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Original Article

Effect of Isolated Phytochemicals from *Hardwickia binata* on Analgesia and Neuroprotection in Dexamethasone-Induced Diabetic Rats

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ABSTRACT

Background: *Hardwickia binata* (Roxb.), also known as Indian Blackwood or Anjan, is a deciduous tree from the *Leguminosae* family with established value in traditional medicine. Its leaves and other tissues have been explored for management of ailments such as diabetes, pain, and cognitive deficits, but evidence on its specific pharmacological potential remains limited. **Materials and Methods:** This study investigated the antidiabetic, analgesic, and neuroprotective activities of *Hardwickia binata* leaves Ethylacetate Fraction (HbL-EAF) in dexamethasone-induced diabetic *Wistar albino rats*. Healthy male rats (150-200 g) were divided into control (*n*=6) and experimental (*n*=36) groups. Type 2 diabetes was induced using dexamethasone (10 mg/kg, i.m.), with fasting blood glucose used for confirmation. Rats received oral fractions at 200 and 400 mg/kg for eight days. Antidiabetic activity was assessed via glucose reduction. Analgesic effects were measured by the hot plate test, with morphine as standard. Neuroprotective effects were evaluated through the Morris water maze, using donepezil for comparison, measuring escape latency as the outcome. **Results:** Treatment with HbL-EAF resulted in significantly improved antidiabetic activity, as well as increased pain thresholds in the hot plate assay versus control. Additionally, spatial learning and memory were significantly improved in Morris water maze testing, indicating neuroprotective efficacy. Statistical analysis confirmed the significance of observed effects. **Conclusion:** The ethyl acetate leaf fraction of *Hardwickia binata* demonstrated potent antidiabetic, analgesic, and neuroprotective activities in rat models. Further research is necessary to elucidate the molecular mechanisms and develop novel therapeutics for diabetes, pain and cognitive disorders based on these findings.

Keywords: Analgesic, Anti-diabetic, Eddy's hotplate, *Hardwickia binata*, Neuroprotection.

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INTRODUCTION

The metabolic disease known as diabetes mellitus is distinguished by high blood glucose levels, is one of the world's most important health problems. 537 million people worldwide are estimated by the World Health Organization (WHO) to have diabetes, and 1.5 million of those cases end in death annually (Mollik *et al*., 2024). Based on the cause of the illness, diabetes can be classified into two major types: Type 1 and Type 2. Insulin resistance and type 2 diabetes are frequently associated with the use of dexamethasone, a glucocorticoid. Pancreatic-cell dysfunction has been suggested as one of the primary causes of steroid-associated diabetes. In the insulin-resistant diabetic rat model, dexamethasone is frequently employed due to its selective cell cytotoxicity and ability to promote hyperinsulinemia and hyperglycemia. Insulin resistance and abrupt hyperglycemia and hyperlipidemia are brought on by short-term high-dosage administration of dexamethasone (Hossain *et al*., 2024).

Herbal medicine, rich in phytochemicals, have been traditionally used to treat various diseases and are often considered safer with fewer side effects compared to synthetic drugs. In managing diabetes, herbal drugs offer advantages like lower cost, fewer complications, and reduced side effects. Many modern drugs are derived from natural sources, highlighting the importance of plant-based medicines in drug development. Synthetic antihyperglycemic drugs like metformin can cause gastrointestinal issues, B_{12} deficiency, Dipeptidyl Peptidase IV (DPP-IV) inhibitors can produce headaches, upper respiratory infections, and sulfonylureas can develop hypoglycemia, weight gain as adverse effects. Unlike synthetic drugs, herbal medicines support the body's natural healing processes, promoting steady

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recovery with minimal side effects (Chaachouay and Zidane, 2024).

A monotypic genus of flowering plants, *Hardwickia binata* is also called Anjan. The tree is large, growing up to 25-30 m tall with drooping branches. It's small, bifoliate leaves are green in colour, alternating, pinnate, and measures 5-7 cm in length and 2.5-6 cm width. Little, pale yellow flowers with a racemose inflorescence are present. *H. binata* possesses a variety of biological activities and therapeutic qualities (Shingade and Kakde, 2021).

