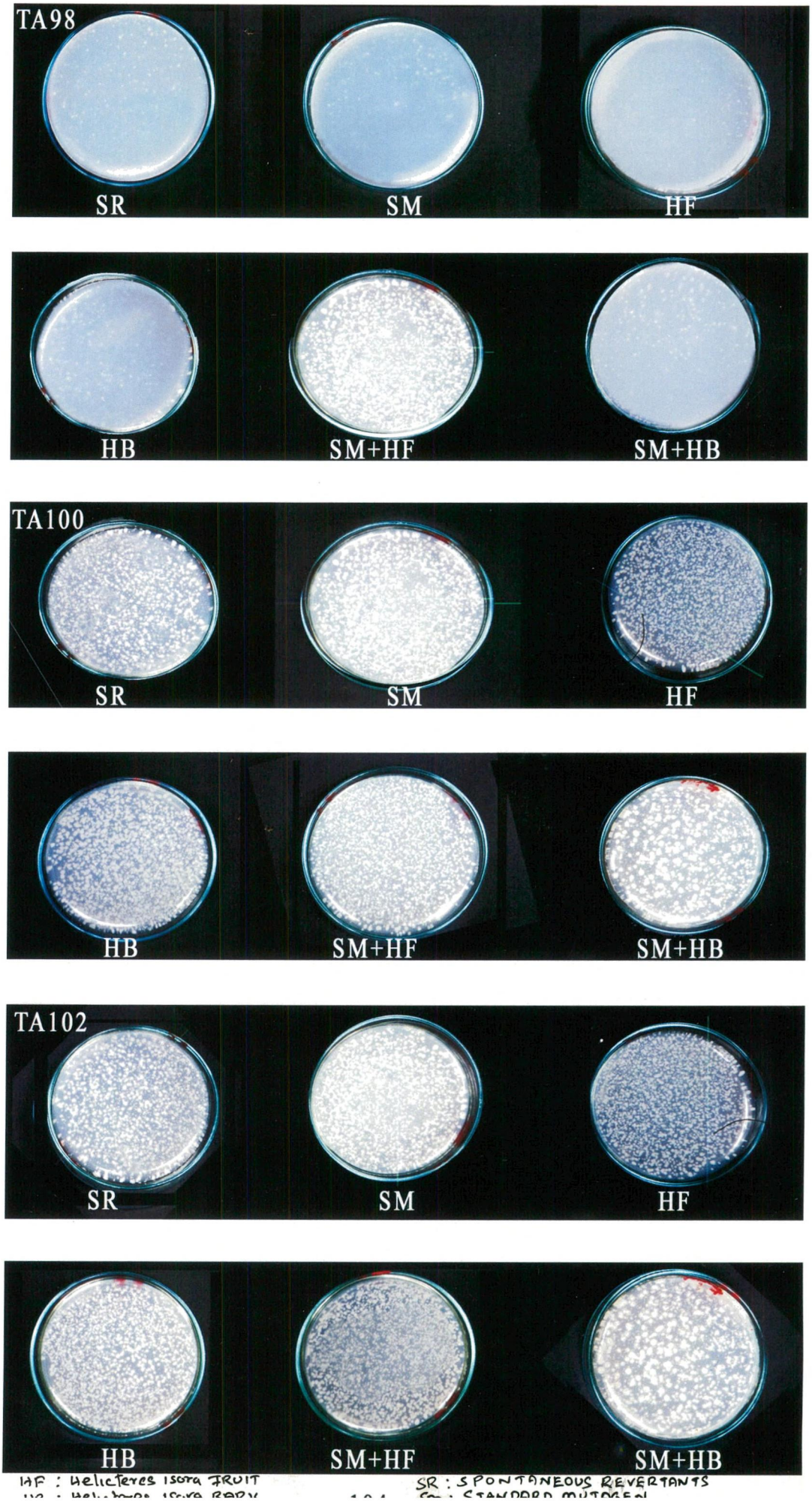
THE REVERSIBILITY OF STANDARD TESTER STRAINS AND MUTAGENICITY OF STANDARD MUTAGENS OF STANDARD TESTER STRAINS TA 98, 100, 102 ON TREATMENT WITH *Helicteres isora*

Sample	Number of His^+ revertants in TA 98		Number of His^+ revertants in TA 100		Number of His^+ revertants in TA 102	
	Extract	Extract + SM	Extract	Extract + SM	Extract	Extract + SM
Fruit	39 ± 4.48	42 ± 4.48	149 ± 5.1	163 ± 6.9	278 ± 5.09	238 ± 4.47
Bark	45 ± 4.12	49 ± 6.68	122 ± 4.7	137 ± 7.6	269 ± 6.04	241 ± 8.06
SR	49 ± 10.6		143 ± 10.6		268 ± 6.70	
SM	267 ± 2.12		274 ± 5.0		366 ± 9.61	
Normal range	30-50		120-200		240-320	

Values are mean of three plates
SR- Spontaneous Revertants SM- Standard Mutagen

PLATE 2

Ames *Salmonella* MICROSOME ASSAY



HF : Helicteres Isora FRUIT

SR : SPONTANEOUS REVERTANTS

The extracts of both the fruit and the bark of *H.isora* did not induce any frame shift mutation or base pair substitution at the dose level tested (100µg/plate). The colony counts in all the three strains were found to be close to the spontaneous revertants frequency showing that both the fruit and the bark powders of *H.isora* were antimutagenic at the dose level tested.

A similar study was conducted by Hayder *et al.* (2007) with the different extracts of leaves of *Myrtus communis* by the *Salmonella typhimurium* assay. The different extracts showed no mutagenicity when tested with *Salmonella typhimurium* strains TA 98 and TA 100. Bhatia *et al.* (2007) have also observed that the methanolic extracts of fruits of Seabuckthorn *(Hippophae rhamnoides* Linn.*)* exhibited strong antimutagenicity against TA 98 and TA 100 strains.

4.1.5 TOXICOLOGICAL EVALUATION OF *Helicteres isora*

Plants contain a large number of biologically active chemicals. Some of these have been found to be extremely useful for treating various human diseases (e.g. digitoxin, colchicines and atropine). However, some plant constituents produce adverse health effects following exposure. The onset of these adverse effects can be quite sudden or take some time to develop. Fortunately, among the thousands of plants in the environment of animals, relatively a few cause acute, life-threatening illnesses when ingested (Bnouham *et al.*, 2006).

Toxicity studies were carried out as a requirement for Ethical Committee clearance. The acute and chronic toxicity of the extracts of *Helicteres isora* in rats was assessed. The changes in selected and hematological and biochemical parameters were also determined after oral administration.

Acute toxicity studies

Two different doses (150 mg and 200 mg/kg body weight) of the fruit and the bark extracts of *Helicteres isora* were administered to eleven groups of ten rats each. One group of rats were taken as untreated control, the other group of rats were given carboxy methylcellulose alone and another group as diabetic control. These rats were given standard diet with distilled water alone. Four groups each of non diabetic and diabetic rats were given either 150mg or 200mg/kg of body weight of both the extracts of *Helicteres isora* fruit (HF) and *Helicteres isora* bark (HB) in 1.0 ml of distilled water. The animals were observed continuously for 3 hours and the mortality, if any, was recorded after 24 hours.

Except one of the diabetic control rat, which died on the 20th day of the experiment, all the other rats remained alive during the entire 45 days of observation.

It is evident from the above study that neither *Helicteres isora* fruit (HF) nor *Helicteres isora* bark (HB) at the doses (150 and 200 mg/kg body weight) administered to the rats has caused any visible toxicity.

Chronic toxicity studies

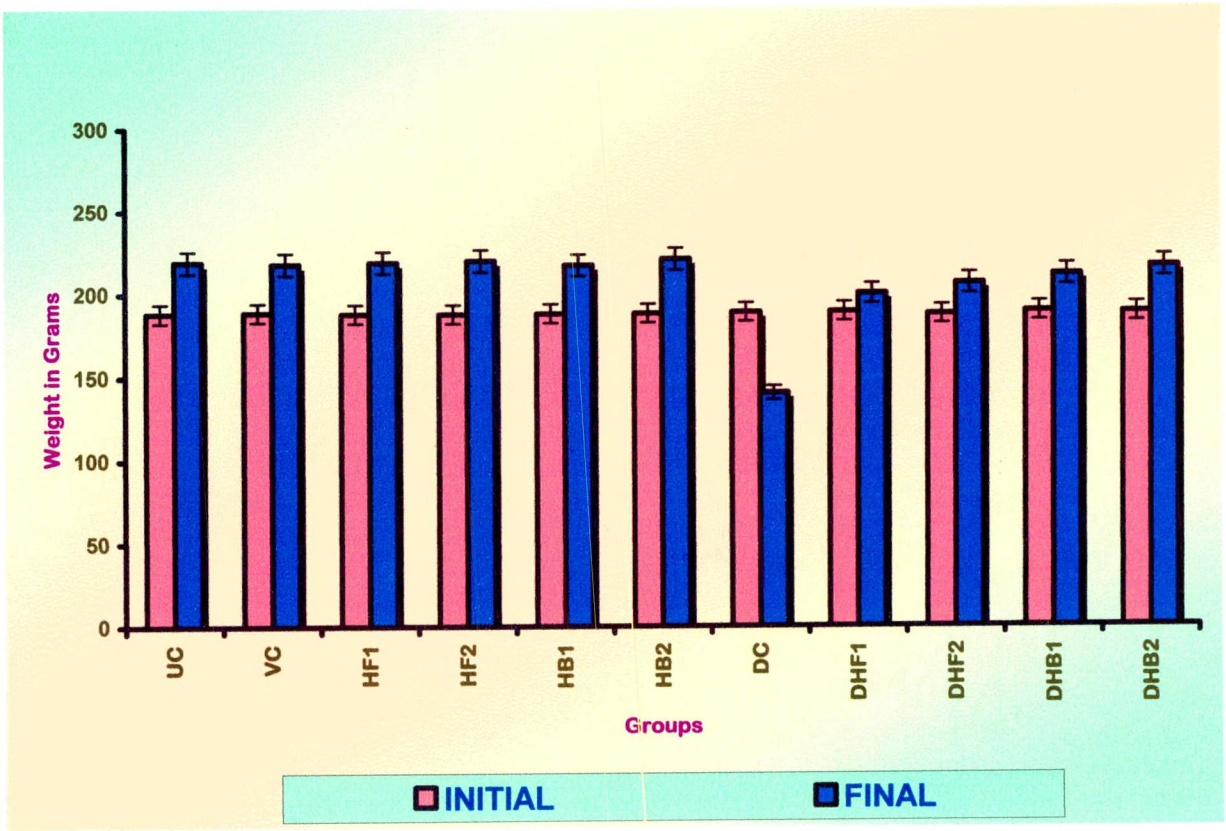
Chronic toxicity, if any, of both the fruit and the bark powders of *Helicteres isora* (150 and 200 mg/kg body weight) was determined in the experimental group of rats in terms of body weight, hematological and biochemical parameters. Food intake of the animals in all the groups was normal during the entire 45 days of experimental period.

Body weight of the rats

The body weight of the rats of the different groups are recorded initially and again at the end of the 45th day. This is illustrated in Figure 3.

FIGURE 3

BODY WEIGHT OF RATS BEFORE AND AFTER THE ADMINISTRATION OF *Helicteres isora* FOR 45 DAYS



Untreated control animals treated with HF or HB of 150 or 200 mg/kg body weight did not produce any weight loss but gained weight ($p<0.05$). There was no significant difference in the increase in the body weight between the controls and those control rats supplemented with HF and HB. A decrease in body weight ($p<0.05$) was noted in streptozotocin - induced diabetic rats. When the diabetic rats were treated with the fruit and the bark extracts of *Helicteres isora* the decrease in body weight was suppressed.

The weight gain of the treated diabetic rats is not above that of the controls even at the maximum dose (200 mg/kg body weight) of HF and HB tried.

Similar results for the body weight of the control and the diabetic rats treated with the extracts of *M.charantia* (bitter gourd), *A. indica* (neem), *O.sativum* (rice) and *O.sanctum* (tulsi) were reported by Mahdi *et al.* (2003). Ananthi *et al.* (2003) also reported a significant increase in body weight in diabetic rats administered with *Eclipta alba* (karichalankanni).

Thus the results reveal that the fruit and the bark extracts of *Helicteres isora* even at 200 mg/kg body weight do not cause any adverse effect on the body weight of the rats.

EFFECT OF *Helicteres isora* FRUIT AND BARK ON THE HEMATOLOGICAL PARAMETERS

The effect of *Helicteres isora* fruit and bark on RBC, WBC, Platelets and hemoglobin of the rats are given in Table 9.

TABLE 9

HEMATOLOGICAL PARAMETERS IN EXPERIMENTAL RATS

Group	Treatment		Hemoglobin (g/dl)	RBC (10^6 / mm^3)	WBC (10^3/ mm^3)	Polymorphs (%)	Lymphocytes (%)	Eosinophils (%)	Platelets/ mm^3
1	Untreated Control	UC	12.7±0.14	8.53±0.46	6.73±0.93	59.2±0.34	47.7±0.14	2.1±0.63	860±6.51
2	Carboxy Methyl Cellulose	VC	12.6±0.35	8.50± 0.46	6.69±0.82	59.4±0.28	47.9±0.64	2.0±0.32	861±4.24
3	Rats+HF (150 mg)	HF1	12.7±0.40	8.52± 0.13	6.71±1.53	59.1±0.44	47.7±0.45	1.9±0.46	860±4.47
4	Rats+HF (200 mg)	HF2	12.8±0.34	8.53± 0.07	6.75±1.37	59.0±0.35	47.6±0.57	2.1±0.40	863±4.56
5	Rats+HB(150 mg)	HB1	12.8±0.31	8.51±0.13	6.73±1.66	59.1±0.44	47.9±0.22	2.1±0.38	862±2.60
6	Rats+HB(200 mg)	HB2	12.9±0.28	8.53± 0.14	6.79±2.36	59.7±0.4	48.0±0.33	2.2±0.46	860±6.63
7	Diabetic (D)	DC	6.8±0.26	6.1± 0.54	5.29±0.24	55.2±0.36	41.2±0.68	2.8±0.23	851±4.00
8	Diabetic+HF (150 mg)	DHF1	11.3±1.08	8.21± 0.07	6.34±0.67	57.0±0.35	46.6±0.42	2.2±0.27	857±2.28
9	Diabetic+HF (200 mg)	DHF2	11.9±0.28	8.29± 0.23	6.49±0.68	57.1±0.26	46.9±0.74	2.0±0.15	859±4.04
10	Diabetic+HB(150 mg)	DHB1	12.0±0.34	8.31± 0.42	6.57±0.66	56.9±0.08	46.9±0.64	2.1±0.54	858±6.32
11	Diabetic+HB(200 mg)	DHB2	12.3±0.20	8.49± 0.60	6.60±0.57	58.9±0.66	47.3±0.44	2.0±0.55	861±6.72
	CD (0.05)		0.50	0.41	1.10	0.97	0.60	0.49	5.41

Values are mean ± SD of ten rats

The hematological parameters of the control and the diabetic rats treated with HF and HB were within the normal limits established for rats: 6-10 (10^6/mm^3) for red blood corpuscles, 5-13 (10^3/mm^3) for white blood corpuscles and 11-17 g/100ml for hemoglobin (Raghuramulu *et al.*, 1983) while in diabetic rats, a significant difference ($p<0.05$) from the values of the control rats was observed.

From the results, it has become clear that *Helicteres isora* fruit and bark did not produce any toxic effect on the hematological parameters.

EFFECT OF *Helicteres isora* FRUIT AND BARK ON THE BIOCHEMICAL PARAMETERS

The biochemical parameters namely creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assessed in serum and urea in blood.

The influence of *Helicteres isora* fruit and bark extracts on selected biochemical parameters in diabetic rats is given in Table 10.

TABLE 10

LEVELS OF BIOCHEMICAL PARAMETERS IN EXPERIMENTAL RATS

Group	Treatment		Urea	Creatinine	Total bilirubin	AST	ALT	ALP
			← (mg/dl) →			← (IU/l) →		
1	Untreated Control	UC	25.3±0.76	0.40±0.022	0.93±0.06	58.3±0.38	29.7±2.77	72.3±1.55
2	Carboxy Methyl Cellulose(CMC)	VC	25.0±0.72	0.41±0.03	0.92±0.02	58.2±0.87	30.2±4.28	72.8±1.50
3	Rats+HF (150mg)	HF1	25.2±0.29	0.40±0.05	0.93±0.02	58.3±0.60	29.9±1.52	73.2±1.42
4	Rats+HF (200mg)	HF2	24.9±0.99	0.42±0.14	0.92±0.03	57.1±4.97	29.8±4.66	72.9±1.52
5	Rats+HB(150mg)	HB1	24.2±0.29	0.39±0.04	0.91±0.02	57.9±1.81	29.1±1.43	72.1±0.94
6	Rats+HB(200mg)	HB2	25.1±0.66	0.40±0.06	0.94±0.03	58.5±1.45	29.9±0.81	73.1±2.35
7	Diabetic	DC	83.7±0.92	0.84±0.03	2.01±0.08	89.3±4.66	40.8±2.80	143.2±2.35
8	Diabetic+HF (150mg)	DHF1	37.8±0.83	0.55±0.01	0.99±0.06	60.1±3.26	30.2±2.80	92.6±1.46
9	Diabetic+HF (200mg)	DHF2	35.9±0.90	0.54±0.03	0.97±0.03	59.8±4.14	29.1±4.07	88.9±2.37
10	Diabetic+HB(150mg)	DHB1	32.2±1.42	0.49±0.02	0.92±0.03	58.1±3.21	29.0±1.53	78.9±2.37
11	Diabetic+HB(200mg)	DHB2	30.1±0.65	0.47±0.06	0.92±0.04	58.9±0.99	29.4±0.94	75.3±1.55
	CD (0.05)		0.96	0.04	0.05	2.87	3.27	2.12

Values are mean ± SD of ten rats AST U=µm oxaloacetate liberated/min/L ALT U=µm pyruvate liberated/min/L

ALP U=mm p-nitrophenol liberated/ min/L

All the biochemical parameters tested namely, total bilirubin, AST, ALT, ALP and creatinine in serum and urea in blood did not show any deviation from the normal range in both the controls and in those rats treated with either doses (150 or 200 mg) of both HF and HB.

The mode of disposal of nitrogen is by the formation of urea and creatinine. Increased urea and creatinine formation in diabetic condition might be due to increased protein catabolism, which would have resulted in increased elimination of urea, nitrogen and creatinine. In diabetes, urea synthesis is normalized by insulin therapy. Administration of *Helicteres isora* fruit and bark to diabetic rats would have reduced the elevated levels of urea and creatinine to the normal level, thus showing the normalizing effect of HF and HB on urea and creatinine synthesis.

The activities of AST and ALT were found to be higher in the diabetic animals when compared to the control rats. The activities of serum AST and ALT of diabetic rats were brought back to near normal in HF and HB treated diabetic rats. No significant change was noticed in the activities of these enzymes in HF and HB treated diabetic rats when compared to that of respective control rats.

Bolkent *et al.* (2008) have also reported an increase in the activities of aspartate transaminase and alanine transaminase in the diabetic animals, on the administration of vitamin B6 for 15 days, streptozotocin induced biochemical alterations were restored to near normal levels.

Hence, it could be suggested that oral administration of *Helicteres isora* fruit and bark revert the increased activities of diagnostic marker enzymes found in diabetic rats, also regulate the protein metabolism and urea synthesis, which clearly demonstrates that they have insulin like effect. However, HF and HB extracts do not alter the assayed parameters in

the control rats, thereby indicating their non-toxic nature in the applied dosage.

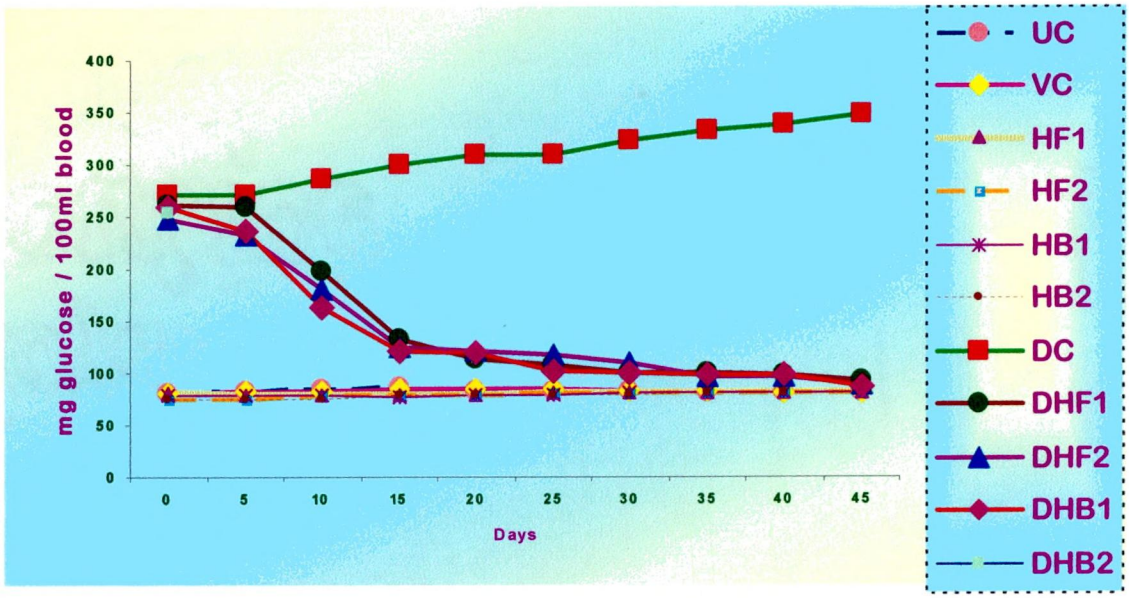
The hematological and the biochemical profile of the experimental animals analysed and observed confirmed the benefits of *Helicteres isora,* which proved to have hypoglycemic effect in the management of diabetes mellitus. Several schools of thought are available for the pharmacological effects of medicinal plants. Some hypotheses relate to their effects on the activity of pancreatic β cells (synthesis, release, cell regeneration / revitalization) or the increase in the protective/ inhibitory effect against insulinase and increasing insulin sensitivity or insulin like activity of the plant extracts. Other mechanisms involved might improve glucose homeostasis, increase of peripheral utilization of glucose, increase in the synthesis of hepatic glycogen and/or decrease of glycogenolysis, inhibition of intestinal glucose absorption, reduction of glycemic index of carbohydrates and reduction in the effect of glutathione. All these actions might be responsible for the reduction or the abolition of diabetic complications (Bnouham *et al.*, 2006).

EFFECT OF *Helicteres isora* FRUIT AND BARK EXTRACT ON BLOOD GLUCOSE LEVELS AT INTERVALS OF 5 DAYS FOR A PERIOD OF 45 DAYS

Blood glucose was determined at intervals of 5 days for a period of 45 days. This is illustrated in Figure 4.

FIGURE 4

LEVELS OF BLOOD GLUCOSE ON ADMINISTRATION OF *Helicteres isora*



As indicated in Fig.4 administration of *Helicteres isora* fruit and bark extracts was found to reduce blood glucose level in the control and the diabetic rats. The maximum reduction in blood glucose was noted on the 15th day.

Oral administration of the extracts significantly reduced the blood glucose level in diabetic rats treated with *Helicteres isora* fruit and bark. Control rats treated with 150 or 200 mg/kg body weight of *Helicteres isora* fruit and bark did not exhibit any significant change in the blood glucose level. Also the levels were within the normal range.

In the diabetic controls, the blood glucose level increased upto 300 ± 0.65 mg/dl in 15 days after the administration of streptozotocin. This increase continued throughout the experimental period and it was 348.0 ± 1.61 mg/dl on the 45th day. But administration of 150 mg/kg body weight of HF to diabetic rats showed significant ($p<0.05$) reduction from

the 15th day onwards and produced 69 per cent reduction in the elevated glucose level on the 45th day. With the dosage of 200 mg/kg body weight, HF caused a significant ($p<0.05$) reduction of the blood glucose level from the 15th day onwards and produced 75 per cent reduction in the blood glucose level on the 45th day.

Bark extract of *Helicteres isora* (HB) at the dose of 150 mg/kg body weight produced significant reduction in the blood glucose from the 15th day onwards and produced 70 per cent reduction in the glucose level on the 45th day. At the dose of 200 mg HB/kg body weight, there was a 76 per cent reduction from the 15th day onwards and the blood glucose level was on par with those of control rats on the 45th day.

The results indicate that the selected doses of the fruit and the bark powders of *Helicteres isora* reduce the blood glucose level in streptozotocin-induced diabetic rats. The dosage of 200 mg/kg body weight of HF and HB was found to be more effective than the dosage of 150 mg/kg body weight.

On the basis of these studies, the dosage of 200 mg/kg body weight of HF and HB individually and in combination (100 mg HF + 100 mg HB/kg body weight) were selected for further investigations and compared with the effect of selected oral diabetic drugs - glibenclamide (sulfonylurea) and metformin (biguanide) commonly used in the treatment of diabetes mellitus.

PHASE II

4.2 DETERMINATION OF ANTIDIABETIC EFFECT OF THE FRUIT AND THE BARK OF *Helicteres isora*

4.2.1 EFFECT OF *Helicteres isora* FRUIT AND BARK ON BLOOD GLUCOSE AND LIVER GLYCOGEN

The effect of HF and HB on blood glucose and liver glycogen levels are presented in Table 11.

TABLE 11

LEVELS OF BLOOD GLUCOSE AND LIVER GLYCOGEN IN DIFFERENT GROUPS OF RATS

Groups	Treatment		Blood glucose (mg/dl)	Liver Glycogen (mg/g tissue)
1	Untreated Control	C	80.1±1.46	62.6±1.46
2	Rats + HF (200mg)	HF	80.5±0.50	62.1±0.99
3	Rats + HB(200mg)	HB	80.3±0.50	62.4±0.45
4	Rats + HF (100 mg) + HB (100 mg)	HF+HB	80.7±0.96	62.9±1.44
5	Diabetic	DC	344.5±1.45	29.0±1.18
6	Diabetic + HF (200mg)	DHF	87.7±0.55	59.2±1.42
7	Diabetic + HB (200mg)	DHB	80.9±0.85	60.7±1.39
8	Diabetic + HF (100 mg) + HB (100 mg)	DHF+DHB	82.4±0.80	61.4±1.49
9	Diabetic + Glibenclamide	DG	90.2±0.86	58.4±1.41
10	Diabetic + Metformin	DM	102.0±1.34	52.3±1.45
	CD (0.05)		9.75	8.25

Values are mean ± SD of ten rats

The blood glucose levels were found to have neither increased nor decreased in rats on the administration of HF and HB individually and in combination (Group 2 to 4). It indicates that the HF and the HB do not have hypoglycemic effect on normoglycemic rats.

The oral administration of HF and HB individually and in combination showed a significant ($p<0.05$) decrease in blood glucose level in streptozotocin-induced diabetic rats. The results were compared with the effects of the administration of glibenclamide and metformin (Group 9 and 10 respectively). Administration of the herbal extracts showed a hypoglycemic effect on the blood glucose levels similar to that of the allopathic drugs tested. The extracts reduced the elevated blood glucose level in the streptozotocin-diabetic rats to a level on par with those to the untreated controls. Therefore the pharmacological effect of the herbal extracts could be compared to that of glibenclamide and metformin.

Insulin and sulfonylurea drugs cause hypoglycemia when taken in excessive doses and overt hypoglycemia is the most worrisome effect of these drugs. However the extracts of HF and HB did not cause any adverse effect of hypoglycemia.

Several studies have reported the antidiabetic action of a number of medicinal plants. Animal researches have indicated that *Ginseng* lower blood glucose by possibly decreasing the rate of carbohydrate absorption in the portal hepatic circulation and possibly increasing glucose transport and uptake (Luo and Luo, 2008).

The fruit and the seeds of *Momordica charantia* (bitter melon) were thought to exert hypoglycemic effects in normal and diabetic animal models (Shetty *et al.*, 2003).

Murraya koenigii (curry leaves) showed varying hypoglycemic and antihyperglycemic effect (Kesari *et al.*, 2005). Oral route of *Cucurbita ficifolia* (fig leaved gourd) juice also showed highly significant reduction of glycemia after 14 days of treatment (Xia and Wang, 2006).

Zhang *et al.* (2003) have also reported that treatment of alloxan induced diabetic rats with *Rehmannia glutinosa* (Chinese Foxglove) oligosaccharide for 15 days resulted in a significant decrease in blood glucose level and increase in hepatic glycogen content.

The results of the present study are corroborative with the report of Lemhadri *et al.* (2004), who showed that administration of the aqueous extract of *Origanum vulgare* (oregano) to STZ-induced diabetic rats caused a significant reduction in the blood glucose level.

Aqueous extracts of other plants such as *Carum carvi* and *Capparis spinosa* (Eddouks *et al.*, 2004), *Withania coagulans* (Hemalatha *et al.*, 2004) and *Fraxinus excelsior* (Eddouks and Maghrani, 2004) has also been found to be beneficial in the treatment of diabetes mellitus.

Liver glycogen levels

Administration of HF and HB individually and in combination did not produce any change in liver glycogen levels in normal rats. There was a marked reduction in the liver glycogen levels of the streptozotocin induced diabetic rats. Treatment with the herbal extract remarkably attenuated this reduction in glycogen content. This effect of the herbal drug is better than that of the allopathic drugs tested.

Increase in liver glycogen can be brought about by an increase in glycogenesis and/or decrease in glycogenolysis. Babu *et al.* (2003) reported

that the extract of *Cassia kleinii* has stimulated glycogenesis and/or inhibited glycogenolysis in the diabetic rat liver.

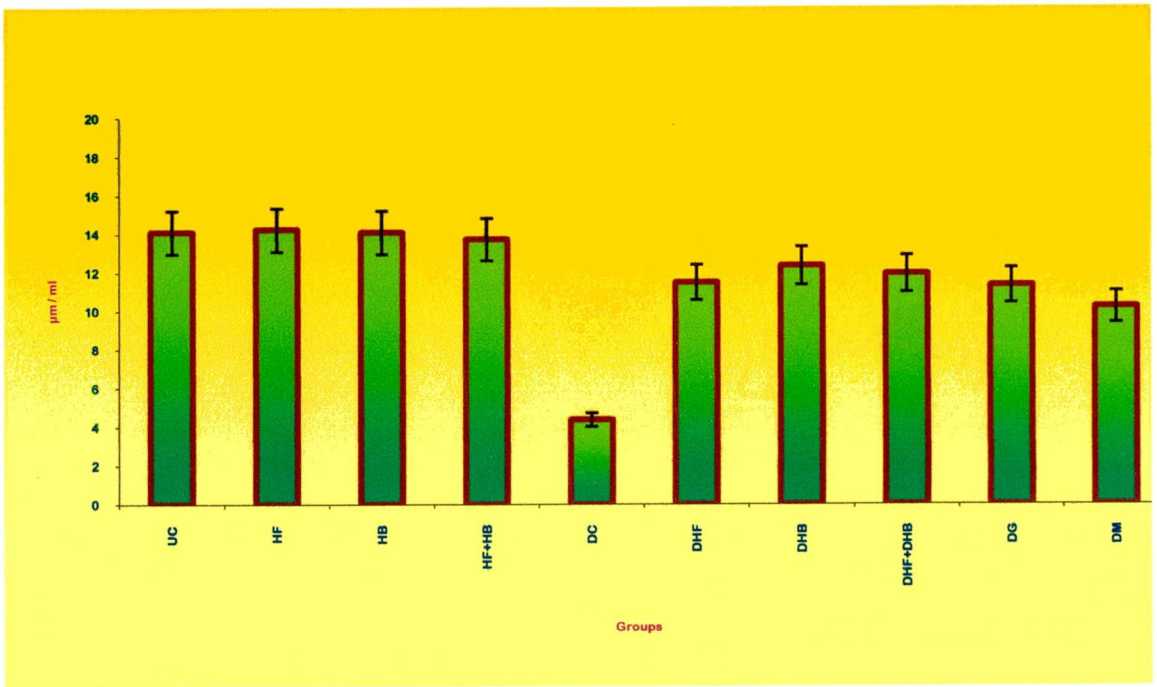
Another study by Sridhar *et al.* (2005) showed that decreased liver glycogen in diabetes had been increased significantly after the administration of *Eugenia jambolana* (jamun) seed powder. The results of the present study are in corroboration with this report. HF and HB extracts could have stimulated glycogenesis or inhibited glycogenolysis in the liver of the diabetic rats.

4.2.2 EFFECT OF *Helicteres isora* FRUIT AND BARK ON PLASMA INSULIN CONCENTRATION

The plasma insulin levels of the normal and the diabetic rats were assessed by conducting a sub study and the results are illustrated in Figure-5.

FIGURE 5

LEVELS OF INSULIN IN *Helicteres isora* TREATED RATS



Plasma insulin concentrations were not significantly different in rats treated with HF and HB individually and in combinations as compared to untreated control rats. Administration of HF and HB did not produce any significant increase in plasma insulin level of normoglycemic rats, whereas the HF and HB treated diabetic rats have shown increased levels of plasma insulin when compared to the diabetic control rats. From the results of the present study, it appears that still insulin producing cells are functioning and stimulation of the insulin release could be possible by the treatment of the extracts of HF and HB. The increased plasma insulin may be due to the activation of beta cells of islets of langerhans.

The results are in accordance with that of Rajagopal and Sasikala, (2008) who have reported that administration of hydro-ethanolic extracts of *Nymphaea stellata* (water lily) flowers significantly decreased the levels of plasma insulin in diabetic rats.

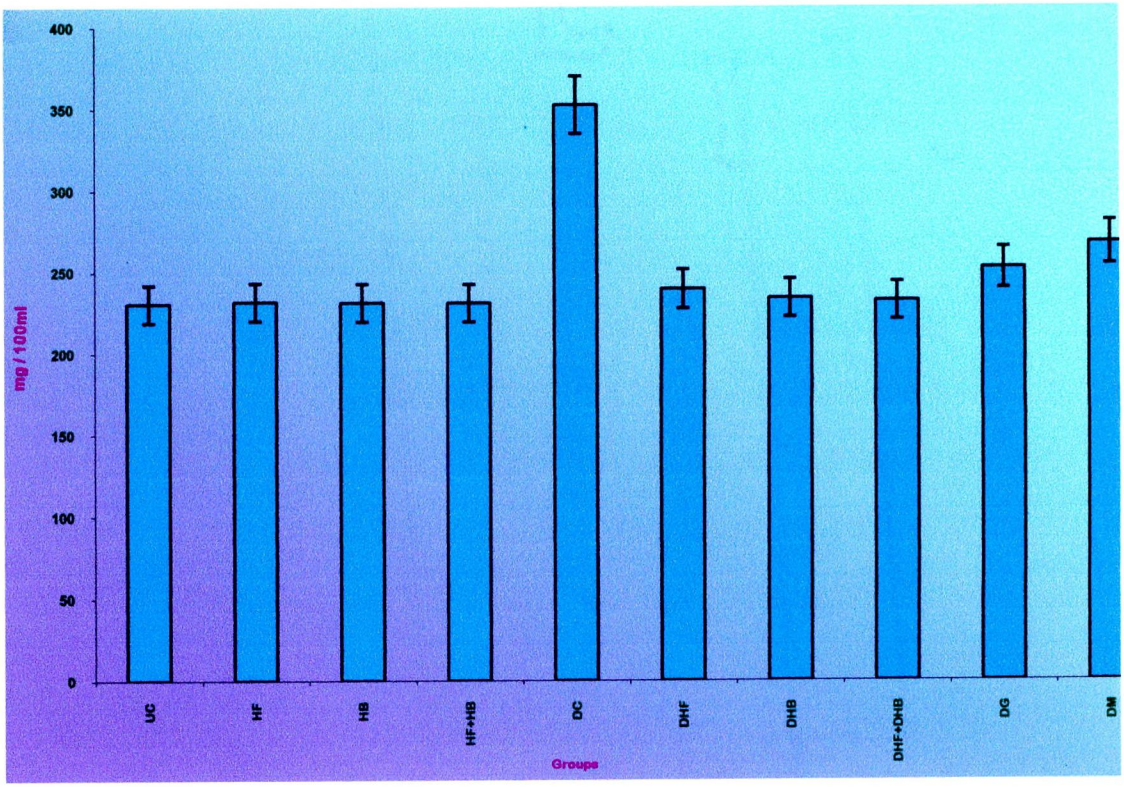
Babu *et al.* (2007) also reported that the administration of cinnamaldehyde to diabetic rats also showed increased levels of plasma insulin.

4.2.3 INFLUENCE OF *Helicteres isora* FRUIT AND BARK ON THE SERUM FRUCTOSAMINE LEVELS

Serum fructosamine concentration in the control and the experimental groups of rats is presented in Figure 6.

FIGURE 6

SERUM FRUCTOSAMINE LEVELS IN RATS



Serum fructosamine concentrations were not significantly different in the untreated control rats and the rats treated with HF and HB individually and in combinations. There was a significant ($p<0.05$) increase in the serum fructosamine level in the streptozotocin-induced diabetic rats. A significant ($p<0.05$) reduction of fructosamine (glycosylated plasma proteins) in the diabetic rats treated with HF, HB, glibenclamide and metformin treatment confirmed the effect of these in controlling the level of blood glucose. The antihyperglycemic effect produced by HF and HB in this respect was not significant ($p<0.05$) than the effect produced by the well known oral hypoglycemic agents glibenclamide and metformin.

Hosoda *et al.* (2003) have reported that fructosamine was reduced by Oolong tea in diabetic patients. The results of the present investigation are consistent with that of Lee *et al.* (2008) who have also reported that the level

of fructosamine was lowered in streptozotocin - induced diabetic rats that were given *Fomitopsis pinicola* extracts.

4.2.4 EFFECT OF *Helicteres isora* FRUIT AND BARK ON HEPATIC KEY ENZYMES OF CARBOHYDRATE METABOLISM

The effect of HF and HB individually and in combinations on hepatic key enzymes of carbohydrate metabolism glucokinase, glucose-6-phosphate dehydrogenase, fructose-1,6-bisphosphatase and glucose-6-phosphatase is shown in Table 12.

TABLE 12

ACTIVITIES OF GLUCOKINASE, GLUCOSE-6-PHOSPHATE DEHYDROGENASE, FRUCTOSE-1, 6- BISPHOSPHATASE AND GLUCOSE-6-PHOSPHATASE (U/mg protein)

Groups	Treatment		Glucokinase	Glucose-6-phosphate dehydrogenase	Fructose-1,6-bisphosphatase	Glucose-6-phosphatase
1	Untreated Control	UC	50.9±2.74	149.9±5.1	268.3±5.99	416.5±2.37
2	Rats + HF (200mg)	HF	50.2 ±4.41	149.8±2.27	268.7±2.32	417.6±3. 8
3	Rats + HB(200mg)	HB	51.0±2.16	148.9±2.35	269.1±1.46	416.8±1.50
4	Rats HF (100 mg) + HB (100 mg)	HF+HB	50.5±2.85	149.3±2.02	268.4±1.11	417.9±2.31
5	Diabetic	DC	24.7±2.32	73.2±4.0	642.5±3.75	762.2±3.87
6	Diabetic + HF (200mg)	DHF	49.1±2.05	145.0±3.41	279.4±2.37	449.5±1.73
7	Diabetic + HB (200mg)	DHB	49.7 ±2.05	148.3±3.74	274.5±3.22	447.3±2.03
8	Diabetic HF (100 mg) + HB (100 mg)	DHF+DHB	50.1±2.49	149.1±2.38	269.1±2.37	419.8±1.50
9	Diabetic + Glibenclamide	DG	45.7±3.19	141.8±1.78	299.7±3.99	458.7±4.23
10	Diabetic + Metformin	DM	47.5 ± 2.40	143.7±2.61	268.3±4.54	449.7±2.26
	CD (0.05)		3.11	3.64	30.09	3.23

Values are mean ± SD of ten rats
Glucokinase: Units: µmoles of glucose phosphorylated/hr
Glucose-6-phosphate dehydrogenase : Units: 50 per cent reduction in NADP
Fructose-1,6 bisphosphatase: Units: µmoles of phosphate liberated/hr
Glucose-6-phosphatase: Units: µmoles of phosphate liberated/hr

In control rats, the oral administration of HF (200mg/kg b.w.) and HB (200mg/kg b.w.) individually and in combinations [HF (100mg) + HB (100mg)/kg b.w.] did not change the activities of glycolytic enzyme, glucokinase, pentose phosphate pathway enzyme glucose-6-phosphate dehydrogenase and gluconeogenic enzymes, glucose-6-phosphatase and fructose-1, 6-bisphosphatase.

In streptozotocin-induced diabetic rats, a significant decrease in the activity of glucokinase and glucose-6-phosphate dehydrogenase was observed. The gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase activities ($p<0.05$) were found to have significantly increased in diabetic rats.

Petrolonis *et al.* (2004) observed that the activities of lipogenic enzyme and glucokinase had significantly decreased, whereas the activities of gluconeogenic enzymes had significantly increased in the diabetic liver. Glucose-6-phosphatase activity in pancreatic islets could be an important factor in the control of glucose metabolism and consequently of glucose dependent insulin secretion.

This is in line with the findings of Ugochukwu and Babady (2003), who have reported that diabetic rats showed a significant decrease in the activities of both the hepatic glucose-6-phosphate dehydrogenase and glucokinase.

Coccinia indica was found to alter the abnormal activities of key hepatic enzymes in the experimental diabetic rats. The glycolytic enzyme glucokinase is expressed only in the liver and the pancreatic β-cells and has a key role in the regulation of glucose homeostasis. In hepatocytes, the phosphorylation of glucose by glucokinase facilitates the uptake and

metabolism of glucose by maintaining a gradient for glucose transport into these cells (Mukherjee *et al.*, 2006).

In pancreatic β-cells, glucokinase appears to make up part of the glucose sensing mechanism and to be involved in the regulation of insulin secretion (Coghlan and Leighton, 2008).

Seo *et al.* (2008) have confirmed that the treatment of diabetic rats with either turmeric or curcumin had drastically reduced glucose-6-phosphatase and had elevated the activities of liver glucokinase and glucose-6-phosphate dehydrogenase. Treatment with aqueous extract of *Enicostemma littorale* blume in diabetic rats significantly decreased liver glucose-6-phosphatase activity (Maroo *et al.*, 2003).

The results of this study are also in agreement with Prakasam *et al.* (2002) who have reported that oral administration of aqueous extract of *Casearia esculenta* root for 45 days in diabetic rats have resulted in a significant reduction in blood glucose, glucose-6-phosphatase and fructose-1,6-bisphosphatase and an increase in the activity of liver glucokinase. Pari and Satheesh (2004) have shown that the activity of the hepatic enzymes such as glucokinase has significantly increased and glucose-6-phosphatase, fructose 1,6-bisphosphatase have been significantly decreased in diabetic rats after the administration of *Boerhaavia diffusa* extract.

Treating the diabetic rats with *Helicteres isora* extracts has been found to be beneficial as all the three carbohydrate-metabolizing enzymes assessed were found to be on par with the non-diabetic controls. This goes to prove that *Helicteres isora* is an antidiabetic medicinal plant.

4.2.5 EFFECT OF *Helicteres isora* FRUIT AND BARK ON LIPID PROFILE

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40 per cent of diabetic patients (Ravi *et al.*, 2005). Effect of administration of HF and HB on serum and liver lipid profile of untreated control, treated control and streptozotocin-induced diabetic rats is depicted in Tables 13 and 14 respectively.

TABLE 13

SERUM LIPID PROFILE (mg/dl) IN THE EXPERIMENTAL RATS

Group	Treatment		Total cholesterol	HDL cholesterol	VLDL cholesterol	LDL cholesterol	Triglycerides	Phospholipids
1	Untreated Control	C	88.7±0.52	38.9±0.34	16.2±1.89	33.9±0.23	70.6±0.60	179.0±0.63
2	Rats+HF (200mg)	HF	88.1±0.62	37.5±0.54	15.7±1.18	34.08±0.45	68.4±0.49	180.8±0.60
3	Rats+HB (200mg)	HB	87.9±0.46	35.1±1.07	16.5±1.86	36.4±0.38	69.5±0.60	180.3±0.74
4	Rats HF (100 mg) + HB (100 mg)	HF+HB	88.2±0.77	39.4±0.60	15.4±1.38	33.4±1.42	69.8±0.29	181.1±0.62
5	Diabetic	DC	158.3±0.74	34.6±0.76	25.1±5.63	98.8±0.62	123.2±0.68	243.5±0.90
6	Diabetic+HF (200mg)	DHF	102.3±0.40	43.9±0.65	19.6±0.96	38.7±0.28	84.5±1.50	197.3±0.57
7	Diabetic+HB (200mg)	DHB	99.8±0.65	46.2±0.89	18.4±1.12	35.3±0.40	83.6±0.46	192.8±1.12
8	Diabetic HF (100 mg) + HB (100 mg)	DHF+DHB	102.7±0.54	45.8±0.38	20.0±3.25	36.9±0.57	78.4±0.41	190.1±0.63
9	Diabetic+Glibenclamide	DG	109.0±0.83	33.9±0.65	17.3±1.21	57.8±0.39	83.5±0.62	191.4±0.94
10	Diabetic+Metformin	DM	112.2±4.42	47.7±0.79	18.7±2.27	46.0±0.60	88.9±0.63	198.0±0.60
	CD (0.05)		0.8	0.82	2.23	0.72	0.81	0.88

Values are mean ± SD of ten rats

TABLE 14

LIVER LIPID PROFILE (mg/dl) OF THE RATS

Group	Treatment		Total cholesterol	HDL cholesterol	VLDL cholesterol	LDL cholesterol	Triglycerides	Phospholipids
1	Untreated Control	UC	3.71±0.014	2.10±0.014	0.15±0.042	1.45±0.072	4.8±0.34	27.2±0.77
2	Rats+HF (200 mg)	HF	3.69±0.034	2.13±0.02	0.19±0.032	1.37±0.77	4.5±0.57	26.3±0.773
3	Rats+HB (200 mg)	HB	3.61±0.040	2.09±0.034	0.17±0.054	1.35±0.028	4.9±0.63	24.1±0.65
4	Rats HF (100 mg) + HB (100 mg)	HF+HB	3.70±0.063	2.15±0.028	0.16±0.042	1.41±0.084	4.7±0.52	22.3±0.93
5	Diabetic	DC	4.20±0.032	2.14±0.038	0.18±0.032	1.96±0.108	6.8±0.46	37.3±0.96
6	Diabetic+HF (200 mg)	DHF	3.89±0.69	2.15±0.049	0.19±0.068	1.55±0.068	5.0±0.37	30.1±0.65
7	Diabetic+HB (200 mg)	DHB	3.81±0.045	2.40±0.06	0.15±0.046	1.26±0.054	4.9±0.34	26.4±0.66
8	Diabetic HF (100 mg) + HB (100 mg)	DHF+DHB	3.79±0.034	2.15±0.028	0.15±0.016	1.49±0.110	4.5±0.40	29.4±0.59
9	Diabetic+Glibenclamide	DG	3.75±0.014	2.13±0.023	0.16±0.014	1.46±0.034	4.9±0.52	27.2±0.63
10	Diabetic+Metformin	DM	3.78±0.696	2.11±0.038	0.17±0.014	1.50±0.085	4.8±0.60	28.1±0.95
	CD (0.05)		0.06	0.40	0.04	0.09	0.56	0.91

Values are mean ± SD of ten rats

The treatment of the control rats with HF (200mg/kg b.w.) and HB (200mg/kg b.w.) individually and in combinations [HF (100mg) + HB (100mg)/kg b.w.] for 45 days did not alter the serum and the liver lipid profile.

The values of the different fractions of lipids of the untreated control rats administered with HF and HB individually and in various combinations were almost similar to those of the control rats.

Mean serum and liver levels of total cholesterol, VLDL cholesterol, LDL-cholesterol, triglycerides (TGL) and phospholipids were significantly ($p<0.05$) higher and HDL cholesterol levels were significantly ($p<0.05$) lower in streptozotocin-induced diabetic rats than in the controls.

The results of this investigation were in accordance with Sharma *et al.* (2008) who have reported that the extracts of *Eugenia jambolona* treatment have effected a significant dose dependent reduction in total lipids, LDL and VLDL cholesterol and phospholipids with a concomitant rise in HDL-cholesterol in the diabetic rats.

Kameswararao *et al.* (2003) reported that the serum cholesterol, triglyceride, LDL and VLDL cholesterol levels were significantly higher in the diabetic rats compared to those in the normal rats, while HDL cholesterol level was found to be decreased in the diabetic rats. On treatment with *Momordica cymbalaria* to the diabetic rats, a significant reduction in serum total cholesterol, LDL cholesterol, VLDL cholesterol and triglyceride and a significant increase in HDL cholesterol were observed. The root of *Withania somnifera* has been shown to be a highly potential hypocholesterolemic and hypoglycemic agents (Anwer *et al.*, 2008).

Hyperlipidemia is often associated with diabetes and could induce cardiovascular problems. Studies have shown that curcumin has been an

effective hypolipidemic agent. It has been observed that the LDL-VLDL fractions and the serum levels of TGL get reduced by dietary curcumin in the diabetic rats (Manjunatha and Srinivasan, 2007).

Basch *et al.* (2003) reported that the administration of fenugreek decreased TGL level and increased HDL cholesterol levels, presumably due to enhanced insulin sensitivity.

Ipomoea batatas (Caiapo) lowered total and LDL cholesterol levels, as well as blood glucose by increasing insulin sensitivity without affecting insulin secretion (Ludvik *et al.*, 2004). In the present study, *Helicteres isora* might have increased insulin sensitivity or insulin secretion.

In the present study when compared to the untreated control, the phospholipid level was found to be higher in the diabetic rats and it has been significantly reduced in those diabetic rats treated with *Helicteres isora.* These findings are in line with those of Pari and Murugan (2007) who have reported that the level of phospholipids has been reduced by dietary curcumin in diabetic rats.

4.2.6 EFFECT OF *Helicteres isora* FRUIT AND BARK ON OXIDATIVE STRESS

Changes in the levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides in diabetic rats

The levels of TBARS and hydroperoxides in the liver of untreatedcontrol and the diabetic rats consequent to the administration of HF and HB individually and in selected combinations is depicted in Figures - 7 and 8.

FIGURE 7

LEVELS OF THIOBARBITURIC ACID REACTIVE SUBSTANCES IN THE LIVER OF THE RATS

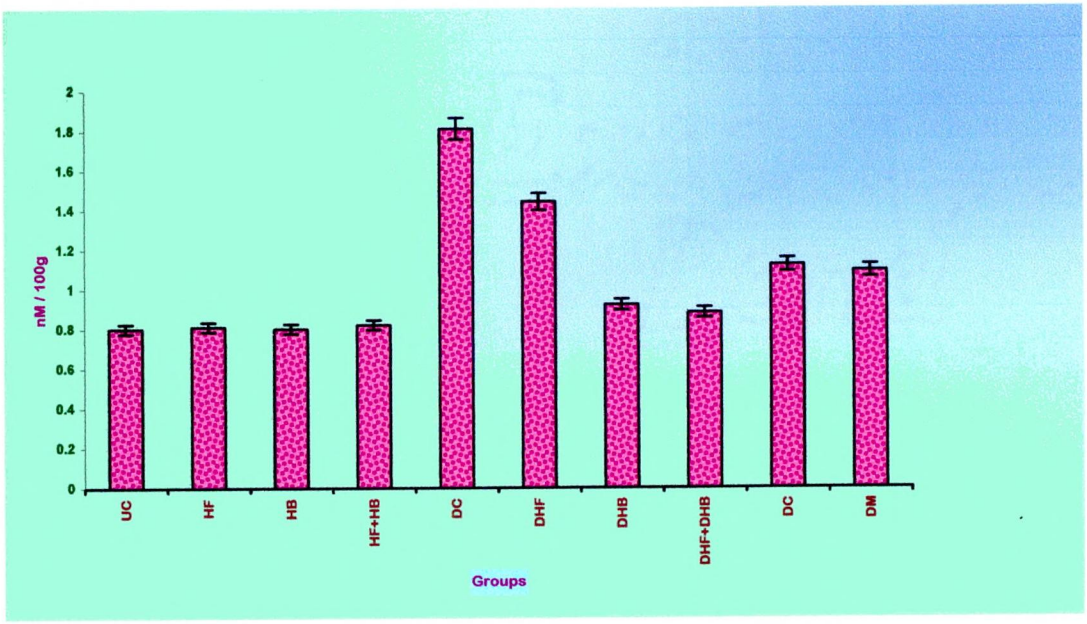
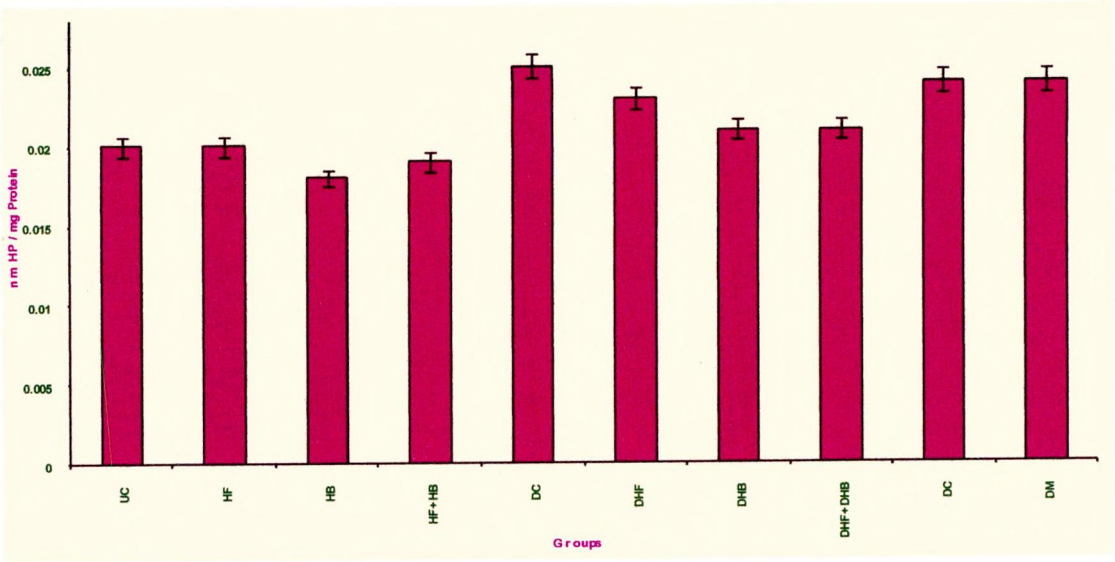


FIGURE 8

LEVELS OF HYDROPEROXIDES IN THE LIVER OF THE RATS



The levels of TBARS and hydroperoxides in the liver samples of the rats were significantly ($p<0.05$) higher in streptozotocin-diabetic rats when compared to the untreated control rats. Oral administration of both HF and HB individually and in combinations in streptozotocin-diabetic rats caused a significant ($p<0.05$) decrease in the level of TBARS and hydroperoxides in the liver as compared to that of the diabetic rats. This might be due to the anti-peroxidative effect of the components present in HF and HB. The reference drugs glibenclamide and metformin also decreased the level of TBARS and hydroperoxides in the liver of streptozotocin-induced diabetic rats significantly ($p<0.05$). Both HF and HB showed a significant effect than that of glibenclamide and metformin in this respect.

Earlier, there have been many reports documenting elevated serum lipid peroxide level and diminished antioxidant status in diabetic subjects (Bukan *et al.*, 2003).

Lipid peroxidation (LPO) refers to the reaction of oxidative deterioration of polyunsaturated lipids. Peroxidation involves the direct reaction of oxygen and lipid to form radical intermediates and to produce semistable peroxides, which in turn damage the enzymes, nucleic acids, membranes and proteins. Increased lipid peroxidation under diabetic condition may be due to the increased oxidative stress in the cells as a result of the depletion of antioxidant scavenger systems. Catalase and Glutathione peroxidase are considered to be essential antioxidants in the reduction of H_2O_2 (Gupta *et al.*, 2003). The increased levels of the antioxidants present in the extracts of *Helicteres isora* might be responsible for the significant reduction in TBARS and hydroperoxides in treated diabetic rats.

Kamalakannan and Prince (2003) have reported that the oral administration of *Aegle marmelos* has decreased the concentration of TBARS in the heart and pancreas of streptozotocin-induced diabetic rats. The inhibition of antioxidant enzymes in diabetes results in over production of reactive oxygen species that might lead to the accumulation of lipid peroxide products. The increased level of TBARS is an index of lipid peroxidation. The increased levels of LPO in diabetic rats indicate the degenerative status in diabetes.

Aslan *et al.* (2007) have also reported that feeding of ethanol extracts of *Helichrysum graveolens* Capitulums proved to have hypoglycemic effect in rats, lowered lipid peroxidation and altered superoxide dismutase and catalase enzymes in erythrocytes and tissues.

From the results of the present study, it could be understood that the harmful effects of TBARS in diabetes could be drastically reduced by the administration of *Helicteres isora*, which is an excellent source of antioxidants.

Changes in the activities of enzymic antioxidants in diabetic rats

Figures 9-12 represent the activities of antioxidant enzymes catalase, glutathione peroxidase, superoxide dismutase and glutathione-S-transferase in the diabetic rats treated with HF and HB as well as oral hypoglycemic drugs.

FIGURE 9

CATALASE ACTIVITY IN THE LIVER OF THE RATS

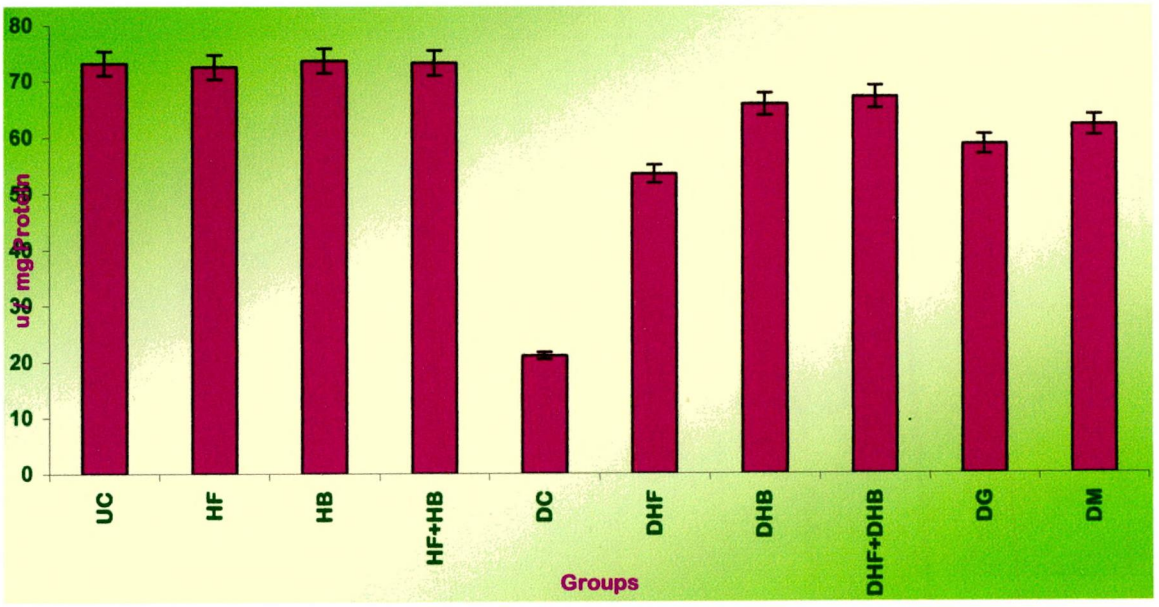


FIGURE 10

GLUTATHIONE PEROXIDASE ACTIVITY IN THE LIVER OF THE RATS

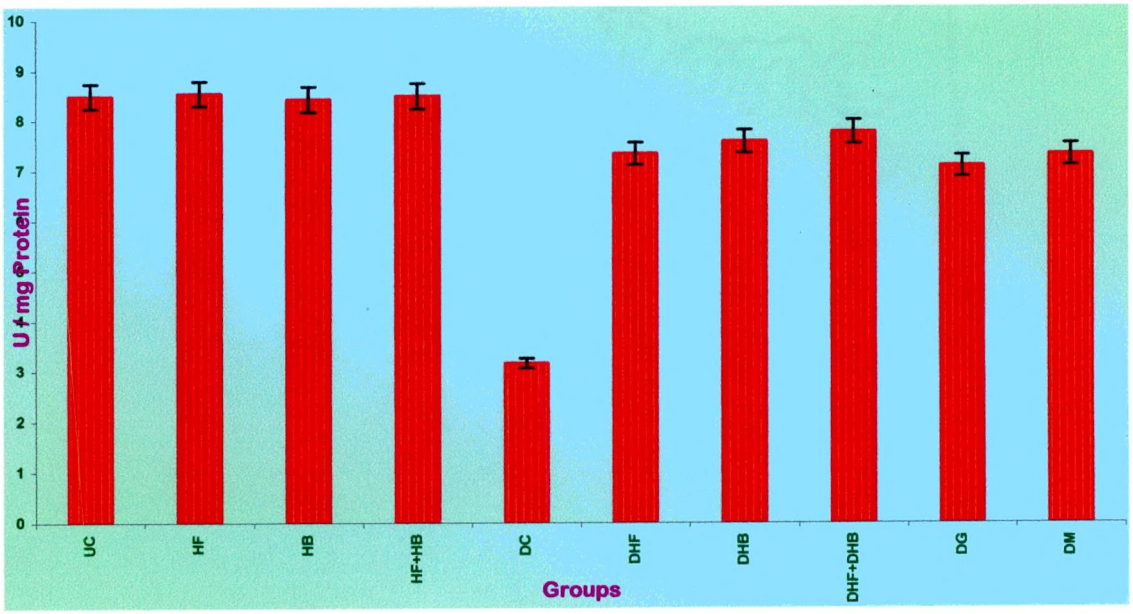


FIGURE 11

SUPEROXIDE DISMUTASE ACTIVITY IN THE LIVER OF THE RATS

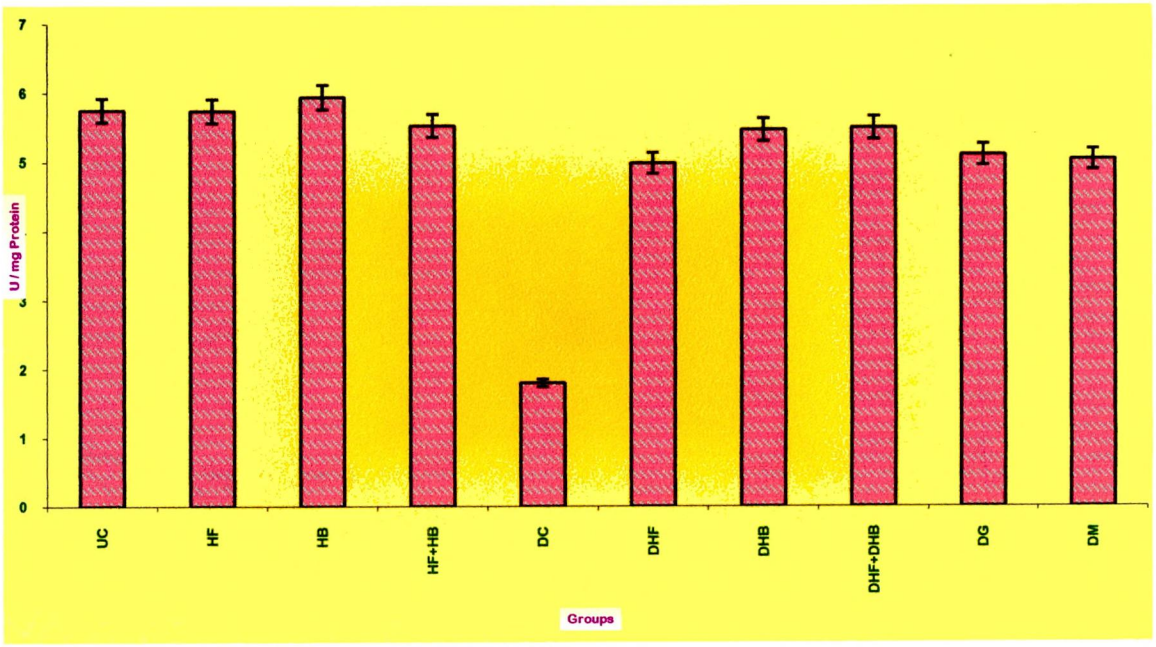
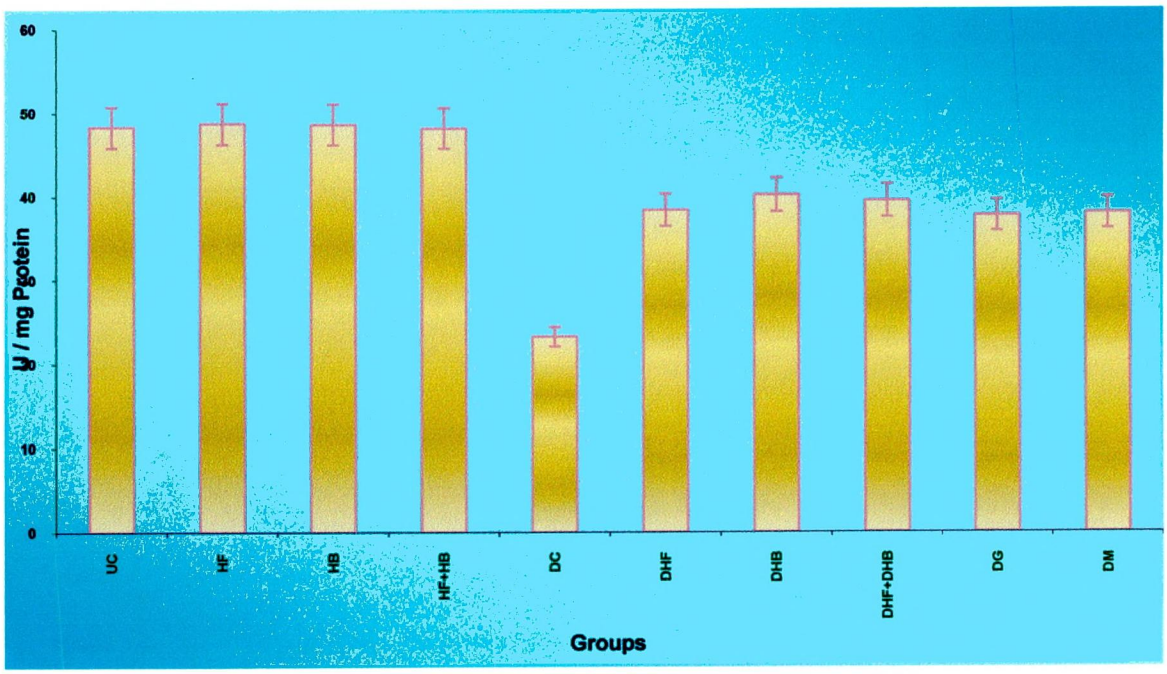


FIGURE 12

GLUTATHIONE -S-TRANSFERASE ACTIVITY IN THE LIVER OF THE RATS



In the rats treated with HF and HB, individually and in various combinations, the activities of the antioxidant liver enzymes were found to be not significantly altered. However, the activities of catalase, glutathione peroxidase, superoxide dismutase and glutathione S - transferase in the liver of diabetic rats were found to be significantly decreased when compared with those of the untreated control rats.

Scavenging enzymes namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) metabolize the free radicals formed during normal conditions to harmless intermediates. A change in this normal process in diabetes would have led to the overproduction of superoxide ion and hydrogen peroxide.

Somani and Singhai (2008) assessed the activities of SOD and CAT in the serum of the diabetic and the *Myristica fragrans* treated diabetic rats and found that these enzymes were significantly low in the diabetic control rats.

CAT, GPx and SOD activities in the erythrocytes were found to be low in the alloxan-treated diabetic rats when compared with the untreated control rats. When with 75 per cent methanolic extracts of *A. marmelos* was administered to diabetic rats, it significantly ($P< 0.001$) increased the activities of SOD, CAT and GPx (Sabu and Kuttan, 2004). HF and HB showed a protective effect against free radical damage. Administration of HF and HB tend to bring back the activities of antioxidant enzymes to normal levels. Hence the levels of enzymic antioxidants found in this study are in accordance with those studies mentioned above proving that *Helicteres isora* has an excellent capacity to scavenge free radicals.

Changes in the levels of non-enzymic antioxidants in diabetic experimental animals

Figures 13-16 show the levels of vitamin C, vitamin E, vitamin A and reduced glutathione in the liver of the control and the diabetic experimental rats.

FIGURE 13

LEVELS OF VITAMIN C IN THE LIVER OF THE RATS

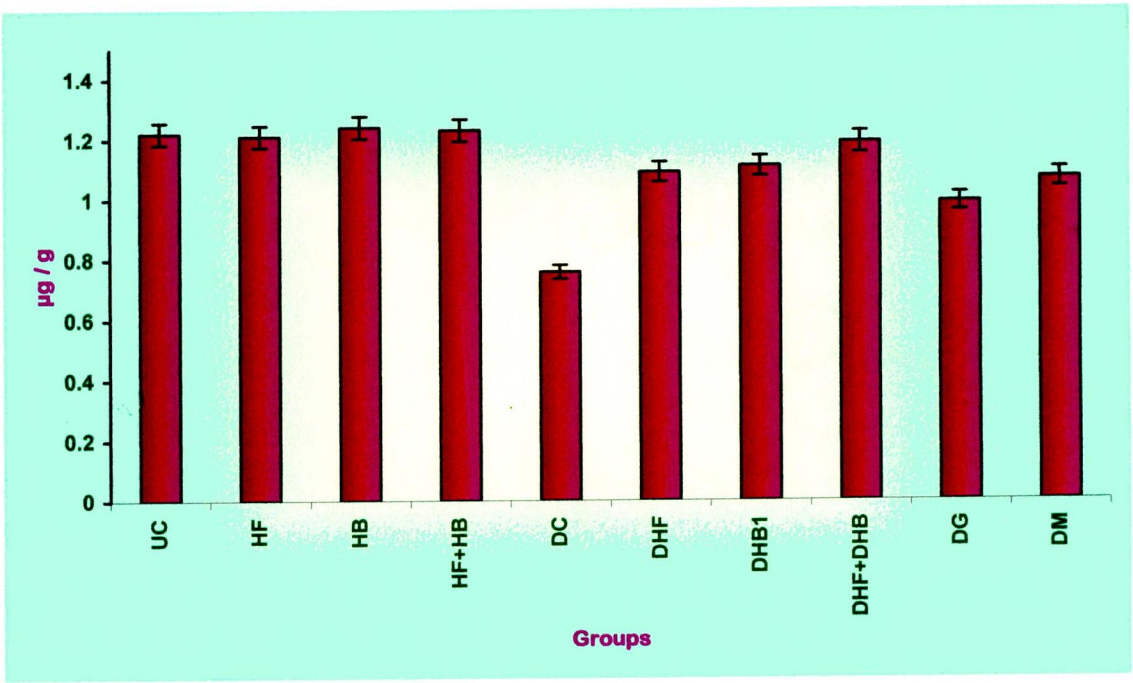


FIGURE 14

LEVELS OF VITAMIN E IN THE LIVER OF THE RATS

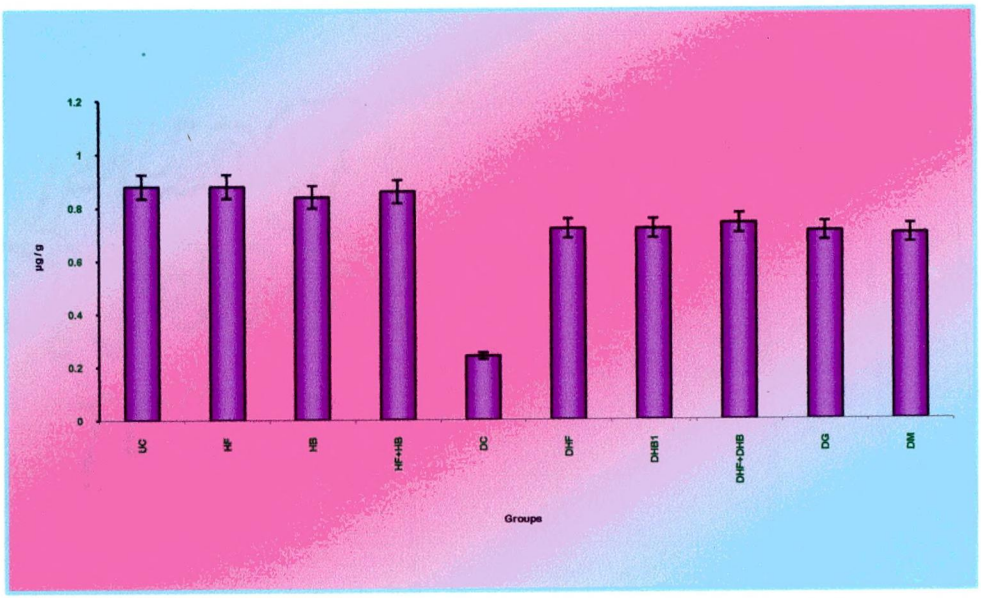


FIGURE 15

LEVELS OF VITAMIN A IN THE LIVER OF THE RATS

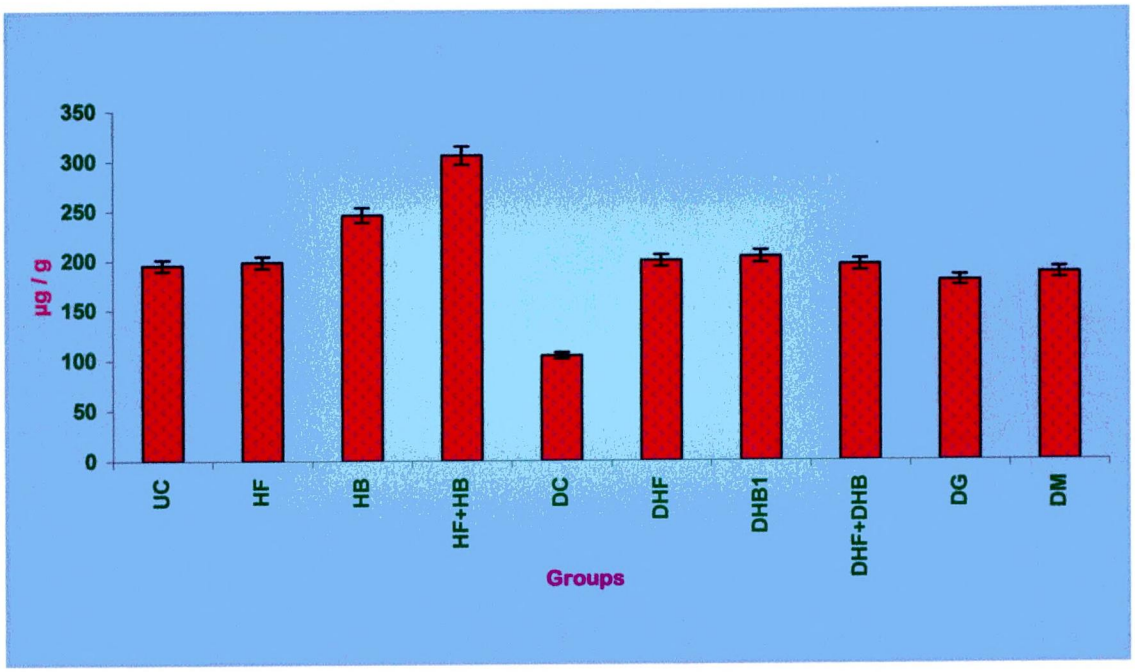
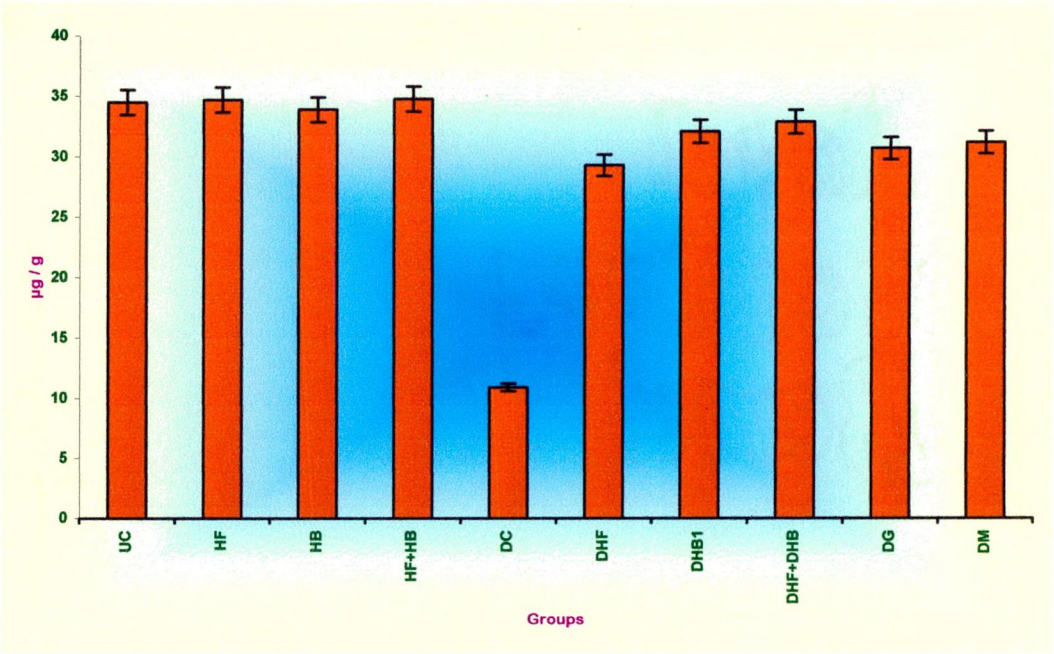


FIGURE 16

LEVELS OF REDUCED GLUTATHIONE IN THE LIVER OF THE RATS



There was no significant change recorded in the levels of vitamin C, vitamin E, vitamin A and reduced glutathione in the control rats treated with both HF and HB individually and in combinations. Administration of both HF and HB individually and ins combinations to streptozotocin-induced diabetic rats prevented a significant decrease in the levels of ascorbic acid, α-tocopherol and vitamin A and reduced glutathione in the liver of the rats. Vitamin C has several important biological functions. Inhibition of insulin glycation in pancreatic β- cells by vitamin C treatment is largely responsible for the improvement of insulin sensitivity and glycemic control.

Vitamins C and E are centrally involved against reactive oxygen species attack (Wahab *et al.*, 2002).

Studies have shown that the rats not supplemented with vitamin E, demonstrated exacerbated free radical production and excessive lipid peroxidation level and decrease in reduced glutathione levels and vitamin E supplementation is found essential for probable tissue antioxidant storage. Decreased glutathione levels in diabetics have been considered to be an index of increased oxidative stress. Diabetic rats treated with vitamin E exhibited increased level of glutathione and this might be one of the factors responsible for the inhibition of lipid peroxidation (Madhu *et al.*, 2005; Olanlokun, 2008). Similarly, administration of the extracts of *Helicteres isora* improved the levels of vitamins C, A and E and glutathione levels in the liver of the diabetic rats. This goes to prove that *Helicteres isora* has remarkable antioxidant potential.

4.2.7 EFFECT OF *Helicteres isora* FRUIT AND BARK ON SERUM PROTEIN

The effect of HF and HB on serum protein levels is presented in Table 15.

TABLE 15

TOTAL PROTEIN, ALBUMIN, GLOBULIN (g/dl) AND ALBUMIN/GLOBULIN RATIO

Groups	Treatment		Total protein	Albumin	Globulin	A/G ratio
1	Untreated Control	UC	7.62±2.161	3.30±0.13	4.32±0.04	0.76
2	Rats + HF (200 mg)	HF	7.65±0.06	3.31±0.03	4.34±0.03	0.76
3	Rats + HB(200 mg)	HB	7.61±0.02	3.34±0.03	4.27±0.06	0.78
4	Rats HF (100 mg) + HB (100 mg)	HF+HB	7.69±0.03	3.32±0.02	4.37±0.03	0.76
5	Diabetic	DC	5.39±1.844	2.12±0.88	3.27±0.96	0.64
6	Diabetic + HF (200 mg)	DHF	7.17±0.08	2.62±0.65	4.56± 0.04	0.57
7	Diabetic + HB (200 mg)	DHB	7.20±0.04	3.28±0.03	3.92±0.05	0.84
8	Diabetic HF (100 mg) + HB (100 mg)	DHF+DHB	7.45±0.04	3.17±0.02	4.28±0.05	0.74
9	Diabetic + Glibenclamide	DG	6.03±0.72	2.83±1.08	3.20±0.03	0.88
10	Diabetic + Metformin	DM	6.01±1.1	2.67±0.36	3.34±1.01	0.80
	CD (0.05)		0.86	0.24	0.27	

Values are mean ± SD of ten rats

The levels of protein, albumin and globulin and A/G ratio were found to be reduced in the diabetic rats when compared to the control rats. The levels of the protein were found to have returned to normalcy in diabetic rats treated with *Helicteres isora.*The present study investigation was also in accordance with that of Kumar *et al.* (2007b). They have also reported that the aqueous bark extract of *H. isora* could influence the protein metabolism and marker enzymes in streptozotocin-induced diabetic rats. The bark extract of *H. isora* was also found to ameliorate the impaired renal function and inhibits the liver damage associated with diabetes in rats.

The results are also similar to that of Palanivel *et al.* (2001) who had reported that the level of protein and albumin/globulin ratio was lower in the STZ induced diabetic rats than in the untreated control rats. Increased protein catabolism in diabetes might have induced a direct adverse effect on the synthesis and the secretion of albumin.

Mori *et al.* (2003) have detected proteinuria in streptozotocin-induced diabetic rats, decreased serum level of protein than that in normal non-diabetic rats.

Thus the fruit and the bark extracts of *Helicteres isora* were found to be as effective as glibenclamide and metformin in restoring the protein level in the diabetics.

4.2.8 HISTOPATHOLOGICAL STUDY OF RAT LIVER, PANCREAS AND KIDNEY

The results of the histopathological study of the rat liver, pancreas and kidney are presented in Table 16 and plates 3-8

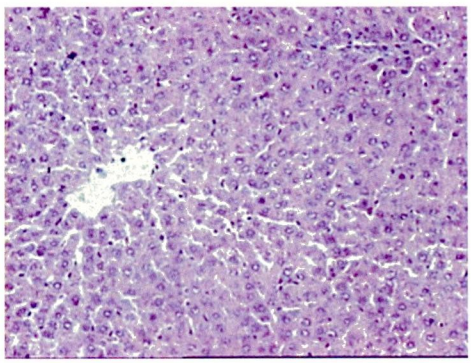
TABLE 16

HISTOPATHOLOGY OF RAT LIVER, PANCREAS AND KIDNEY

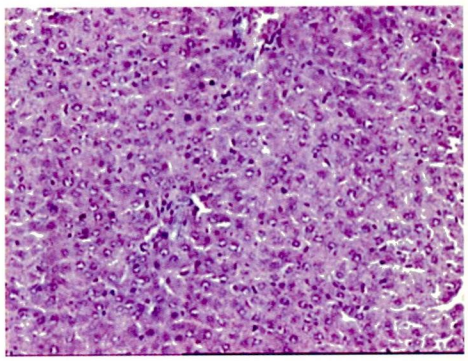
Group	Treatment		Liver	Pancreas	Kidney
1	Untreated Control	UC	normal histology	normal histology	normal histology
2	Rats+HF (200 mg)	HF	normal histology	normal histology	normal histology
3	Rats+HB (200 mg)	HB	normal histology	normal histology	normal histology
4	Rats + HF (100 mg) + HB (100 mg)	HF+HB	normal histology	normal histology	normal histology
5	Diabetic	DC	normal histology	insulitis	mild, focal interstitial nephritis.
6	Diabetic+HF (200 mg)	DHF	normal histology	normal histology	normal histology
7	Diabetic+HB (200 mg)	DHB	normal histology	normal histology	normal histology
8	Diabetic + HF (100 mg) + HB (100 mg)	DHF+DHB	normal histology	normal histology	normal histology
9	Diabetic+Glibenclamide	DG	normal histology	normal histology	normal histology
10	Diabetic+Metformin	DM	normal histology	normal histology	normal histology

Values are mean of ten rats

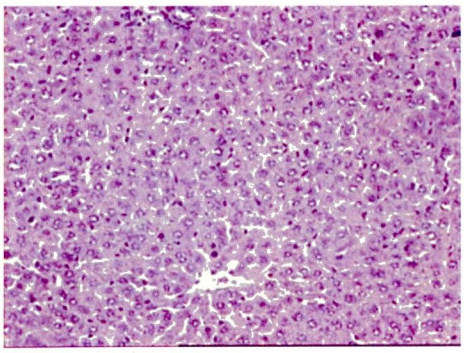
PLATE 3
LIVER SECTIONS OF THE RATS



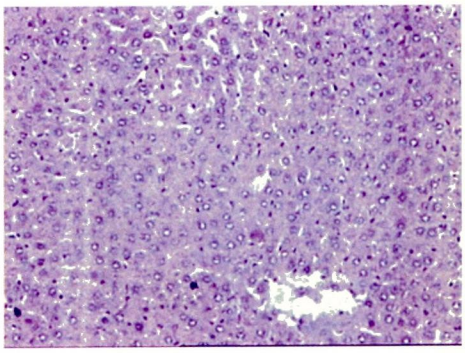
G 1



G 2



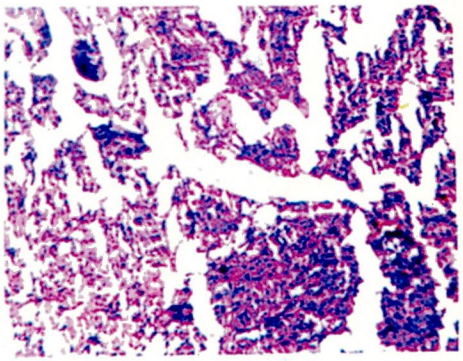
G 3



G 4

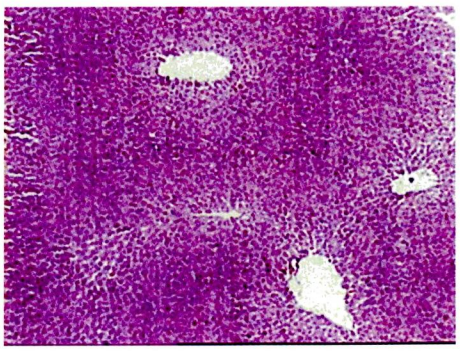
G 1 -4 Normal liver showing normal radiating hepatocytes

G 5 Diabetic control -Parenchyma with oedema, areas of congestion, mildly dilated sinusoids, congested portal triads and infiltration by chronic inflammatory cells.

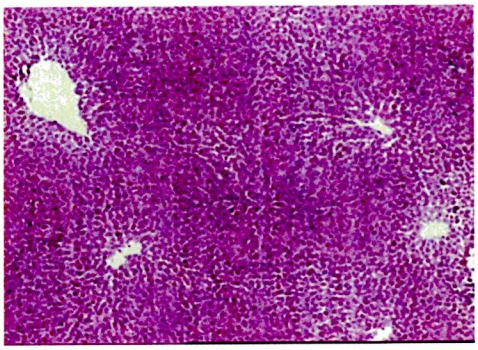


G 5

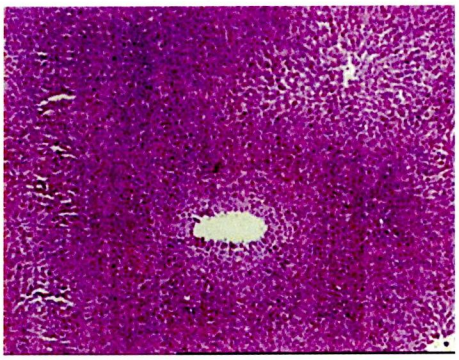
PLATE 4
LIVER SECTIONS OF THE RATS



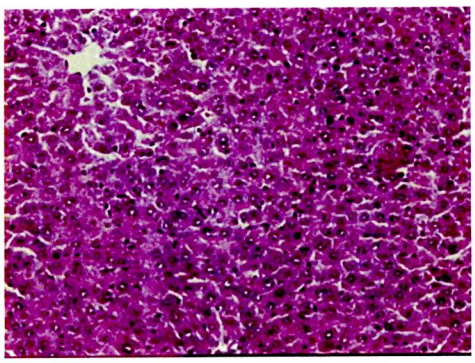
G 6



G 7

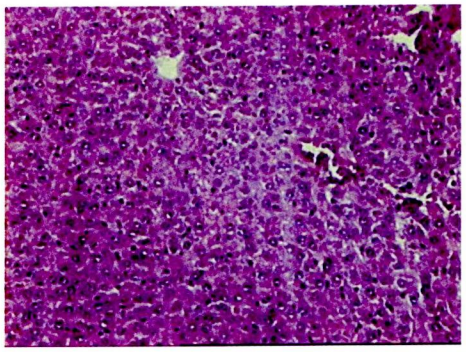


G 8



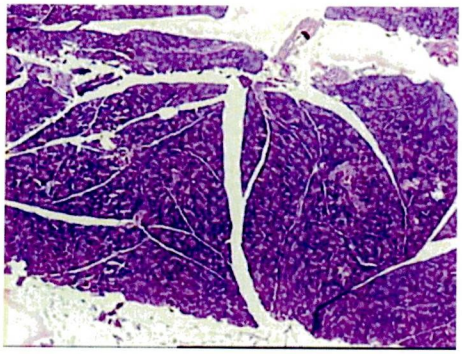
G 9

G 6-10 Liver showing normal radiating hepatocytes

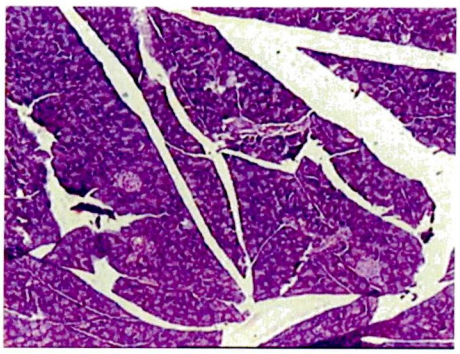


G 10

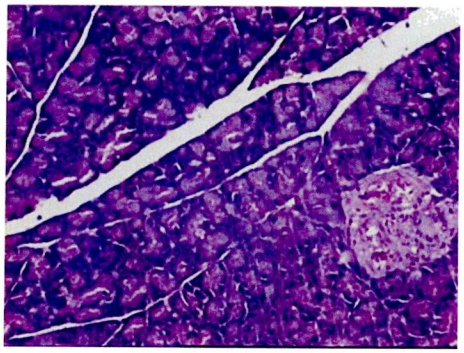
PLATE - 5
PANCREAS SECTIONS OF THE RATS



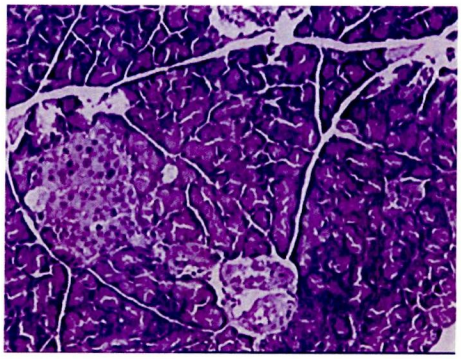
G 1



G 2



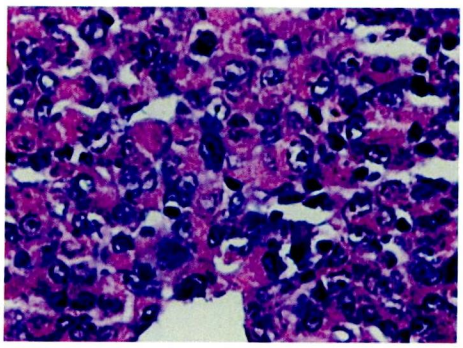
G 3



G 4

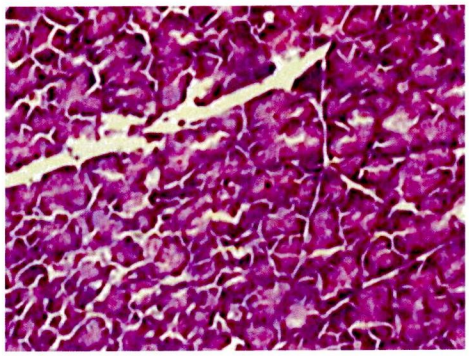
G 1-4 Normal parenchyma islets recognizable.No oedema. Histologically normal.

G 5 - Diabetic control shows parenchyma moderately congested, islets visualized, infiltrated mildly with chronic inflammatory cells. Chronic venous congestion of pancreas. Insulitis.

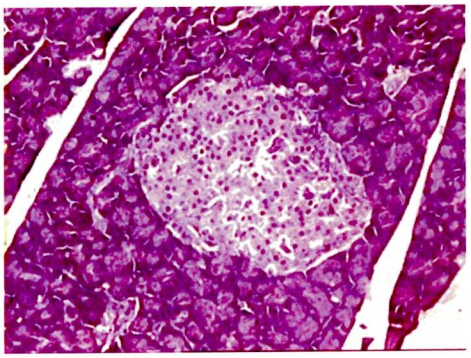


G 5

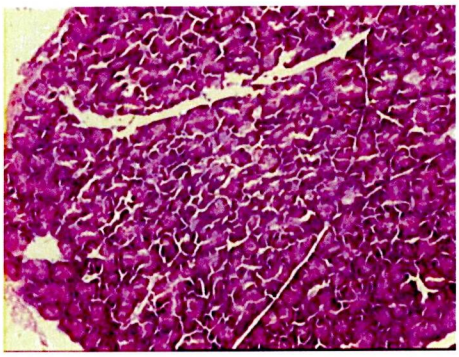
PLATE - 6
PANCREAS SECTIONS OF THE RATS



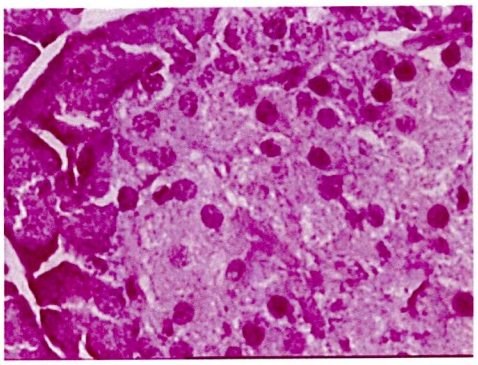
G 6



G 7



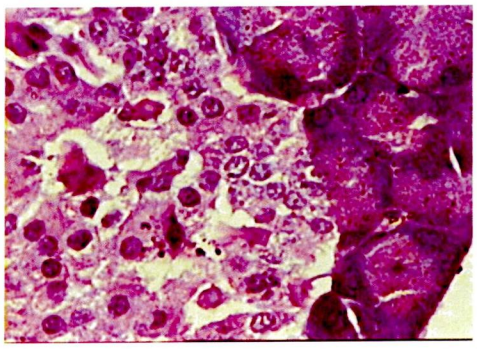
G 8



G 9

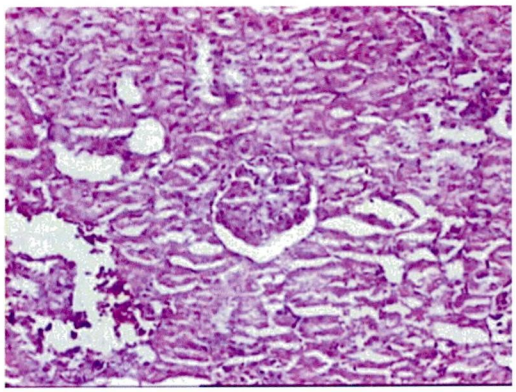
G 6-9 Normal pancreas architecture

G 10 - Mild insulitis

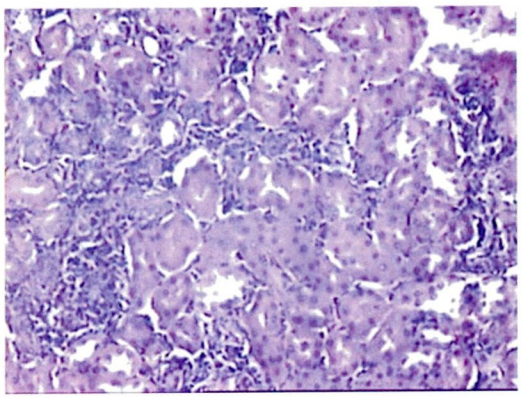


G 10

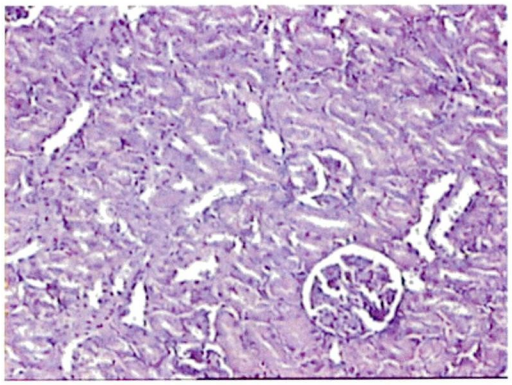
PLATE 7
KIDNEY SECTIONS OF THE RATS



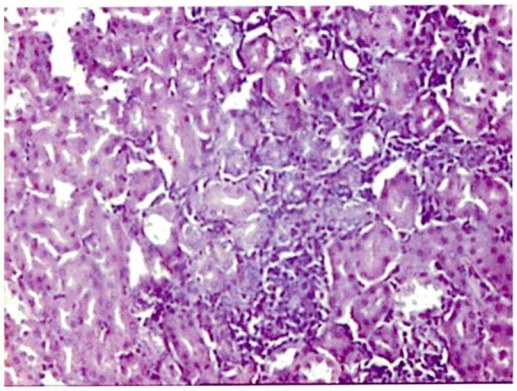
G 1



G 2



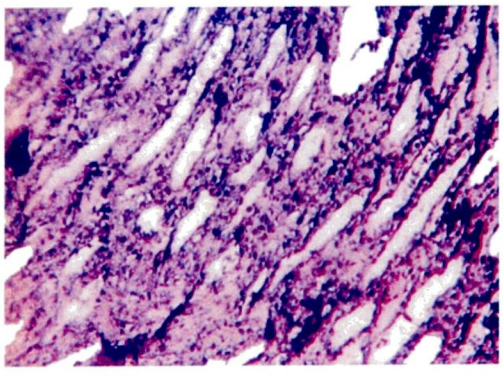
G 3



G 4

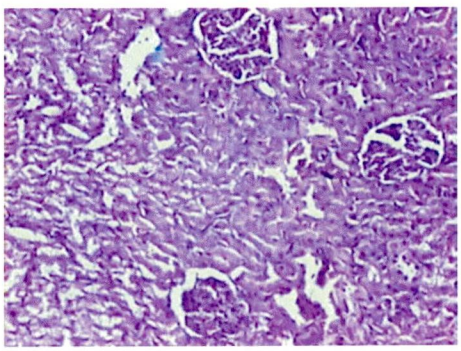
G 1-4 Normal -Glomeruli and Bowman's Capsule normal. Shows normal renal tissue.

G5 Diabetic control -shows congested glomeruli and bowman's capsule, dilated tubules and parenchyma congested - chronic venous congestion of kidney

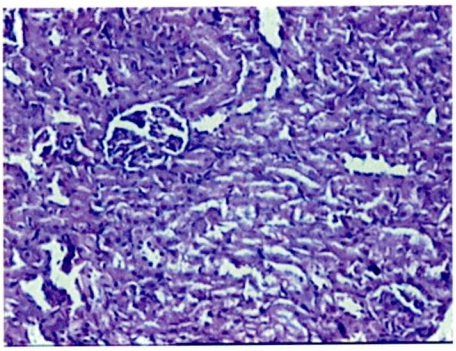


G 5

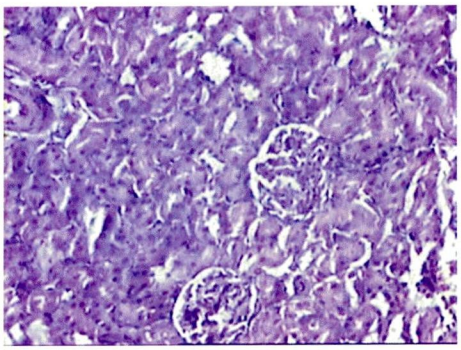
PLATE 8
KIDNEY SECTIONS OF THE RATS



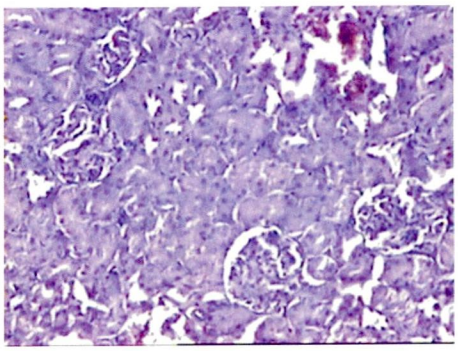
G 6



G 7



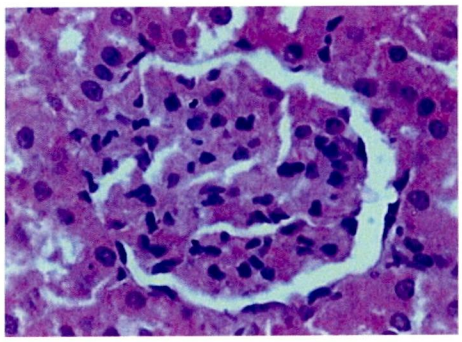
G 8



G 9

G6-G9 Normal Kidney architecture

G 10 - shows mildly congested parenchyma, glomeruli and Bowman's capsule. No inflammatory cells



G 10

Histopathology results of group 1 to 4 indicate that the fruit and the bark extracts of *Helicteres isora* given singly or in combination did not produce any detectable pathological changes in liver, pancreas and kidney, while the pancreas of streptozotocin-induced rats showed insulitis and the kidney has shown effects of hyperglycemia in the form of focal glomerular basement membrane thickening and hyaline arteriosclerosis which might be due to the deposition of increased amounts of circulating glycosylated end products. The administration of extracts of *Helicteres isora* (group 6 to 10) would have reverted the histological changes that had occurred due to diabetes. A study by Kumar *et al.,* (2006c) had shown that the diabetic group of rats treated with *Terminalia chebula* extract, an increase in the number of islets shows that they were regenerated.

The histopathological studies of Ravi *et al.* (2004) also revealed the protective effect of *Eugenia jambolana* seed kernel on pancreatic β-cells.

PHASE III

4.3 IDENTIFICATION OF THE PHYTOCHEMICALS IN THE FRUIT AND THE BARK OF *Helicteres isora*

4.3.1 QUALITATIVE DETECTION OF THE PHYTOCHEMICALS

The fruit and the bark of *Helicteres isora* were screened qualitatively for the presence of phytochemicals such as alkaloids, flavonoids, phenols, terpenoids, tannins and saponins.

The results of the qualitative detection of phytochemicals in the extracts of *Helicteres isora* are given in Table 17.

TABLE 17

PHYTOCHEMICALS IN THE EXTRACTS OF

Helicteres isora

Phytochemicals	Fruit	Bark
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Terpenoids	+	+
Tannins	+	+
Saponins	-	-

+ indicates presence - indicates absence

The fruit and the bark of *Helicteres isora* contain all the phytochemicals tested except saponin. The results of the present study are similar to that of Akilandeshwari *et al.* (2001) that the leaf extract of *Justicia tranquebariensis* L.F was found to contain flavonoids, glycosides and phytosterols in the alcohol, acetone and benzene extract.

4.3.2 SCREENING OF THE PHYTOCHEMICALS BY THIN LAYER CHROMATOGRAPHY

The petroleum ether, benzene, chloroform, acetone, ethanol and methanol extracts of the fruit and the bark were prepared and subjected to thin layer chromatography in different solvent systems. The results of the phytochemicals identified by thin layer chromatography are shown in Table 18.

TABLE 18

IDENTIFICATION OF THE PHYTOCHEMICALS BY THIN LAYER CHROMATOGRAPHY

Extracts	Phytochemicals	Fruit	Bark
Petroleum ether	-	-	-
Benzene	Terpenes	+	+
Chloroform	-	-	-
Acetone	Terpenes	+	+
	Flavonoids	+	+
Ethanol	Terpenes	+	+
	Flavonoids	+	+
	Phenols	+	+
Methanol	Terpenes	+	+
	Flavonoids	+	+
	Phenols	+	+
	Alkaloids	+	+

+ indicates presence – indicates absence

Table 18 reveals that the acetone extracts of *Helicteres isora* were found to contain terpenes and flavonoids and only terpenes were extracted by benzene. The petroleum ether and chloroform extracts do not show the presence of any of the phytochemicals whereas the terpenes, flavonoids and phenols could be isolated by ethanolic extract. The methanol extracts of *Helicteres isora* was found to contain terpenes, flavonoids, phenols and alkaloids.

Oleszek and Stochmal (2002), have reported that triterpene, saponin and flavonoids were isolated from the seeds of *Trifolium* species. An

alkaloid trigonelline, a methyl derivative of nicotinic acid is an active ingredient in *Trigonella foenum-graecum*, which increases the glucose uptake from blood and its subsequent oxidation (Jachak, 2002). The two triterpenoids and their glycosides from *Olea europaea* were found to exert hypoglycemic effect (Somova *et al.*, 2003).

Hence the presence of alkaloids, flavonoids and terpenes might be responsible for the antidiabetic and antioxidant activities of *Helicteres isora*.

4.3.3 ISOLATION AND QUANTIFICATION OF THE ACTIVE CONSTITUENTS OF *Helicteres isora* BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The HPLC analysis of the fruit and the bark extracts of *Helicteres isora* was carried out to identify the nature of the components present. The major components present in the fruit and the bark extracts were found to be rosmarinic acid and scutellarein as indicated in Table 19.

TABLE 19

RETENTION TIME AND CONCENTRATION OF ROSMARINIC ACID AND SCUTELLAREIN IN *Helicteres isora*

Sample	Rosmarinic acid		Scutellarein	
	Retention time	Concentration (µg / ml)	Retention time	Concentration (µg / ml)
Fruit	2.816	0.397	3.150	0.600
Bark	2.829	1.002	3.172	0.417
Standard	2.836	-	3.235	-

Of the several selected standards run in HPLC in the same experimental set up, the retention time (Rt) values of the fruit and the bark samples of *Helicteres isora* were closer to those of the two standards rosmarinic acid and scutellarein. Rosmarinic acid eluted at retention time of 2.8 and scutellarein at 3.23. The UV properties of the retention time were matching the UV spectra of authenticated standards. The peaks were well resoluted.

On the basis of spectroscopy and hydrolysis experiments the structures of five flavonoid glucuronides were obtained from the fruit of *Helicteres isora*, three of which were previously unknown compounds, isoscutellarein 4'-methyl ether 8-O-beta-D-glucuronide 6"-n-butyl ester, isoscutellarein 4'-methyl ether 8-O-beta-D-glucuronide 2", 4"-disulfate and isoscutellarein 8-O-beta-D-glucuronide 2",4"-disulfate (Kamiya *et al.*, 2001).

Satake *et al.* (1999) have isolated three new compounds; 49-O-b -D-glucopyranosyl rosmarinic acid, 4,4' 9- O -di- b - D- glucopyranosyl rosmarinic acid and 2 R – O - (49 – O - b - D- glucopyranosyl caffeoyl) -

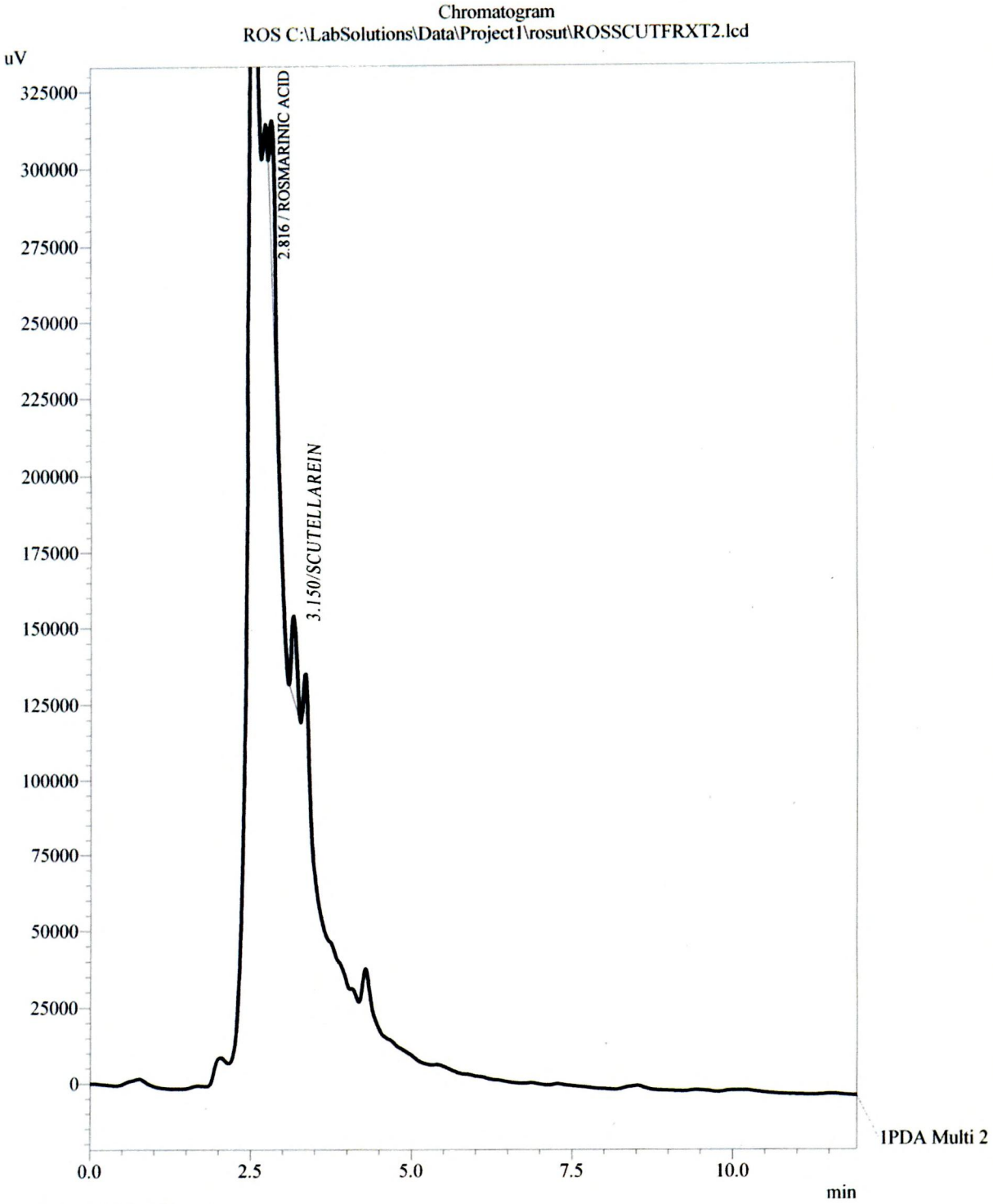
3 - (4-hydroxyphenyl) lactic acid named as 49-O-b –D glucopyranosyl isorinic acid together with rosmarinic acid from the fruit of *Helicteres isora* by spectroscopic and chemical analysis. They were found to have greater scavenging activity against superoxide anion produced with xanthine and xanthine oxidase (XOD).

Three neolignans, helisterculins A and B and helisorin were also elucidated from the aqueous extracts of the fruit of *Helicteres isora* (Tezuka *et al.*, 1999).

Plates 9, 10 and 11 give the chromatogram of the fruit and the bark extracts of *Helicteres isora* and the standard.

PLATE 9

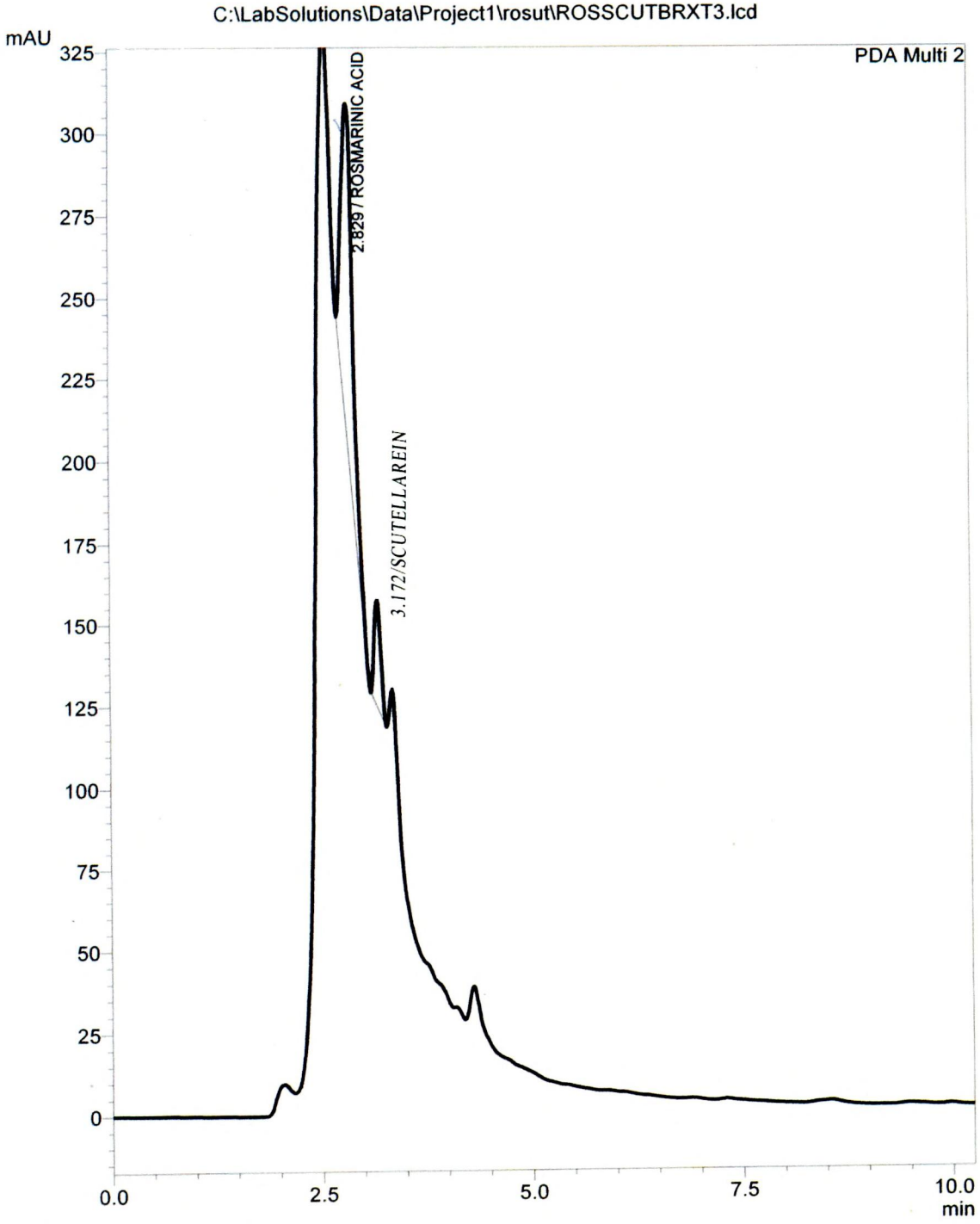
CHROMATOGRAM OF THE FRUIT EXTRACT



1 PDA Multi 2 / 325nm 4nm

PLATE 10

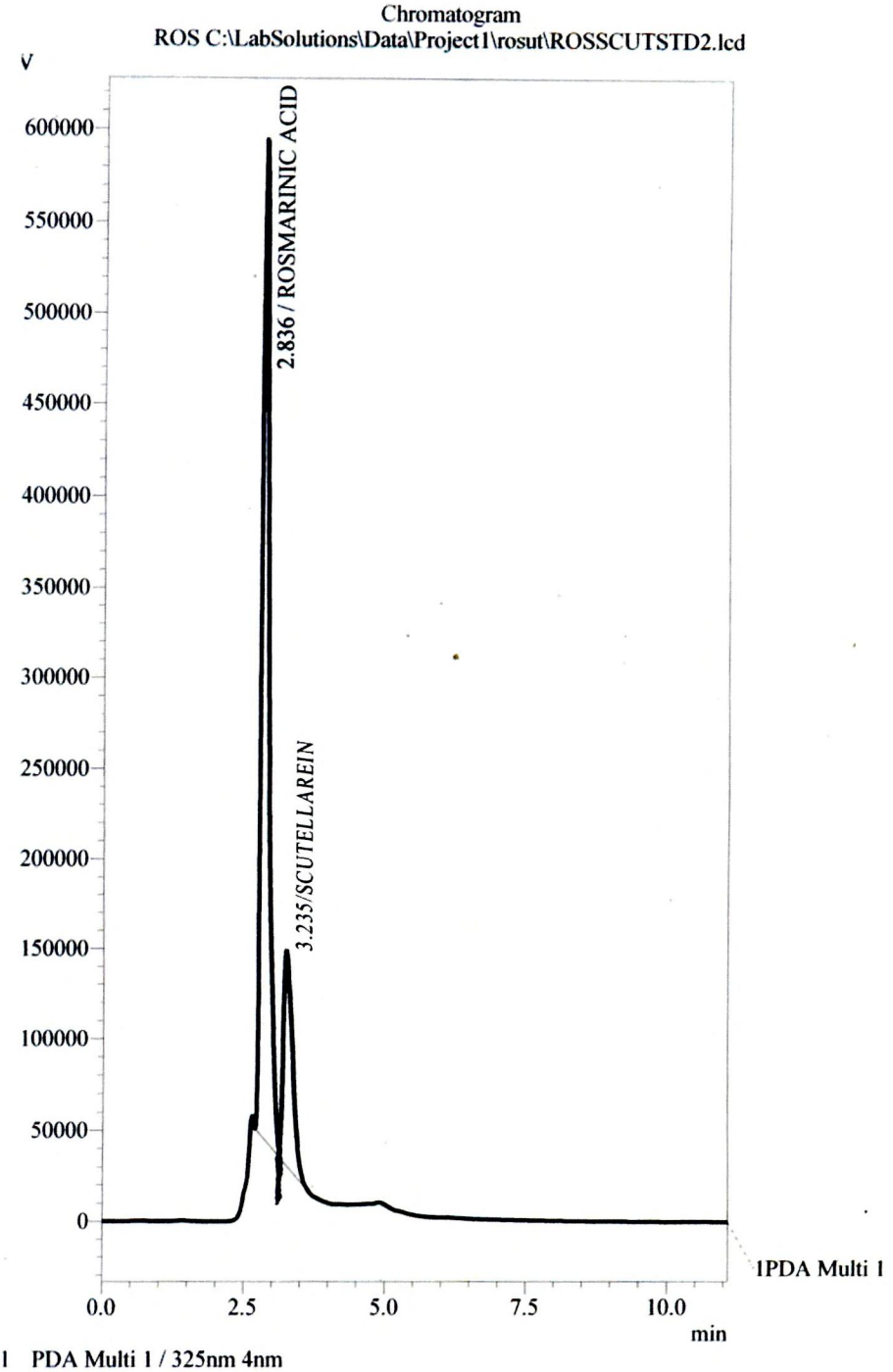
CHROMATOGRAM OF THE BARK EXTRACT



1 PDA Multi 2/325nm 4nm

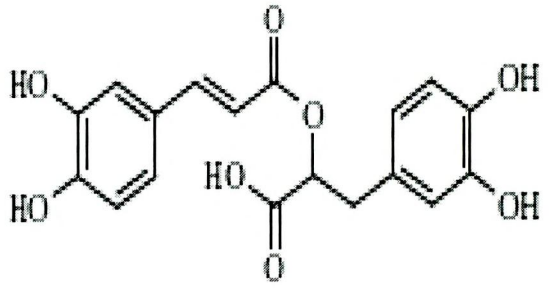
PLATE 11

CHROMATOGRAM OF THE ROSMARINIC ACID AND SCUTELLAREIN



1 PDA Multi 1 / 325nm 4nm

Rosmarinic acid



Molecular weight: 360.31

Formula: $C_{18}H_{16}O_8$

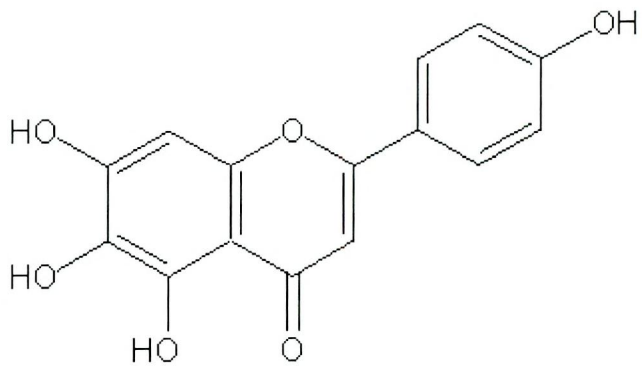
It is a tannin-like compound, sometimes described as a depside of caffeic acid. Originally identified in rosemary (*Rosmarinus officinalis* L.), the structure was elucidated as an ester of caffeic acid and 3- (3, 4 dihydroxyphenyl) lactic acid. The compound has been reported to occur in several taxonomically non-related families of the plant kingdom (Toth *et al.*, 2003).

Rosmarinic acid has a number of interesting biological activities, e.g. antiviral, antibacterial, anti-inflammatory and antioxidant. The presence of rosmarinic acid in medicinal plants, herbs and spices has beneficial and health promoting effects. In plants, rosmarinic acid is supposed to act as a preformed constitutively accumulated defence compound (Petersen and Simmonds, 2003).

Tepe *et al.* (2007) have studied the *in vitro* antioxidant activities and rosmarinic acid levels of the methanol extracts of *Salvia verticillata* subsp. *verticillata* and *S. verticillata* subsp. *amasiaca*. The extracts were screened for their possible antioxidant activity by two complementary test systems.

Their results have revealed that there is a strong correlation between the rosmarinic acid level and the antioxidant activity potential. Rosmarinic acid and its derivatives are more likely to be responsible for most of the observed antioxidant activities of *Salvia* species.

Scutellarein



Molecular weight : 286.241

Formula: $C_{15}H_{10}O_6$

Scutellarin is widely used in treating various cardiovascular diseases (Chen *et al.,* 2006). In a study conducted by Zhu *et al.* (2000) scutellarein was found to prevent vascular endothelial dysfunction in streptozotocin induced diabetic rats, and also potentiated the contraction induced by phenylepherine.

Rosmarinic acid and the scutellarein derivatives were found to have IC_{50} = 440 µg/ml and 1 mg/ml for DPPH inhibition activity respectively. These compounds were also have antioxidant and antiacetylcholinesterase activity (Fale *et al.*, 2008).

Thus it could be concluded that the two peaks of the fruit and the bark extracts of *Helicteres isora* samples referred to as rosmarinic acid and scutellarein and could be suggested to be the active principle that has potential antioxidant and antidiabetic activity.