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ANTI-OXIDANT AND ANTI DIABETIC ACTIVITY OF CHLOROFORM EXTRACT OF *HELICTERES ISORA*

Sunanda Sabbithi[1], Narendra Babu Ankem[2]

[1] Malla Reddy Institute of Pharmaceutical Sciences, Kompally, Hyderabad- 500014, Research Scholar, Acharya Nagarjuna University College of Pharmaceutical Sciences, Guntur, Andhra Pradesh- 522510

[2] Sir C. R. Reddy College of Pharmaceutical Sciences, Eluru, Andhra Pradesh-534007

Corresponding author Mrs. Sunanda Sabbithi Email id: sunandapharma@gmail.com
Phone no: 9491137158 Malla Reddy Institute of Pharmaceutical Sciences, Kompally, Hyderabad-500014

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ABSTRACT

Diabetes mellitus, a metabolic disorder is responsible for the death of millions of people across the globe. Poor efficacy, high cost and adverse side effects associated with available synthetic anti-diabetic drugs have necessitate the need to search for anti-diabetic drugs of natural origin. Therefore, in this study, anti-diabetic and antioxidant activities of chloroform fraction of *Helicteres isora* were evaluated. Phytochemical composition, *in vitro* antioxidant and *in vivo* antidiabetic activity of the fraction were evaluated following standard protocols. Hyperglycemia was induced via intraperitoneal injection of 120mg/kg b. wt of alloxan monohydrate. Male Wister rats weighing between 120.20±15.25g was randomly distributed into five groups consisting of six rats each and administered 200 and 400mg/kg b. wt of the fraction, 10mg/kg b. wt of glibenclamide, and 2ml/kg b. wt of normal saline respectively. The quantitative phytochemical screening revealed the presence of phytochemicals such as phenols, flavonoids, tannins, saponins and alkaloids. The fraction exhibited antioxidant activity in a concentration-dependent manner with percentage inhibition against DPPH radicals compared with ascorbic acid. At 400 mg/kg b. wt the fraction reduced the blood glucose concentration of rats compared with the glibenclamide treated group after 21st day. The results of this study showed that chloroform fraction of *Helicteres isora* possess significant antioxidant and anti-diabetic activities.

***Key words*:** *Helicteres isora*, glibenclamide, antioxidant and anti-diabetic activities

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease with chronic hyperglycemia characterized by alterations in carbohydrate, protein, and lipid metabolism due to complete or partial insulin deficiency accompanied by organ system dysfunction [1]. Factors such as changes in social status, dietary habits, smoking, and alcohol consumption may be responsible for the long-standing increase in the prevalence of this disease in recent years [2]. Diabetes poses a serious threat to global health, transcending socioeconomic status and borders. According to recent data from the International Diabetes Foundation (IDF), 463 million adults are currently living with diabetes [3]. It is reported that if sufficient measures are not taken to contain this pandemic, 578 million people will have diabetes by 2030, and by 2045 this number could increase to approximately 700 million, with the largest proportion in Africa and Asia [3]. In addition to the increasing prevalence of the disease, the burden of DM complications is also increasing [1]. Currently, DM-related amputations, cerebrovascular diseases, cardiac diseases, and renal diseases occur at high rates in populations not recently associated with these diseases [2]. There is growing concern over the increasing prevalence of DM in sub-Saharan Africa (SSA), including Nigeria. Recent studies indicate that approximately 5. 8% of adult Nigerians (approximately 6 million people) are living with DM [4]. This figure does not accurately represent the current reality, as two-thirds of the estimated diabetes cases in Nigeria are undiagnosed [5]. There is a link between diabetes and oxidative stress, which plays a key role in the development of both microvascular and cardiovascular diseases.

In many diseases characterized by tissue damage, oxidative stress is either the cause or the consequence of this damage. In the diabetic state, steady-state oxidative damage is increased as a result of an increase in oxidizable substrates, an increased rate of autoxidation of substrates, a decrease in antioxidant defenses, or a combination of all these processes [6]. Accurate knowledge and understanding of oxidative stress in diabetes and the adaptive response to it includes knowledge on the timing of its manifestation, its characterization in terms of oxidative damage to biomolecules such as lipids and the antioxidant enzymes affected and their control at the level of transcription or activity, or both. Traditional herbal remedies have been used for centuries to treat diabetes, but few have been scientifically evaluated [7]. According to a World Health Organization report, medicinal plants continue to be an alternative to traditional

chemotherapy and may become the most important source of daily medicines for people in developing countries who do not have access to basic medical facilities[8].The use of medicinal plants for the treatment of diseases dates back to human history, as evidenced by archives of traditional herbal remedies from ancient China and Babylonia[9].Traditional Chinese medicine has been used in drug and dietary therapy for thousands of years[10]. The earliest Chinese medical text in existence, the Yellow Emperor's Medical Canon, 475-221 BC, uses the term for "tumor"[10].

Chinese medicine has its own unique approach to disease prevention and treatment, and includes recipes that include not just one but many medicinal plants.

Over the course of many years of folk practice, various Chinese anticancer herbs and numerous related recipes have been tested and used for the treatment and prevention of various malignant tumors [10]. Medicinal phytotherapy against cancer is still widely used today, especially in rural and remote mountainous areas, and even in urban areas [11]. It has been documented that phytochemicals such as phenols, flavonoids, tannins, saponins, and alkaloids have been used to treat various diseases [12]. Phytochemicals have proven to be novel therapeutic agents for the treatment of chronic diseases [13]. Due to their different classes, phytochemicals are used to treat various diseases such as cancer, inflammation, and neurodegenerative diseases, and have become a useful source of similar compounds for the formulation of novel chemotherapeutic agents [13]. Phenols and flavonoids are natural human antioxidants used to treat degenerative diseases such as diabetes and cancer.[13] Plant extracts rich in tannins are used as diuretics for upset stomach and diarrhoea. [14] They are also used as anti-inflammatory, antiseptic, antioxidant and hemostatic drugs. [15] Alkaloids are a group of phytochemicals that contain nitrogen in a heterocyclic ring. They are naturally synthesized by numerous organisms including animals, plants, bacteria and fungi. The medical importance of the alkaloids includes antibacterial, antifungal, antihypertensive (some indole alkaloids), antiarrhythmic (quinidine, spearine), antimalarial (quinine), and anticancer (dimeric indoles, vincristine, vinblastine) effects. Saponins have also been reported to be used as membrane permeabilizing agents, immune stimulants, cholesterol lowering agents, and anticancer agents. They have also been shown to have a significant effect on animal growth, feed intake, and reproduction. [15]

The present study was investigating the anti-oxidant and in vivo anti diabetic activity of chloroform leaf extract of *Helicteres isora.*

MATERIALS AND METHODS

Plant material- collection and authentication:

The leaves of *Helicteres isora* were collected from native species growing in deciduous forests of Tirumala region, Andhra Pradesh, India. The leaves have been identified taxonomically and authenticated by Dr. S. Madhava Chetty, Associate Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India.

Preparation of the extracts:

The collected leaves were washed thoroughly with water and dried in the shade at room temperature. The dried leaves were ground well to coarse powder (400gms). Chloroform extract was obtained by extracting powder with methanol using Soxhlet apparatus by continuous hot percolation method for 72hr. After completion of the extraction the solvent was removed by using rotary evaporator. Thus, the chloroform extract was obtained. The % yield was found to be 21.17% w/w. From this chloroform extract was prepared by subsequent fractionation. The % yield of the chloroform extract thus obtained was found to be 6% w/w. The extracts were preserved in a refrigerator for further study. The chloroform (CEHI) extracts of *Helicteres isora* were subjected to further investigations [16].

Preliminary phytochemical screening:

Various Preliminary phytochemical like carbohydrates, amino acids, tannins, flavonoids, alkaloids, steroids, glycosides, saponins and Inulin [17, 18]

Estimation of *in-vitro* antioxidant activity:

***In-Vitro* Anti-oxidant activity:** The anti-oxidant activity of the *Helicteres isora* plant extracts has been determined by using the following *In-vitro* methods: DPPH free radical scavenging activity, Nitric oxide (NO) free radical scavenging method

Anti-diabetic activity:

Animals:

Male Wistar Albino rats (240±20gms) were procured and a total of 42 rats (n=42) were used for the experiment. They were divided into 7 groups of 6 animals each. They were randomly housed in standard polypropylene cages and maintained under the standard conditions: room temperature 25±3^{o}C, humidity 45%-55%, 12/12hr light/dark cycle. They were fed with commercially available normal pellet diet and water was allowed *ad libitum.* The animals were acclimatized to the laboratory conditions at least one week prior to the behavioral experiments. Each animal was used only once. The animal handling was performed according to the Good Laboratory Practice (GLP) guidelines. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Institutional Animal Ethics Committee of College. [19].

Induction of diabetes:

Following an overnight fast, fasting blood glucose levels of 30 rats were determined and 24 rats were injected intraperitoneally, with freshly prepared Alloxan monohydrate (2%solution, dissolved in 0.9% sodium chloride) in a dose of 120mg/kg body weight. Animals were carefully observed for first 24hours following the injection for any evidence of allergic reactions, behavioural changes, convulsions and hypoglycemic attacks. No untoward reaction was observed in any animal [19].

Grouping:

Group I: Served as normal control.

Group II: Served as diabetic control and received alloxan (120 mg/kg) and vehicle.

Group III: Alloxan (120 mg/kg) + Glibenclamide (10 mg/kg p. o.) and served as standard.

Group IV: Alloxan (120 mg/kg) + CEHI extract (200 mg/kg, p. o.)

Group V: Alloxan (120 mg/kg) + CEHI extract (400 mg/kg, p. o.)

After 3 days (72 hours) of alloxan injection, the animals developed stable hyperglycemia. Only those animals with blood glucose level more than 250mg/dl were selected for the study. Later they were divided into seven groups each comprising of 6 rats. Normal control, diabetic control and standard groups kept same for anti-diabetic activity of plant extracts. The treatment (p. o.) was started from the same day except normal control and diabetic control groups for a period of 14 days. During this period, animals in all groups had free access to standard diet and water.

Body weight and blood glucose levels were measured on '0' day, 7th, 14th and 21st day of the post treatment in overnight fasted animals. On 7th, 14th and 21st days, other biochemical parameters (SGOT, SGPT, Cholesterol and Triglycerides) were estimated in all animals.

Collection of blood:

The blood for the estimation was collected from the retro orbital plexus into the centrifuge vials. Later serum was separated by centrifugation of blood at a speed of 2000 rpm for 10 minutes. The serum was collected and quantitatively analyzed for blood glucose, triglycerides, cholesterol, SGOT (Serum glutamate oxaloacetate transaminase) and SGPT (Serum glutamate pyruvate transaminase) by using their respective kits.

IV. Estimation of *In-Vivo* Antioxidant Activity

Preparation of Post Mitochondrial Supernatant (PMS):

The Pancreas were perfused with ice cold saline(0.9% sodium chloride) and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 rpm for 5 minutes at 4^{o}C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 rpm for 20 minutes at 4^{o}C to get the PMS**.**

***In-vivo* Antioxidant activity**

The anti-oxidant activity of the *Helicteres isora* plant extracts has been determined by subjecting the PMS to the different *In-vivo* methods Lipid Peroxidation method (LPO), Reduced Glutathione method (GSH) and Catalase method (CAT)

Statistical analysis:

The data obtained from the present study were subjected to statistical analysis. All the results were expressed as Mean ± Standard Error (SEM). Data obtained from various groups was subjected to one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Significant values were set accordingly.

RESULTS

Preliminary phytochemical screening:

Results of the preliminary phytochemical screening on chloroform leaf extracts of *Helicteres isora were* shown in Table 1.

Table 1: Preliminary phytochemical screening

S. No.	TEST	Chloroform Extract

1.	Carbohydrates	++
2.	Proteins and Amino acids	+++
3.	Tannins	++
4.	Flavonoids	+
5.	Steroids	++
6.	Cardiac glycosides	+
7.	Saponins	++

+ indicates Presence, ++ indicates clarity, +++ indicates better response

***In-vitro* Anti-oxidant activity:**

DPPH free radical scavenging activity:

As the concentration of the drug increased, the % inhibition also increased. The highest % inhibition of Ascorbic acid (74.12±0.93), CEHI (51.90±0.43) was recorded at a concentration of 125µg/ml. IC_{50} and R^2 values were calculated. The results were presented in **Table 2**.

Nitric oxide (NO) free radical scavenging method:

As the concentration of the drug increased, the % inhibition also increased. The highest % inhibition of Ascorbic acid (92.82 ± 1.16) and CEHI (49.58 ± 1.04) was recorded at a concentration of 125 µg/ml. IC_{50} and R^2 values were calculated. The results were presented in **Tables 3.**

Table 2: DPPH free radical scavenging activity

Concentration (µg/ml)	**Ascorbic acid**	**CEHI**
	%Inhibition (Mean ± SEM)	**%Inhibition (Mean ± SEM)**
5	12.62 ± 0.13	2.42 ± 0.96
10	18.09 ± 0.14	8.50 ± 0.39
15	24.56 ± 1.08	14.75 ± 0.46
25	40.06 ± 0.16	26.22 ± 1.02
50	55.65 ± 0.77	38.77 ± 0.93
75	66.52 ± 0.53	42.60 ± 0.52
100	68.04 ± 0.57	45.44 ± 0.67
125	72.12± 0.93	49.90 ± 0.43

R^2 Value & IC50	IC_{50} = 58.498 R^2 = 0.9914	IC_{50} = 116.19 R^2 = 0.9835

Table 3: Nitric oxide (NO) free radical scavenging method

Concentration (µg/ml)	**Ascorbic acid**	**CEHI**
	%Inhibition (Mean ± SEM)	**%Inhibition (Mean ± SEM)**
5	5.03 ± 0.81	2.62 ± 1.62
10	12.12± 1.25	6.48 ± 1.40
15	24.81 ± 0.67	11.01 ± 1.54
25	43.33 ± 1.09	19.54 ± 1.39
50	68.28 ± 1.46	33.94 ± 1.04
75	78.56 ± 0.74	40.55 ± 1.36
100	80.23 ± 1.21	46.44 ± 1.51
125	88.82 ± 1.16	50.58 ± 1.04
R^2 Value & IC50	IC_{50} = 41.89 R^2 = 0.9813	IC_{50} = 147.42 R^2 = 0.9653

Anti-diabetic study:

The animals did not show any gross behavioral changes.

Effect of CEHI on Morphological parameters:

(i) Effect on Body weight:

In diabetic control group treated with alloxan (120 mg/kg, i. p, single dose) a decrease in the body weights of animals on the 7^{th}, 14^{th} and 21^{st} day (224.1±3.99, 216.4±6.07 and 205.1±3.27)

was observed when compared to the 0th day body weight (250.7±6.58). This indicates that alloxan reduced the body weights persistently.

The rats treated with chloroform leaf extracts of *Helicteres isora* (CEHI) prevented reduction in body weights compared to diabetic control group. The CEHI effect on body weight data was presented in **Table 4**.

Table 4: Effect of CEHI on Body weights in Alloxan induced diabetic rats:

Treatment Group	**Body weight of animals (g)**			
	0 day	**7th day**	**14th day**	**21st day**
Normal control	248.1±4.21	250.5±5.81	252.4±3.58	254.2±4.42
Diabetic control	250.7±6.58	224.1±3.99	216.4±6.07	205.1±3.27
Alloxan+ Glibenclamide (10 mg/kg)	246.4±5.40	218.4±6.09	224.5±4.58	232.5±5.06
Alloxan + CEHI (200 mg/kg)	248.4±6.60	221.7±4.50	228.4±3.61	231.4±5.43
Alloxan + CEHI (400 mg/kg)	247.2±5.42	224.4±3.76	232.6±5.13	236.5±3.63

Values are expressed as Mean ± SEM (n=6)

CEHI: Chloroform extract of *Helicteres isora*

Effect of CEHI on Biochemical parameters:

Effect on Serum Glucose levels:

In diabetic control group treated with alloxan (120mg/kg, single dose) a significant increase ($p<0.001$) in the serum glucose levels on the 7th, 14th and 21st day (290.07±10.47, 312.67±11.04 and 348.14±9.71) was observed when compared to the normal control group (84.68±9.01,

92.68±7.00 and 95.81±8.05) respectively. This indicates that alloxan induces persistent diabetes mellitus. In the group receiving standard drug (Glibenclamide, 10 mg/kg, p. o, once daily) there was a significant decrease ($p<0.01$) in the serum glucose levels on the 7^{th}, 14^{th} and 21^{st} day (348.56±8.26, 250.44±7.72 and 132.97±6.84) respectively when compared to the diabetic control group. In standard group, the serum glucose levels decreased significantly by day 21.

Rats treated with low dose chloroform extract of *Helicteres isora (*200mg/kg, p.o, once daily) there was a significant ($p<0.05$ and $p<0.01$) decrease in the serum glucose levels on 7^{th}, 14^{th} and 21^{st} day (365.80±9.50, 275.27±9.70 and 167.04±9.64) when compared to diabetic control group respectively. The group receiving high dose chloroform extract of *Helicteres isora* (400mg/kg, p. o, once daily) also showed a significant decrease ($p<0.01$) in serum glucose levels on 7^{th}, 14^{th} and 21^{st} day (373.50±8.43, 260.89±8.314 and 152.94±8.51) when compared to diabetic control group. A dose related decrease in serum glucose levels was observed in the CEHI (200mg/kg and 400mg/kg). This result suggests the anti-diabetic activity of *Helicteres isora.* The effect of CEHI on serum glucose levels was given in **Table 5**.

Effect on SGOT:

Rats treated with alloxan (diabetic control) showed a significant increase ($p<0.001$) in SGOT levels (128.98±6.112, 138.09±5.25 and 148.63±5.48) when compared to normal control (54.07±3.62, 56.70±4.15 and 56.34±4.17) when measured on days 7^{th}, 14^{th} and 21^{st}. The group treated with standard drug showed a significant decrease ($p<0.01$) in SGOT levels (151.41±4.07, 92.46±3.50 and 71.105±3.91) when compared to diabetic control. The groups receiving chloroform leaf extracts of *Helicteres isora* (CEHI) at doses of 200mg/kg and 400mg/kg, p. o, once daily) showed a significant decrease ($p<0.01$) in SGOT levels (158.58±5.58, 111.73±4.01 and 99.44±5.40) and (150.53±4.14, 104.22±4.66 and 85.26±3.87) when measured on days 7^{th}, 14^{th} and 21^{st} respectively when compared to diabetic control group. The effect of CEHI on serum SGOT was given in **Table 6**.

Effect on SGPT:

Rats treated with alloxan (diabetic control) showed a significant increase (0.001) in SGPT levels (74.67±5.60, 97.31±4.63 and 102.02±4.93) when compared to normal control (39.08±3.47, 39.88±3.82 and 37.54±3.32) when measured on days 7^{th}, 14^{th} and 21^{st}. The group treated with standard drug showed a significant decrease ($p<0.01$) in SGPT levels (98.39±3.87, 61.5±3.46

and 51.63±3.21) when compared to diabetic control. The groups treated with different doses of chloroform leaf extract of *Helicteres isora* (CEHI) such as 200mg/kg and 400mg/kg showed a significant decrease ($p<0.01$) in SGPT levels (96.44±5.42, 79.5±5.08 and 77.12±5.07) and (108.41±4.41, 68.57±4.49 and 68.29±3.79) when measured on days 7th, 14th and 21st respectively when compared to diabetic control group. The effect of CEHI on serum SGPT was given in **Table 7**.

Effect on Serum cholesterol:

A significant increase in serum cholesterol ($p<0.001$) was observed in rats treated with alloxan (diabetic control) (122.33±5.16, 130.37±4.71 and 141.38±5.92) respectively when measured on days 7th, 14th and 21st when compared to normal control (55.04±3.65, 54.02±3.18 and 55.88±4.51). The group treated with standard drug (glibenclamide, 10mg/kg, p. o, once daily) showed a significant decrease in serum cholesterol levels (143.58±3.90, 101.26±3.57 and 79.66±4.25) when measured on days 7th, 14th and 21$^{st\ h}$ when compared to the diabetic control group. The groups receiving chloroform leaf extract of *Helicteres isora* (CEHI) at a dose of 200mg/kg and 400mg/kg showed a significant decrease ($p<0.01$) in serum cholesterol levels (147.29±5.04, 110.25±5.53 and 102.31±4.44) and (151.97±4.24, 107.35±3.52 and 92.75±3.91) respectively when compared to diabetic control group. The serum cholesterol levels were decreased from day 7th to day 21st in groups treated with standard drug and chloroform leaf extracts of HI. These values suggest that Glibenclamide, CEHI had cholesterol lowering activity. The effect of CEHI on serum cholesterol was given in **Table 8**.

Effect on serum triglycerides:

Rats treated with alloxan (diabetic control) showed a significant increase in ($p<0.001$) serum TG levels on 3rd, 7th and 14th day (118.02±5.09, 139.64±4.98 and 139.09±3.98) respectively when compared to normal group (77.06±4.00, 78.14±3.10 and 77.58±2.51). The group treated with standard drug (glibenclamide, 10mg/kg, p. o, once daily) had a triglyceride levels of (140.33±3.91, 100.18±3.96 and 84.45±3.41) when measured on days 7th, 14th and 21st respectively. This was significantly lower ($p<0.01$) when compared to diabetic control. The group treated with CEHI (200mg/kg, p. o, once daily) showed a significant decrease ($p<0.01$) in triglyceride levels on 7th, 14th and 21st day (147.19±4.52, 118.13±3.61 and 102.18±3.21) when compared to the diabetic control respectively. The other group receiving high dose CEHI

(400mg/kg) also showed a significant decrease ($p<0.01$) in serum TG on 7th, 14th and 21st day (138.10±4.25, 112.64±3.77 and 94.50±2.74) when compared to diabetic group respectively. The leaf extract of *Helicteres isora* (CEHI) on serum triglycerides was given in **Table 9**.

Table 5: Effect of CEHI on Blood Glucose levels in Alloxan induced diabetic rats

Treatment Group	**Serum Glucose levels (mg/dL)**			
	0 day	**7th day**	**14th day**	**21st day**
Normal control	84.75±3.22	84.68±9.01	92.68±7.00	95.81±8.05
Diabetic control	90.24±2.70	290.07±10.47**	312.67±11.04**	348.14±9.71**
Alloxan + Glibenclamide (10mg/kg)	85.55±3.41	348.56±8.26**	250.44±7.72**	132.97±6.84**
Alloxan + CEHI (200mg/kg)	83.84±2.71	365.80±9.50**	275.27±9.70*	167.04±9.64**
Alloxan + CEHI (400mg/kg)	91.81±2.72	373.50±8.43**	260.89±8.31**	152.94±8.51**

Values are expressed as Mean ± SEM (n=6)

*P < 0.05 and ** P < 0.01 Vs Diabetic control

Table 6: Effect of CEHI on Serum SGOT levels in Alloxan induced diabetic rats

Treatment	**Serum SGOT (IU/L)**

Group	**0 day**	**7th day**	**14th day**
Normal control	54.07±3.62	56.70±4.15	56.34±4.17
Diabetic control	128.98±6.11**	138.09±5.25**	148.63±5.48**
Alloxan + Glibenclamide (10mg/kg)	151.41±4.07**	92.46±3.50**	71.105±3.91**
Alloxan + CEHI (200mg/kg)	158.58±5.58**	111.73±4.01**	99.44±5.40**
Alloxan + CEHI (400mg/kg)	150.53±4.14*	104.22±4.66**	85.26±3.87**

Values are expressed as Mean ± SEM (n=6)

*P < 0.05 and ** P < 0.01 Vs Diabetic control

Table 7: Effect of CEHI on Serum SGPT levels in Alloxan induced diabetic rats

Treatment Group	**SGPT levels**		
	7th day	**14th day**	**21st day**
Normal control	39.08±3.47	39.88±3.82	37.54±3.32
Diabetic control	74.67±5.60**	97.31±4.63**	102.02±4.93**
Alloxan + Glibenclamide (10mg/kg)	98.39±3.87**	61.5±3.46**	51.63±3.21**
Alloxan + CEHI (200mg/kg)	96.44±5.42**	79.5±5.08*	77.12±5.07**
Alloxan + CEHI (400mg/kg)	108.41±4.41**	68.57±4.49**	68.29±3.79**

Values are expressed as Mean ± SEM (n=6)

*P < 0.05 and ** P < 0.01 Vs Diabetic control

Table 8: Effect of CEHI on Serum Cholesterol levels in Alloxan induced diabetic rats

Treatment Group	**Serum Cholesterol (mg/dL)**		
	7th day	**14th day**	**21st day**

Normal control	55.04±3.65	54.02±3.18	55.88±4.51
Diabetic control	122.33±5.16**	130.37±4.71**	141.38±5.92**
Alloxan + Glibenclamide (10mg/kg)	143.58±3.90**	101.26±3.57**	79.66±4.25**
Alloxan + CEHI (200mg/kg)	147.29±5.04**	110.25±5.53**	102.31±4.44**
Alloxan + CEHI (400mg/kg)	151.97±4.24**	107.35±3.52**	92.75±3.91**

Values are expressed as Mean ± SEM (n=6)

*P < 0.05 and ** P < 0.01 Vs Diabetic control

Table 9: Effect CEHI on Serum Triglyceride levels in Alloxan induced diabetic rats

Treatment Group	**Serum Triglycerides (mg/dL)**		
	7th day	**14th day**	**21st day**
Normal control	77.06±4.00	78.14±3.10	77.58±2.51
Diabetic control	118.02±5.09**	139.64±4.98**	139.09±3.98**
Alloxan + Glibenclamide (10mg/kg)	140.33±3.91**	100.18±3.96**	84.45±3.41**
Alloxan + CEHI (200mg/kg)	147.19±4.52**	118.13±3.61**	102.18±3.21**
Alloxan + CEHI (400mg/kg)	138.10±4.25**	112.64±3.77**	94.50±2.74**

Values are expressed as Mean ± SEM (n=6)

*P < 0.05 and ** P < 0.01 Vs Diabetic control

***In vivo* Anti-oxidant activity:**

In the present study various antioxidant parameters were assessed in the pancreas at the end of the study on 21st day.

Estimation of Malondialdehyde by Lipid peroxidation method:

Rats treated with only alloxan had MDA levels of (0.411±0.038) when measured on 14th day. This was significantly higher ($p<0.01$) when compared to MDA levels in normal control group (0.056±0.007).

Diabetic rats treated with standard drug (Glibenclamide, 10 mg/kg, p. o, once daily) had MDA levels of (0.062±0.006) when measured on day 14. This was significantly lower ($p<0.01$) when compared to the diabetic control group.

The groups treated with CEHI-200 mg/kg and CEHI-400 mg/kg) exhibited a significant decrease ($p<0.01$) in the MDA levels. The results were given in **Table 10**.

Estimation of GSH by Reduced Glutathione method:

A significant decrease in the levels of GSH was observed in the diabetic control group (0.009±0.002) when compared to the normal control group (0.171±0.004). The group receiving the standard drug (Glibenclamide 10 mg/kg, p. o, once daily) had significant increase ($p<0.01$) in the GSH levels (0.080±0.004) when compared to the diabetic control group.

The groups treated with CEHI (200 mg/kg) and CEHI (400 mg/kg) exhibited a significant increase ($p<0.01$) in the GSH levels when compared to the diabetic control group. The results were given in **Table 10**.

Estimation of Catalase levels:

A significant decrease in the levels of Catalase was observed in the diabetic control group (0.008±0.001) when compared to the normal control group (0.056 ± 0.002). The group receiving the standard drug (Glibenclamide 10 mg/kg, p. o, once daily) had significant increase ($p<0.01$) in the Catalase levels (0.084 ± 0.002) when compared to the diabetic control group.

The groups treated with CEHI-200 mg/kg and CEHI-400 mg/kg exhibited a significant increase ($p<0.01$) in the Catalase levels when compared to diabetic control group. The results were given in **Table 10**.

Table 10: *In-vivo* antioxidant activity of CEHI

Group	**Lipid Peroxidation**	**Reduced Glutathione (GSH)**	**Catalase**
Control	0.056±0.007	0.171±0.004	0.056±0.002
Diabetic control	0.411±0.038**	0.009±0.002**	0.008±0.001**

Standard	0.062±0.006**	0.080±0.004**	0.084±0.002**
CEHI (200mg/kg)	0.092±0.004**	0.054±0.006**	0.038±0.002**
CEHI (400mg/kg)	0.059±0.008**	0.071±0.007**	0.069±0.002**

Values are expressed as Mean ± SEM for six animals

** P < 0.01 Vs Diabetic control

DISCUSSION

Diabetes mellitus is a complex and diverse group of diseases that disrupts carbohydrate, fat, and protein metabolism. The prevalence of diabetes has increased dramatically worldwide in recent decades, affecting almost 10% of the population. Diabetes is accompanied by the development of micro- and macrovascular complications, which contribute significantly to the morbidity and mortality of the disease. The high number of therapeutic errors, unpleasant side effects, and enormous costs of oral anti diabetic drugs has created an urgent need and desire for alternative treatments. In recent years, the field of herbal medicine has experienced exponential growth and these drugs have become increasingly popular in both developing and developed countries due to their natural origin and fewer side effects [20, 21]. In this study, the leaves of the plant *Helicteres isora* are used as an herbal medicine.

Phytochemical studies of *Helicteres isora* (CEHI) leaf extract revealed the presence of various pharmacologically active compounds such as tannins and flavonoids. Alloxan-treated diabetic rats administered *Helicteres isora* (CEHI) leaf extract at doses of 200 mg/kg and 400 mg/kg rapidly normalized blood glucose levels compared to the diabetic control group. This may be because some beta cells may still survive for *Helicteres isora* to act and exert its insulin releasing effect. Furthermore, a dose-dependent reduction in blood glucose levels was observed.

In our study, the study of liver enzymes SGOT and SGPT shows that their activity is decreased in both glibenclamide and *Helicteres isora* (CEHI) leaf extract treated groups. Increases in these liver enzymes are a sign of liver damage. The results of this study showed that *Helicteres isora* (CEHI) leaf extract and glibenclamide have a hepatoprotective effect. The study also found that serum cholesterol levels were lower after treatment with glibenclamide and *Helicteres isora* (CEHI) leaf extract compared to the diabetic group. High cholesterol levels are associated with coronary heart disease (CHD) in diabetic patients.

In our study, a reduction in serum triglyceride levels was observed in the glibenclamide and leaf extract (CEHI) treated groups compared to the diabetic group. Oxidative stress is a condition in which the levels of toxic reactive oxygen intermediates (ROIs) exceed the host's endogenous antioxidant defenses and occurs in the early stages of diabetes. Hyperglycemia exacerbates endothelial ROS generation through many mechanisms, including activation of protein kinase C isoforms, increased formation of advanced glycation end products, and increased glucose influx via the aldose reductase pathway.

These are some of the known biochemical mechanisms of tissue/organ damage caused by hyperglycemia. The reactive species are scavenged by various enzymatic and non-enzymatic antioxidants, protecting tissue/organ damage from oxidative stress. In this study, we measured both enzymatic and non-enzymatic antioxidants in the pancreas in vivo. Malondialdehyde (MDA) is an end product of lipid peroxidation and a non-enzymatic antioxidant that scavenges hydroxyl radicals at low concentrations.

In our study, we observed an increase in MDA in diabetic rats compared to the glibenclamide and *Helicteres isora* leaf extract (CEHI) treated groups, indicating that *Helicteres isora* leaf extract has antioxidant properties. Glutathione is an enzymatic antioxidant.

Glutathione peroxidase, a selenium-containing enzyme, detoxifies H_2O_2 to significant concentrations of H2O through the oxidation of reduced glutathione.

In this study, the decreased glutathione activity observed in diabetic rats was shown to be an important adaptive response to increased oxidative stress.

The increased activity of reduced glutathione observed in diabetic rats treated with glibenclamide and *Helicteres isora* leaf extract (CEHI) indicates that these extracts have antioxidant properties.

Catalase is an enzymatic antioxidant. Catalase is a heme protein that catalyzes the reduction of hydrogen peroxide and protects tissues from the highly reactive hydroxyl radical.

In our study, a decrease in pancreatic catalase activity was observed in diabetes, which may be adversely affected by the accumulation of superoxide anion radicals and hydrogen peroxide. An increase in catalase activity was observed in alloxan-induced diabetic rats treated with glibenclamide and *Helicteres isora* (CEHI) leaf extract, suggesting that these extracts have antioxidant properties. In our study, the decreased levels of enzymatic antioxidants (catalase, decreased GSH) in diabetic animals were significantly restored by treatment with *Helicteres isora* (CEHI) leaf extract.

The non-enzymatic antioxidant (MDA) levels, which were increased in diabetic animals, were significantly decreased in diabetic animals treated with glibenclamide and *Helicteres isora* leaf extract (CEHI). This means that the potential antioxidant defenses are reactivated by the active ingredients of *Helicteres isora,* improving the scavenging of oxyradicals and increasing the detoxification ability. In this study, an in vitro antioxidant study was also conducted to evaluate the scavenging ability of *Helicteres isora.* DPPH is the most commonly used method to screen the antioxidant activity of many Chinese herbal medicines. The method is based on the reduction of the colored free radical DPPH in chloroform solution by radical scavengers.

It measures the decrease in absorbance of DPPH at its absorption maximum at 516 nm, which is proportional to the concentration of radical scavenger added to the DPPH reagent solution.

From the results of this study, it can be concluded that the active principles of *Helicteres isora* (CEHI) leaf extract are hydrogen donors and scavenge free radicals. Nitric oxide is classified as a free radical due to its unpaired electron and shows significant reactivity with certain types of proteins and other free radicals. In vitro inhibition of nitric oxide radical is also a measure of antioxidant activity.

In the present study, nitrite produced by incubating sodium nitroprusside solution in standard phosphate buffer at 25°C was reduced by *Helicteres isora* (CEHI) leaf extract. This is likely because the antioxidant active components in the leaf extract compete with oxygen to react with nitric oxide, suppressing the production of nitrite. In the present study, *Helicteres isora* (CEHI) leaf extract inhibited NO scavenging, but not as much as ascorbic acid. These results prove that

Helicteres isora (CEHI) leaf extract has anti diabetic and antioxidant properties. Thus, these results support the claim of using this plant for diabetic diseases.

Further work is obviously required to fractionate, purify, identify and to elucidate the exact mechanism of action of the hypoglycemics principles present in the leaf extracts of *Helicteres isora (*CEHI).

CONCLUSION

The present study clearly indicated a significant anti diabetic activity with the leaf extracts of *Helicteres isora* (CEHI) supports the traditional usage of leaves by the Ayurvedic physicians for the control of diabetes. Hence, it was concluded that the leaf extracts have anti-diabetic activity. The phytochemicals like tannins, flavonoids present in the extracts may be responsible for anti-diabetic activity. The leaf extracts of *Helicteres isora* also showed a significant antioxidant activity; hence it might help in preventing diabetic complications.

REFERENCES

1. A.E. Uloko, B.M. Musa, M.A. Ramalan, I.D. Gezawa, F.H. Puepet, A.T. Uloko, M.M. B orodo, K.B. Sada, Prevalence and risk factors for diabetes mellitus in Nigeria: a systematic review and meta-analysis, Diabetes Therapy, 9 (3) (2018), pp. 1307-1316
2. C.M. Chikezie, O.A. Ojiako, A.A. Emejulu, P.C. Chikezie, Atherogenicity of diabetic rats administered single and combinatorial herbal extracts. *Bulletin of Faculty of Pharmacy*, Cairo niversity, 56 (2) (2018), pp. 169-174
3. P. Saeedi, I. Petersohn, P. Salpea, B. Malanda, S. Karuranga, N. Unwin, S. Colagiuri, L. Guariguata, A.A. Motala, K. Ogurtsova, J.E. Shaw, Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, Diabetes Res. Clin. Pract., 157 (2019), Article 107843
4. I.U. Ezeani, E.T. Ugwu, F.O. Adeleye, I.D. Gezawa, I.O. Okpe, M.I. Enamino, Determinants of wound healing in patients hospitalized for diabetic foot ulcer: results from the MEDFUN study, Endocrine Regulation, 54 (2020), pp. 207-216
5. N. Ghouri, S. Hussain, S.H. Ahmed, S.A. Beshyah, R. Rashid, E. Al-Ozairi, S. Waqar, A. Dabhad, R. Mohammed, changing how we risk-categorise in

Ramadan: does the IDF-DAR scoring system achieve the requirements for people with diabetes internationally, Diabetes Res. Clin. Pract., 175 (2021), Article 108835

6. P.V. Thai, H.A. Tien, H. Van Minh, P. Valensi, Triglyceride glucose index for the detection of asymptomatic coronary artery stenosis in patients with type 2 diabetes, Cardiovasc Diabetol, 19 (1) (2020), 1-0
7. Batubara, M.E. Prastya, Potential Use of Indonesian Medicinal Plants for Cosmetic and Oral Health: a Review, Jurnal Kimia Valensi, 6 (1) (2020), pp. 120-134
8. E. Esfandiari, A. Dorali, M. Ghanadian, B. Rashidi, F.S. Mostafavi, Protective and therapeutic effects of Phoenix dactylifera leaf extract on pancreatic β-cells and diabetic parameters in streptozotocin-induced diabetic rats, Comp Clin Path, 29 (2020), pp. 847-854
9. Y. Cai, Q. Luo, M. Sun, H. Corke, Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, Life Sci., 74 (17) (2004), pp. 2157-2184
10. Y.Z. Cai, M. Sun, J. Xing, Q. Luo, H. Corke, Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants, Life Sci., 78 (25) (2006), pp. 2872-2888
11. M.A. El Hadi, F.J. Zhang, F.F. Wu, C.H. Zhou, J. Tao, Advances in fruit aroma volatile research, Molecules, 18 (7) (2013), pp. 8200-8229
12. K.S. Kang, Phytochemical constituents of medicinal plants for the treatment of chronic inflammation, Biomolecules, 11 (5) (2021 May), p. 672
13. S. Kumar, A.K. Pandey, Chemistry and biological activities of flavonoids: an overview, The scientific world journal (2013), Oct;2013
14. M. Saxena, J. Saxena, R. Nema, D. Singh, A. Gupta, Phytochemistry of medicinal plants, J Pharmacogn Phytochem, 1 (6) (2013)
15. Jude E, Okokon, Bassey S, Antia and Emem omoh. Analgesic and Anti-inflammatory Effects of Methanolic extract of *Hippocratea Africana*. International J of Pharmacology. 2008; 4(1): 51-55.
16. Kokate CK. Practical Pharmacognosy. Delhi: Vallabh Prakashan. 1994; 4th ed.
17. Khandelwal KR. Practical Pharmacognosy, Pune: Nirali Prakashan. 2004; 11: 149-56.

18. Clement Jackson, Herbert O.C. Mbagwu, Indongesit Jackson, Godwin Ekpe, Udeme Eyaekop and Grace Essien. Evaluation of the hypoglycemic effect of aqueous extract of *Phyllanthus amarus* in alloxan induced diabetic albino rats. Int J Pharm Biomed Res. 2011; 2(3): 158-160.
19. S Badami, S Mahendran and V Maithili. Evaluation of antidiabetic effect of embelin from *Embelia ribes* in alloxan induced diabetes in rats. Biomedicine & Preventive Nutrition. 2011; 1: 25-31.
20. Grover J.K, Yadav S and Vats V. Medicinal plants of India with antidiabetic potential. J. Ethnopharmacol. 2002; 81: 81–100
21. Sumeet Gupta, Kritika Mehla, Devesh Chauhan, Satish Kumar and Anroop Nair**.** Morphological changes and Antihyperglycemic effect of *Michaelia Champaca* leaves extract on Beta-cell in Alloxan induced diabetic rats. Recent Research in Science and Technology. 2011; 3(1): 81-87.