Antibacterial and antifungal properties were demonstrated by the leaf extract. The bark of the roots exhibited anticancer properties. The tree's resin exhibited antidiuretic properties. Additionally, the resin combined with cubes and sandal is used to treat Sexually Transmitted Diseases (STDs) such as gonorrhoea, leucorrhea, and chronic cystitis. Additionally, it is the source of oleo-resin, a balsam, a fibre, and a resin with regional medical applications (Manimegalai *et al*., 2023).

However, diabetic and neuroprotective effects haven't been evaluated on leaves of *Hardwickia binata*. Thus, the purpose of this study was to examine the impact of leaf extract of *Hardwickia binata* on blood glucose levels and neuroprotection in an experimental diabetic rat model induced by dexamethasone.

MATERIALS AND METHODS

Plant Materials

Hardwickia binata plant leaves were collected from the medicinal garden of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, Andhra Pradesh, India. For the plant specimen authentication was done by Dr. P. Vijetha, Associate Professor, Department of pharmacognosy at Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, with voucher specimen No. 01/2024.

Preparation of Fraction

The leaves were collected dried in the shade, and then roughly ground into powder. Coarse powder was macerated with petroleum ether up to 24 hr to remove the lipids and fats. After defatting, the soxhlet extraction was done in a mixture of ethanol and water in 80:20 ratio. The extract was collected, concentrated and fractionated with ethylacetate to isolate the specific phytochemicals of the plant. Finally, the ethylacetate fraction was separated and concentrated as *Hardwickia binata* leaves Ethylacetate Fraction (HbL-EAF).

Phytochemical Screening

Standard preliminary phytochemical qualitative analysis of the 80% ethanol extract and HbL-EAF was done for detection of biologically active compounds like alkaloids, glycosides, phenols, flavonoids, terpenes, steroids, saponins, proteins and carbohydrates using standard procedures (Tamada *et al*., 2022).

In vitro Pharmacological Screening

Anti-oxidant Activity

Reducing Power Assay Method

The HbL-EAF was combined with phosphate buffer (5 mL, 0.2 M, pH 6.6) and 1 mL of ethanol at different doses. After adding 5 mL (1%) of potassium ferricyanide to the combination above, it was incubated for 20 min at 50°C. The resultant solution was centrifuged for 10 min at 3000 rpm after 5 mL of 10% trichloroacetic acid were added. After adding distilled water (5 mL), ferric chloride (1 mL, 0.1%), and the surface layer of the solution (5 mL), the absorbance was determined at 700 nm. Ascorbic acid at varying quantities (10 to 100 µg/mL) served as the standard (Umamahesh *et al*., 2019). Every test was conducted in triplicate, and the average of the three results was noted.

Anti-Diabetic Activity

Increase of Glucose Uptake in Yeast Cells

A 10% (v/v) suspension was prepared in distilled water by centrifuging one gram of commercial baker's yeast in distilled water at 3000 rpm (Revolution Per Minute) for 5 min to produce a clear supernatant fluid. After dissolving different amounts of separated ethyl acetate fraction in Dimethyl Sulfoxide (DMSO) (25 and 50 mg/ml), the mixture was transferred to 1 mL of glucose solution (5, 10, and 20 mM) and incubated for 10 min at 37°C. After adding 100 µL of yeast suspension, the reaction was swirled and incubated for an additional 60 min at 37°C. After 60 min, the tubes were centrifuged at 2500 rpm for 5 min, and the amount of glucose in the supernatant was calculated. The standard medication was glibenclamide (Nuannoi *et al*., 2018). The formula was used to determine the percentage increase in yeast cells absorption of glucose.

$$Increase\ in\ glucose\ uptake\ (\%) = \left[\frac{AbsControl - Abs\ Sample}{AbsControl}\right] x100$$

Animals Used in Study

Male *Wistar rats* weighed between 200 to 250 g were purchased from Mahaveer Enterprises, Hyderabad. The animals were acclimated to a constant temperature of 25±2°C and percentage humidity of 45 to 55% for two weeks, with a 12-hr light/dark cycle. During the acclimatisation phase, the animals had unrestricted supply to water and pellet meal. Following the quarantine period, animals were divided based on body weight to conduct experiments. The Institutional Animal Ethical Committee (IAEC) has accepted the protocol for conducting animal studies (1529/PO/Re/S/11/CPCSEA/CHIPS/IAEC11/PRO-01/2023-24).

Dexamethasone Induced Hyperglycemia in Rats

Five groups of six rats each were put together from the animals. The animals were given with treatment for a period of eight days and on day nine, various parameters were evaluated. