CHAPTER – I

EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF HELIOTROPIUM INDICUM WHOLE PLANT IN STZ INDUCED DIABETIC RATS.

INTRODUCTION

Diabetes is a chronic disease that occurs when the pancreas fails to produce sufficient insulin, or when the body cannot effectively use the insulin it produces. Since insulin is the main hormone that regulates blood glucose levels, hyperglycemia is a common result of uncontrolled diabetes, which over time can lead to serious damage to many of the body's systems especially the nerves and blood vessels (WHO, 2006; Simeon et al., 1991). A chronic hyperglycemic condition in diabetes is associated with long term damage, dysfunction, and failure of various organs, such as eyes, kidneys, nerves, heart, and blood vessels (Jadhav and Puchchakayala, 2012). Effective blood glucose control is the key to preventing or reversing diabetic complications and improving quality of life in patients with diabetes (De Fronzo et al., 1999). Diabetes mellitus has been identified by the Indian Council of Medical Research as one of the refractory diseases for which satisfactory treatment is not available and suitable herbal preparations need to be investigated (Maniyar and Bhixavatimath, 2012).

Currently available therapies for the management of diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors, and thiazolidinediones, which are used as monotherapy or in combination to achieve better glycemic regulation. There are many synthetic antidiabetic agents currently available; however, these have a number of adverse side effects on the body (Jung et al., 2006; Mondal et al., 2012). Oral antidiabetic agents have a number of serious adverse effects, thus managing diabetes without any side effects is still a challenge (Saxena and Kishore, 2004). The treatment of diabetes with synthetic drugs is costly and chances of side effects are high.

In India, indigenous remedies have been used in the treatment of DM, since the time of Charaka and Sushruta (6th century BC) (Grover et al., 2001). India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethno botanical information reports
about 800 plants that may possess anti-diabetic potential (Alarcon-Aguilara et al., 1998). Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques (Mukherjee et al., 1972; Jafri et al., 2000). There have been several reviews on the hypoglycemic medicinal plants (Ivorra et al., 1989; Atta-Ur-Rahman and Zaman, 1989), more particularly use of Indian botanicals for hypoglycemic activity (Grover et al., 2002; Saxena and Vikram, 2004; Pulok and Mukherjee, 2006).

In view of reducing the cost of treatment for diabetes mellitus, there is considerable research for herbal production as anti-diabetics and around 1200 plants have been reported to possess anti-diabetic property (Marles and Fransworth, 1995). Many Indian medicinal plants have been found to be successfully used to manage diabetes and some of them have been tested and the active principles were isolated. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. Further, after the recommendations made by WHO on diabetes mellitus, (World Health Organization, 1980) investigation on hypoglycemic agents from medicinal plants have become more important. Traditional medicine plays a crucial role in healthcare and serves the health needs of a vast majority of people in developing countries. 80% of the world population relies on traditional medicine, encouraging the use of indigenous forms of medicine rather than expensive imported drugs.

Before the introduction of insulin and other pharmaceutical preparations traditional medicine mainly derived from plants were used to treat diabetes mellitus. Uses of herbs has been practiced for centuries in all parts of the world in different systems of medicine like Ayurveda, Siddha, Unani, naturopathy and others (Kameswara Rao and Appa Rao, 2001). Natural products and their derivatives have been a successful source of bioactive molecules in medicines much before the advancement of other modern therapeutics in the post-genomic era (Jadhav and Puchchakayala., 2012). Due to an increase in demand by patients to use natural products with antidiabetic activity, investigations on hypoglycemic agents derived from medicinal plants have gained popularity in recent years. Laboratories are conducting research on these medicinal plants in a scientific manner for the development of alternative drugs and strategies for better management of diabetes.
In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named Rasayana/Unani are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes (Sangeeta et al., 2011). The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world (Maurya and Srivastava., 2011). Therefore, with the rising number of diseases lately, many researchers have evaluated the medicinal plants as alternative therapeutic agents. The effectiveness and safety of drugs derived from the medicinal plants require scientific evaluation to establish the profiles of therapeutic effectiveness and toxicity of plant products. One example of such products is antihyperglycemic agents for use in the treatment of diabetes mellitus.

Okvirk et al., (2013) reported a list of 37 medicinal plants used in the traditional medicine for the treatment of diabetes in Bangladesh. Among 37 medicinal plants listed, Heliotropium indicum is one of the plants used in the traditional medicine for the treatment of diabetes (Okvirk et al., 2013). Ethno-botanical information on 73 species of medicinal plants belonging to 46 families used to treat diabetes was reported by Devi et al., (2011). Among 73 species of medicinal plants listed, Heliotropium indicum is one of the medicinal plants used as a traditional medicine for the treatment of diabetes in Manipur, India (Devi et al., 2011).

Tirumala hills, which lie geographically in the South- Eastern Ghats, are known for the rich heritage of the flora. A number of plants with known and unknown medicinal values are available here (Thammanna et al., 1990; 1994). The area is inhabited by a number of tribes which include the Chenchus, Nakkalas, Sugalis, Yanadis and Yerukalas. In an extensive ethno botanical survey of the medicinal plants of Rayalaseema region (Tirumala hills), 35 species were found to be used for antidiabetic treatment by local people and tribals, which have to be explored scientifically for their use in the effective treatment of diabetes (Madhavachetty et al., 2008). Heliotropium indicum (Fig. 1) is one of those plants which belong to the family Boraginaceae.
**Heliotropium indicum** Linn.

**Botanical name:** Indian Heliotrope.

**Kingdom:** Plantae

**Family:** Boraginaceae.

**Genus:** Heliotropium

**Species:** indicum

**Vernacular name/ Telugu Name:** Nagadanti, Nagadongi, Telukondi.

**Locality:** Tirumala Hills, Chittoor (District), Andhra Pradesh, India.

**Habit:** Herb

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Fig.1 *Heliotropium indicum* Linn.
Heliotropium indicum Linn is a wild herbaceous plant which belongs to the family Boraginaceae and is found in barren lands during summer however scattered plants may be seen in the late September. This plant is characterized by the presence of deep green leaves with borage or rough surface, white flowers arranged on the curved inflorescence axis, which appears like an elephant trunk, hence the common name is “Hathi Sur”. Heliotropium indicum Linn is a coarse foetid herb, up to 2 ft. high, with ascending hirsuit branches found throughout India in sunny localities, on waste lands and anthropogenic habitats in periodically desiccating pools and ditches and anthropogenic habitats, generally below 800 m altitude, widely considered as a weed of fields. The leaves are simple, alternate or sub-opposite, 4.5 to 10 cm/2.5 to 5 cm, ovate or ovate oblong, margin undulate, sparsely strigose along nerves on either side, serulate or undulate with cordate, minutely pilose beneath nerves and veins conspicuous on the lower side.

Heliotropium indicum may flower throughout the year; the flowering season is very long and new flowers develop apically within the cyme while mature nutlets are already present at the base of the inflorescence. The flowers are white or violet coloured, regular, sessile, two ranked pentameric, extra axillary. Sepals-5, 2.5 mm long, bristly with a few long hairs outside, free, green, linear lanceolate and unequal. Numerous branched, more or less densely hirsuit with spreading hairs are found in the stem and the root system is tap root and branched.

Distribution:

Heliotropium indicum Linn commonly known as ‘Indian heliotrope’ is very common in India and some parts of Africa and Bangladesh, but also found in other countries.

Flowering and fruiting season: Throughout the year.

Reported medicinal activities: Heliotropium indicum has been used in different traditional and folklore systems of medicine for curing various diseases. Several activities of this plant have been reported, which include;

- Antitumor (Kugelman et al., 1976),
- Antimicrobial (Rao et al., 2002);
- Anti-inflammatory (Srinivas et al., 2000),
- Wound healing (Reddy et al., 2002),
- Anti proliferative (Moongkarndi et al., 2004),
- Anti tuberculosis (Machinan et al., 2005),
- Gastro protective (Adelaja et al., 2008),
- Immuno stimulant (Ashoka et al., 2009),
- *In-vitro* antioxidant and antibacterial (Rajeswara Rao et al., 2012)
- Antihyperglycemic (Aqheel et al., 2013), and
- *In-vitro* antihelmintic activities (Kabita et al., 2014).

From the survey of literature and from the discussion with the older people in the tribal belts, it is observed that *Heliotropium indicum* has been used in different Traditional and folklore system of medicine to cure variety of diseases. The tribal of Jamaica use decoction of entire plants of *Heliotropium indicum* for the treatment of fever, venereal diseases, sore throat through oral uptake. This plant is highly valued in the folklore medicine and is believed to be used in treating Malaria, Abdominal Pain, fever, dermatitis, venereal diseases, insect bites, menstrual disorders, uriticaria and sore throat (Duttagupt and Dutta, 1977).

Similarly in Philippines and Senegal, the decoction of the plant is used orally as diuretic and for the treatment of kidney stones (Quicumbing, 1951 and Berhault, 1974). Some tribals use the decoction of the entire plant for treating herpes and the paste of fresh plant is used externally for the dressing of wounds. The decoction of stem is used orally by females for treating dysmenorrhea (Gurip et al., 2000). The decoction of leaves and young shoots are used to treat nettle-rash. The juice of the leaves is antiseptic and anti-inflammatory. Dodehe et al., (2011), reported that n-butanol fraction of *Heliotropium indicum* was very efficacious in the treatment of wounds. Similarly, the skin diseases are cured with the fresh paste prepared from the plant.

Decoction of leaves is used for washing of cuts and wounds as well as for the treatment of cholera. Juice of leaves is used for facial acne and wounds. Sap of leaves mixed with salt is used for clearing vision. It is also used for ear and skin infections. Decoction of leaves and flowers is used as gargle for sore throat and tonsillitis. The leaf juice is given to infants for cough and colds. The leaf paste is applied externally to cure rheumatism and skin infections (Barrett 1994). Different workers have reported that alcoholic or aqueous extract of *Heliotropium indicum* has wound healing and analgesic activity (Meher et al., 2011; Dash and Murthy 2011, Dodehe et al., 2011a). The plant contained several secondary metabolites such as volatile oil,
Indicine-N-oxide, esters and terpenes, so it has potent wound healing, anti-tumor and anti-leukemic activities (Yasukawa et al., 2002; Kupchan et al., 1976).

In addition to the above wound healing activity, the volatile oil extracted from the aerial parts revealed activity against *Mycobacterium tuberculosis*. The anti– tuberculosis activity has been reported by Theeraphan et al., 2006. The antifungal activity has been reported by Singh et al., 1994, from the aqueous extract of fresh leaves. The methanol extracts work against bacteria viz. *Klebsiella spp.*, *Pseudomonas aeruginosa*, *P.mirabilis*, *Bacillus subtilis* and *E.coli*. Oluwatoyin et al., 2011 reported that methanol extract of leaves of *Heliotropium indicum* was active against *Streptomonas aureus*, *S.pyogenes*, *S. pneumonia*, *K.pneumonia*, *E.coli* and *Shigella dysentriae* respectively. Andhiwal and Has, (1985) reported antifertility activity of petroleum ether extract of the plant of *Heliotropium indicum*. The antitumor activity of *Heliotropium indicum* has been reported by Kugelman et al., 1976, and Perdue, 1982. These workers obtained the active principle as N-oxide of the alkaloid, indicine. Ohnuma et al., (1982) observed the impact of indicine –N-oxide for the treatment of leukemia and tumours. Adelaja et al., 2006 reported that aqueous extract of *Heliotropium indicum* possesses gastro protective effects. On the basis of the fact that the aqueous extract of the dried leaves of the *Heliotropium indicum* after administration had important impact on indomethacine induced gastric ulcers. The alcoholic extract of *Heliotropium indicum* was found to be antimicrobial and 100 µg/ml conc. was significant with respect to inhibition of growth. This confirms the importance of the plant *Heliotropium indicum*, used by the tribals to cure infectious diseases (Rao et al., 2002). Ashok et al., (2009), reported immuno stimulant effect of aqueous extract of dried leaves of *Heliotropium indicum*. They noted an increase in *invitro* phagocytic index and lymphocyte viability. They also observed increased antibody production in rats and delayed type of hypersensitivity in mice. They concluded that the results were dose dependent and immuno stimulant effect was probably due to the alkaloid content or due to the synergistic effect of all the active ingredient of the plants. *Heliotropium indicum* root extract is used for the treatment of night blindness by the tribals (Ghani, 1998).

Recent researches that include phytochemical analysis of the dried roots and leaves, *invitro* test for antitumor, anti microbial, antifungal, antifertility, uterine stimulation, wound healing etc explains the activity of the plant parts used by the tribals. The various alkaloids,
steroids, triterpenes all confirm its use as a drug. The different effects may be correlated with the synergistic impact of active ingredients of the plants. It is used in Ayurvedic medicines for the treatment of different diseases. The use of the plant in Ayurveda and Siddha has been described in *Ayurvedic Pharmacopoeia of India* Part-I, Volume 6, Ministry of Health and Family Welfare Govt. of India, 2008. This neglected plant bears tremendous properties i.e., being exploited for the use of preparation of various Ayurvedic Medicines. Due to the above applications of the plant in Ayurvedic medicines, the plants are harvested brutally by the greedy traders and labours of the Vaidhya. It needs conservation and popularization for its importance among the local people who can protect the species in its natural population because in spite of having all these medicinal values, the plant is not cultivated and it grows in the wild habitat (Meenakshi et al., 2014).

In India and Bangladesh, *Heliotropium indicum* is used as traditional medicine to treat diabetes mellitus (Devi et al., 2011; Okvirk et al., 2013). The methanolic extract of root of *Heliotropium indicum* was reported to have significant antihyperglycemic activity in Streptozotocin and alloxan induced diabetic rats (Aqheel et al., 2013). But there are no further reports on the Phytochemicals responsible for the antihyperglycemic activity of *Heliotropium indicum*. Hence the present study was undertaken to evaluate the antihyperglycemic activity of the whole plant, *Heliotropium indicum* in STZ induced diabetic rats.

**MATERIALS AND METHODS**

**Collection of Plant Material**

The whole plant *Heliotropium indicum* (HI) was collected from Tirumala hills and identified by the Botanist, Department of Botany, S.V. University, Tirupati. A voucher specimen (Herbarium Accession No: 812) was deposited in the herbarium, Department of Botany, S.V. University, Tirupati. These *Heliotropium indicum* were shade dried and powdered.

**Preparation of aqueous suspension of *Heliotropium indicum***

To prepare aqueous suspension, 1kg of shade dried whole plant powder of *Heliotropium indicum* was soaked in 1 liter of water, in a glass jar for 48 hours at room temperature and the solvent was collected until it gave no colouration. The solvent was concentrated to dryness under reduced pressure in Buchi Rota vapour R-200 and finally freeze dried. The yield of the
suspension was 19% (w/w). From this 500 mg of crude suspension/kg bw was used for screening of the antihyperglycemic activity in STZ induced diabetic rats.

**Preparation of different solvent extracts**

The plant powder of *Heliotropium indicum* was extracted in to the solvents of increasing order of polarity. Hexane, ethyl acetate and methanol extracts were prepared by successive solvent extraction of *Heliotropium indicum* powder in soxhlet apparatus at 68°C-70°C. The filtrates obtained were distilled and concentrated under reduced pressure at low temperature (40°C to 45°C) in the Buchi rotavapor R-200 and finally freeze dried. The yields of the hexane, ethyl acetate and methanol extracts were 18%, 29% and 31% (w/w) respectively. All the extracts were stored at 0°C in airtight containers until needed for further studies.

**Preparation of aqueous extract**

To prepare aqueous extract the *Heliotropium indicum* plant powder (200 g) was soaked in distilled water in a glass jar for 2 days at room temperature and the solvent was filtered. This was repeated three to four times until the filtrate gave no colouration. The filtrate was distilled, concentrated under reduced pressure in the Buchi rotavapor R-200 and finally freeze dried. The yield of the extract was 24% (w/w). The extract was preserved in a refrigerator till further use.

**Preparation of alkaloid rich fraction of *Heliotropium indicum* (ARFHI)**

Alkaloid rich fraction of *Heliotropium indicum* (ARFHI) was prepared by a general acid-base extraction method reported earlier (Houghton and Raman., 1998).

**Experimental animals**

Male albino wistar rats aged 3–4 months with body weights approximately 180–200 g procured from Venkateswara Enterprises, Bangalore, were kept at 25 ± 5°C in a well ventilated animal house under 12 h light and dark cycle. The animals were fed with standard pellet diet (supplied by Venkateswara Enterprises, Bangalore) *ad libitum* and had free access to water. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee and was cleared by the same before beginning of the experiment (No. 27/2012-2013/ (i)/a/CPCSEA/IAEC/SVU/CAR-MSA).
**Induction of Diabetes**

Diabetes was induced in healthy male Wistar Albino rats aged 3-4 months, with body weights 180-200g, by single intraperitoneal injection of freshly prepared Streptozotocin (50 mg/kg bw) dissolved in ice cold 0.01M citrate buffer (pH 4.5) after overnight fasting for 12 hours (Sekar et al., 1990). Since STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release due to destruction of pancreatic β cells, 6 hours after STZ administration the rats were kept for next 24 hours on 15% glucose solution to prevent hypoglycemia. Diabetes was assessed by determining the fasting blood glucose after 72 hours of injection of STZ. The blood glucose levels in STZ rats were increased to markedly higher levels than normal. After 72 hours rats with marked hyperglycemia (fasting blood glucose $\geq 250$ mg/dL) were selected and used for the study.

**Experimental design for the evaluation of antihyperglycemic activity of crude aqueous suspension of *Heliotropium indicum* (HI) in STZ induced diabetic rats.**

The animals were divided into three groups and each group consisted of six rats.

**Group 1:** Normal control + Distilled water

**Group 2:** Diabetic control + Distilled water

**Group 3:** Diabetic rats + 500 mg crude aqueous suspension of *Heliotropium indicum*/kg bw.

After an overnight fast the group 1 and group 2 rats received only distilled water. Whereas group 3 rats received 500 mg crude aqueous suspension of *Heliotropium indicum*/kg bw by gastric intubation using a force feeding needle. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2, 3, 4, 5 and 6 hours after feeding the crude aqueous suspension and blood glucose levels were measured by using glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek Active, Roche Diagnostics, Germany).

**Experimental design for the evaluation of antihyperglycemic activity of different solvent extracts of *Heliotropium indicum* (HI) in STZ induced diabetic rats.**

The animals were divided into seven groups of six animals each as given below.

**Group 1:** Normal control + Distilled water
Group 2: Diabetic control + Distilled water

Group 3: Diabetic rats + 500 mg hexane extract of HI/kg bw

Group 4: Diabetic rats + 500 mg ethyl acetate extract of HI/kg bw

Group 5: Diabetic rats + 500 mg methanol extract of HI/kg bw

Group 6: Diabetic rats + 500 mg aqueous extract of HI/kg bw

Group 7: Diabetic rats + 20mg glibenclamide/kg bw.

After an overnight fast the group 1 and group 2 rats received only distilled water. Whereas group 3, group 4, group 5 and group 6 diabetic rats received hexane, ethyl acetate, methanol and aqueous extracts each at a dosage of 500 mg/kg bw respectively by gastric intubation using a force feeding needle. Group 7 rats received 20mg glibenclamide/kg bw by gastric intubation using a force feeding needle, as a reference drug. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2, 3, 4, 5 and 6 hours after feeding the extract/glibenclamide, and blood glucose levels were measured by using glucose oxidase-peroxidase reactive strips and glucometer (Accu-chek Active, Roche Diagnostics, Germany).

**Evaluation of antihyperglycemic activity of alkaloid rich fraction of *Heliotropium indicum* (ARFHI) in STZ induced diabetic rats.**

The animals were divided into seven groups of six animals each as given below.

Group 1: Normal control+ distilled water

Group 2: Diabetic control+ distilled water

Group 3: Diabetic rats+ ARFHI (250 mg/kg bw)

Group 4: Diabetic rats+ ARFHI (500 mg/kg bw),

Group 5: Diabetic rats+ ARFHI (750 mg/kg bw)

Group 6: Diabetic rats+ ARFHI (1000 mg/kg bw)

Group 7: Diabetic rats+ Glibenclamide (20mg/kg bw) a standard oral antidiabetic drug.
After an overnight fast, the ARFHI suspended in distilled water was fed by gastric intubation, using a force feeding needle. Group 1 and group 2 rats were fed distilled water alone. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2, 3, 4, 5 and 6 hours after feeding the fraction. The results were compared with those of the 7th group of rats which were treated with 20 mg glibenclamide/kg bw. Blood glucose levels were measured by using glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek Active, Roche Diagnostics, Germany).

**Effect of ARFHI on fasting blood glucose levels (mg/dL) of normal rats.**

The animals were divided into two groups of six animals each and received the following treatments.

Group 1: Normal control + distilled water

Group 2: Normal rats + ARFHI (750 mg/kg bw).

Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2, 3, 4, 5 and 6 hours after feeding the fraction, and blood glucose levels were measured by using glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek Active, Roche Diagnostics, Germany).

**Effect of ARFHI on oral glucose tolerance of diabetic rats**

Three groups of diabetic rats each group containing six rats were used for this study.

Group 1: Diabetic rats + distilled water

Group 2: Diabetic rats + 20 mg glibenclamide/kg bw

Group 3: Diabetic rats + 750 mg ARFHI/kg bw.

The oral glucose tolerance test (Bonner wier, 1988) was performed in overnight fasted diabetic rats. Glucose (2 g/Kg bw) was administered orally to all the three groups of rats using a force feeding needle at 0 minute. After 30 minutes of oral glucose administration, the group 2 and group 3 diabetic rats received glibenclamide (20 mg/kg bw) and ARFHI (750 mg/kg bw) respectively. Blood samples were collected from tail vein at 0, 30, 60, 90, 120, 150 and 180 min for estimation of blood glucose using glucose oxidase-peroxidase reactive strips and a
glucometer (Accu-chek Active, Roche Diagnostics, Germany). A comparison was made between the ARFHI and antidiabetic drug glibenclamide.

**Effect of ARFHI on oral glucose tolerance of normal rats**

Three groups of normal rats each group containing six rats were used for this study.

Group 1: Normal Control+ distilled water

Group 2: Normal rats+750 mg ARFHI/kg bw

Group 3: Normal rats +20mg glibenclamide/kg bw

After overnight fast group 2 and group 3 were fed with ARFHI and glibenclamide respectively and normal untreated rats (Group 1) were fed with distilled water. Thereafter, following 30 min of post fraction and drug administration all groups of animals were fed with glucose (2g/kg bw). Blood samples were collected from tail vein prior to dosing and then at 30, 60, 90, 120, 150 and 180 min after glucose administration for estimation of blood glucose using glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek Active, Roche Diagnostics, Germany). (Shirwaikar and Rajendran, 2006; Aslan et al., 2007).

**Evaluation of the acute toxicity of the ARFHI in normal rats**

Acute toxicity of ARFHI was evaluated in healthy wistar male albino rats, according to the guidelines set by Organization for Economic Cooperation and Development (OECD) (Bala et al., 2010). The healthy male rats were randomly divided into two groups of six rats each. The animals were fasted overnight, provided only water after which ARFHI was administered to the groups orally at a dose level of 2000, 3000 mg/kg bw respectively by gastric intubation. The animals were observed continuously for 24 hours for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality.

**Preliminary phytochemical analysis**

The different solvent extracts of *Heliotropium indicum* were screened for the presence of various phytochemical constituents using standard conventional protocols (Harborne, 1998).
**Statistical Analysis**

All values are expressed as Mean ± S.D. The data was statistically analyzed by Students ‘t’ test.

**RESULTS**

**Effect of crude aqueous suspension of *Heliotropium indicum* on fasting blood glucose levels (mg/dL) of diabetic rats (Mean ± S.D)**

The effect of crude aqueous suspension of *Heliotropium indicum* (500 mg/kg bw) on the fasting blood glucose levels of diabetic rats is shown in Table 1. Fasting blood glucose levels of diabetic rats (Group 2) were significantly higher than those of normal rats (Group 1). A significant decrease (46%) in fasting blood glucose levels were observed in diabetic treated group (Group 3), when compared to those of diabetic untreated group (Group 2).

**Effect of different solvent extracts of *Heliotropium indicum* on the fasting blood glucose levels (mg/dL) of diabetic rats (Mean ± S.D)**

The effect of different solvent extracts of *Heliotropium indicum* on the fasting blood glucose levels of diabetic rats is shown in Table 2. The diabetic rats treated with aqueous extract at a dosage of 500 mg/ kg bw showed significant (46.8%) reduction in blood glucose levels. No reduction in blood glucose levels was observed in diabetic rats treated with either hexane or ethyl acetate extracts at the same dosage. Whereas the methanol extract has produced 31.5% fall in the FBG level of the diabetic rats.

**Effect of different doses of ARFHI on fasting blood glucose levels (mg/dL) of normal and STZ induced diabetic rats.**

The effect of different doses of alkaloid rich fraction of *Heliotropium indicum* (ARFHI) on the fasting blood glucose levels of diabetic rats is given in Table 3. The fasting blood glucose levels of diabetic untreated rats (Group 2) were significantly higher than the fasting blood glucose levels of normal rats (Group 1). A significant decrease (60%) in fasting blood glucose levels was observed in diabetic rats treated with ARFHI at a dosage of 750 mg/kg bw when compared to the doses 250 and 500 mg/kg bw (fall in FBG 22% and 47% respectively). However, further increase (1000 mg/kg bw) in the dose of ARFHI did not increase the
The hypoglycemic effect of ARFHI was compared with that of glibenclamide (20mg/kg bw) a standard drug. The effect of ARFHI was more prominent (60%) when compared to that of glibenclamide (39.2%). The treatment with ARFHI at a dosage of 750 mg/kg bw in normal rats did not show any hypoglycemic activity (Table 4).

**Effect of ARFHI on oral glucose tolerance in STZ induced diabetic rats**

After 30 minutes of oral glucose administration (2g/kg bw), the intake of 750 mg ARFHI/kg bw or 20 mg glibenclamide/ kg bw has significantly improved the glucose tolerance in the diabetic rats. In the diabetic untreated rats the glucose levels remained higher without much change even at 180 min after glucose load. Oral administration of ARFHI (750 mg/kg bw) and glibenclamide (20 mg/kg bw) for group 3 and group 2 diabetic rats respectively, resulted in a significant fall in blood glucose levels from 30 minutes (after administration of ARFHI or glibenclamide) onwards and continued up to 180 minutes. The effect of ARFHI was more significant when compared to that of glibenclamide. The results are depicted in Fig. 1.

**Effect of ARFHI on oral glucose tolerance in normal rats**

The blood glucose levels of all groups of animals were measured from 0 min to 180 minutes after glucose load. In all the groups the blood glucose levels were raised after 30 min of glucose load but after that there was a significant decrease in the blood glucose levels of group 2 and group 3 when compared with those in group 1. But there was no hypoglycemia in any group of rats. The results are depicted in Fig. 2.

**Evaluation of the acute toxicity of ARFHI in normal rats**

The various observations showed the normal behavior of the treated rats. No toxic effects were observed even at the dose of 3000 mg ARFHI/kg bw. Hence there were no lethal effects in any of the groups, treated with the highest dose of ARFHI.

**Phytochemical analysis**

Phytochemical analysis revealed the presence of steroids, alkaloids, triterpenes, saponins and tannins in *Heliotropium indicum* whole plant. Phytochemical constituents of different solvent extracts of *Heliotropium indicum* are given in Table 5.
Table 1. Effect of crude aqueous suspension of *Heliotropium indicum* on fasting blood glucose levels (mg/dL) in STZ induced Diabetic rats (Mean ± S.D)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Fasting blood glucose level (mg/dL) at different hours after the treatment with crude HI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Normal control</td>
<td>89 ± 8.03</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>321 ± 26.4†</td>
</tr>
<tr>
<td>Diabetic rats + Crude HI</td>
<td>322 ± 12.5†</td>
</tr>
</tbody>
</table>

† P <0.0001 compared with the initial level of blood glucose (0h) of normal rats.

** P<0.0001 compared with the initial level of blood glucose (0h) in the respective group.

* P<0.001 compared with the initial level of blood glucose (0h) in the respective group.

Numbers in parenthesis indicate the percentage of fall in 0h blood glucose.
Table 2. Effect of different solvent extracts of *Heliotropium indicum* on fasting blood glucose levels of STZ induced diabetic rats. Values are given as mean ± S.D

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Fasting Blood Glucose (mg/dl) levels after treatment with different solvent extracts of HI</th>
<th>0h</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>6h</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>82.66±4</td>
<td>83.66±5</td>
<td>81.16±2</td>
<td>82±3</td>
<td>79.1±2</td>
<td>79.5±3</td>
<td>80.66±3</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>303.33±19†</td>
<td>305.16±20</td>
<td>307.66±19</td>
<td>309±19</td>
<td>310.66±18</td>
<td>314.16±18</td>
<td>316.66±16</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>311±19†</td>
<td>308.66±19</td>
<td>306.33±19</td>
<td>304.66±19</td>
<td>303.33±21</td>
<td>300.5±19</td>
<td>294.33±21</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>354.71±12†</td>
<td>349.71±13</td>
<td>348.28±13</td>
<td>345±13</td>
<td>342±14</td>
<td>339.85±14</td>
<td>332.28±14</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>385.66±19†</td>
<td>362.33±19</td>
<td>335.66±15*</td>
<td>325.16±15*</td>
<td>312.33±14**</td>
<td>295.5±14**</td>
<td>264.16±11** (31.5%)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>334±19†</td>
<td>316.83±17</td>
<td>306.5±15</td>
<td>263.5±11.6**</td>
<td>246.66±13**</td>
<td>227.66±11**</td>
<td>177.66±15** (46.8%)</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>328±24†</td>
<td>299±10</td>
<td>271.3±6*</td>
<td>249±5**</td>
<td>228.5±5**</td>
<td>202.8±5**</td>
<td>193.66±7** (40%)</td>
</tr>
</tbody>
</table>

† P <0.0001 compared with the initial level of blood glucose (0h) of normal rats.

** P<0.0001 compared with the initial level of blood glucose (0h) in the respective group.

* P<0.001 compared with the initial level of blood glucose (0h) in the respective group.

Numbers in parenthesis indicate the percentage of fall in 0h blood glucose.
Table 3. Effect of different doses of ARFHI on fasting blood glucose levels of STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Fasting Blood Glucose (mg/dL) levels after treatment with different doses of ARFHI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>1</td>
<td>81.83±2.8</td>
</tr>
<tr>
<td>2</td>
<td>280.16±15†</td>
</tr>
<tr>
<td>3</td>
<td>344±27 †</td>
</tr>
<tr>
<td>4</td>
<td>402.33±42†</td>
</tr>
<tr>
<td>5</td>
<td>384.66±43†</td>
</tr>
<tr>
<td>6</td>
<td>358.16±17†</td>
</tr>
<tr>
<td>7</td>
<td>331.83±14†</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.D from six rats in each group.
† P <0.0001 compared with the initial level of blood glucose (0h) of normal rats.
** P<0.0001 compared with the initial level of blood glucose (0h) in the respective group.
* P<0.001 compared with the initial level of blood glucose (0h) in the respective group.
Numbers in parenthesis indicate the percentage of fall in 0h blood glucose.
Table 4. Effect of ARFHI on fasting blood glucose levels of normal rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Fasting Blood Glucose (mg/dL) levels after treatment with ARFHI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>1</td>
<td>86.8±3.7</td>
</tr>
<tr>
<td>2</td>
<td>79.5±3.8</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.D from six rats in each group.

Table 5. Phytochemical constituents of different solvent extracts of *Heliotropium indicum*.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Phytochemicals</th>
<th>Hexane</th>
<th>Ethyl acetate</th>
<th>Methanol</th>
<th>Water</th>
<th>ARFHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Steroids</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Triterpenes</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Carbohydrates</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

++, major; +, minor; –, no phytochemical.
Fig. 1. Effect of ARFHI on glucose tolerance in diabetic rats

Fig. 2. Effect of ARFHI on glucose tolerance in normal rats
Discussion

The use of plant products in the treatment of diabetes mellitus is becoming advantageous due to the presence of several bioactive compounds with therapeutic potential. Some of the plants with antidiabetic potential are *Azadirachta indica* (Khosla et al., 2000), *Gymnema sylvestre* (Shanmugasundaram et al., 1990a), *Momordica charantia* (Sharma et al., 1960; Kedar and Chakrabarti., 1982), *Syzygium cumini* (Chopra et al., 1958), *Pterocarpus santalinus* (Kameswararao et al., 2001), *Momordica cymbalaria* (Rajasekhar et al., 2009), *Pterocarpus marsupium* (Sheehan et al., 1983; Manickam et al., 1997), *Syzygium alternifolium* (Kameswararao et al., 2001; Ramesh babu kasetti et al., 2010). Indeed, the widely prescribed insulin-sensitizer Metformin was derived from guanidine, molecule isolated from *Galega officinalis* L. (Bailey and Day, 2004; Witters, 2001). Moreover, *Trigonella foenum-graecum* L. (Fenugreek) is a plant long-consumed around the world for its anti-diabetic properties (Srinivasan, 2006).

Considering the significant importance of the antidiabetic agents from natural products against diabetes, development of new effective agents with no side effects is a compelling urgency. The hypoglycemic activity of a large number of plants/plant products has been evaluated and confirmed in hundreds of studies in animal models of diabetes (Maghrani et al., 2004).

The ethno botanical information suggests that several plant extracts may possess antidiabetic potential, among them are *Phyllanthus emblica* (Cuellar et al., 1980), *Gymnema sylvestre* (Shigemasa, 1992), *Catharanthus roseus* (Marles and Farnsworth, 1995), *Aegle marmelos* (Ayodhya et al., 2010), *Allium sativum* (Chauhan et al., 2010) and *Piper longum* (Nabi et al., 2013) etc.

Based on the traditional usage of *Heliotropium indicum* as a herbal drug in Ayurveda and Siddha to cure many diseases including diabetes, *Heliotropium indicum* whole plant was evaluated for its antihyperglycemic activity (*The Ayurvedic Pharmacopoeia of India*, Part-I, Vol. 6; 2008; Okvirk et al., 2013; Devi et al., 2011). The crude aqueous suspension of *Heliotropium indicum* whole plant at a dosage of 500 mg/kg bw showed significant (46%) antihyperglycemic activity. Hence *Heliotropium indicum* whole plant powder was used for preparing different solvent extracts for evaluating their antihyperglycemic activity.

The hypoglycemic effect of orally administered leaf aqueous extract of the *Phyllanthus emblica* has been reported earlier. Hypoglycemic effects were seen in normal mice. A reduced blood glucose level was also observed when fagosterol was injected intra peritoneal to alloxan-
induced hyperglycemic mice (Cuellar et al., 1980). A mixture of triterpenoid saponins extracted from the leaves of *Gymnema sylvestre* has been known not only to suppress selectively the sweet taste sensation in man, but also to inhibit the glucose absorption in the rat small intestine, leading to a reduction in plasma glucose in the oral glucose tolerance test (Shigemasa, 1992). *Catharanthus roseus* (periwinkle) plant extract was widely used as a traditional treatment for NIDDM. Hypoglycemic activity was observed for catharanthine, leurosine, lochnerine, tetrahydroalstonine, vindoline and vindolinine. Administered orally at a dose of 100 mg/kg, leurosine sulfate and vindolinine hydrochloride were more hypoglycemic than tolbutamide (Maries and Farnsworth, 1995). Aqueous leaf extract of *Aegle marmelos* showed antihyperglycemic activity in Streptozotocin induced diabetic rats after 14 days treatment either by increasing utilization of glucose or by direct stimulation of glucose uptake through increased insulin secretion (Ayodhya et al., 2010). The oral administration of ethanol extract of *Allium sativum* has remarkably blood sugar lowering effect in normal and alloxan induced diabetic rats (Chauhan et al., 2010). The oral administration of *Piper longum* root aqueous extract at a dose of 200 mg/kg bw for 30 days in STZ induced diabetic rats resulted in a significant decrease in FBG levels with the corrections of diabetic dyslipidemia, compared to untreated diabetic rats (Nabi et al., 2013).

In the present study 500 mg/kg bw of aqueous extract of HI has shown a maximum fall in blood glucose levels by about 47% in STZ induced diabetic rats, which is significantly higher than the hypoglycemic effect of 20 mg/kg bw of glibenclamide in the diabetic treated rats. The onset of antihyperglycemic action was observed from 1st hour of the treatment and a steady state increase in the action continued up to 6th hour. The methanol extract also produced significant but less antihyperglycemic activity (a maximum of 31.5%) in comparison with that of aqueous extract. No antihyperglycemic action was observed with hexane and ethyl acetate extracts.

Earlier, Aqheel et al., (2013) reported that the oral administration of methanolic extract of root of *Heliotropium indicum* (500 mg/kg bw) for 15 days and 21 days in Streptozotocin and alloxan induced diabetic rats has shown significant (60%) antihyperglycemic activity when compared with standard drug glibenclamide. The other species of this family *Heliotropium Zeylanicum* methanolic extract was reported to possess antidiabetic, antioxidant and antihyperlipidemic activities in STZ induced diabetic rats (Murugesh et al., 2006).
In the present study preliminary phytochemical analysis of different solvent extracts revealed the presence of steroids, triterpenes, alkaloids, saponins and tannins in *Heliotropium indicum* whole plant. Triterpenes constitute a large structurally diverse group of natural compounds possess various biological activities. Many experiments have shown that these compounds have several antidiabetic mechanisms. They can inhibit enzymes involved in glucose metabolism, prevent the development of insulin resistance and normalize plasma glucose and insulin levels (Nazaruk and Borzym-Kluczyk., 2014). These natural compounds, in contrast to synthetic drugs, apart from producing a hypoglycemic effect have also been found to manifest hypolipidemic and anti-obesity activity. Triterpenes are also promising agents in the prevention of diabetic complications. They have strong antioxidant activity and inhibit the formation of advanced glycation end products, implicated in the pathogenesis of diabetic nephropathy, embryopathy, neuropathy or impaired wound healing. Two triterpenes of *Momordica charantia* were reported to show hypoglycemic effects in the alloxan-injected mice at 400 mg/kg (Harinantenaina et al., 2006). Until now very few clinical studies have been concerned with the application of triterpenes in treating diabetes. Saponins have been reported as plant phytochemical having insulin sensitization and antihyperlipidemic effects in diabetic rats. (Bhavsar et al., 2009; Eu et al., 2010; Lee et al., 2011; Elekofehinti et al., 2013).

The alkaloids are well known phytoconstituents responsible for anti-inflammatory (Srinivas et al., 2000; Barbosa-Filho et al., 2006; Idowu et al., 2006), antioxidant (Murugesh et al., 2006; Idowu et al., 2006), antidiabetic (Singh et al., 2001; Ponnachan et al., 1993), anticancer (Kugelman et al., 1976; Jagetia and Baliga., 2006), antibacterial (Zhang et al., 2010), analgesic (Shang et al., 2010) and many other activities. Berberine is known to have potent hypoglycemic activity obtained from *Tinospora cordifolia* (Singh et al., 2003). Alkaloids like catharanthine, vindoline and vindolinine obtained from *Catharanthus roseus* also lower blood sugar level (Chattopadhyay., 1999). Shani et al., (1974) reported that *trigonella* seeds and the major alkaloid component; trigonelline exerted a mild hypoglycemic effect by delaying gastric emptying, slows carbohydrate absorption. A protein-bound polysaccharide, isolated from water soluble substances of pumpkin was investigated for hypoglycemic activity (Quanhong et al., 2005). Cryptolepine an indoloquinolone alkaloid isolated from *Cryptolepis sanguinolenta* significantly lowers glucose when given orally to diabetic mice (Beirer et al., 1998).
In this study the alkaloid rich fraction of *Heliotropium indicum* (ARFHI) at a dose of 750 mg/kg bw produced a significant (60%) fall in the fasting blood glucose levels of diabetic rats, but it has no effect in normal rats. The blood glucose lowering effect of ARFHI is higher than that of the oral hypoglycemic agent, glibenclamide (20 mg/kg bw). No dose-dependent effect was observed on increasing the dose further. Such a phenomenon of low hypoglycemic response at higher dose is common with indigenous plants and has been observed earlier with many plants like *Aegle marmelos* (Sharma et al., 1996), *Murraya koenigii* (Kesari et al., 2005), *Cinnamomum tamala* (Sharma et al., 1996), *Eugenia jambolana* (Rao et al., 2003), *Terminalia pallida* fruit (Alarcon-Aguilar et al., 2000) and *Psacalium decompositum* (Prince et al., 1999). The decreased antihyperglycemic activity at dose higher than 750 mg/kg bw could be due to reduced or no effect of the components present in the extracts at higher doses (Prince et al., 1999) and/or the presence of other antagonistic components in the extract.

The oral glucose tolerance test showed that the ARFHI gave definite blood glucose lowering activity. The onset of antihyperglycemic action was observed from 30 minutes of the treatment and a steady state increase in the action continued up to 180 minutes in diabetic rats. The ARFHI would have enhanced the glucose utilization, so blood glucose levels were significantly decreased in glucose loaded rats. The antihyperglycemic activity of ARFHI was higher than that of glibenclamide, a standard antidiabetic drug.

**In conclusion, the present study showed that the *Heliotropium indicum* has potential antidiabetic activity. The alkaloid rich fraction of *Heliotropium indicum* (ARFHI) has the capacity to reduce the blood glucose levels in the diabetic rats and so further studies are required to elucidate its effects on insulin secretion and other associated changes in carbohydrate, lipid metabolisms and enzymatic changes.**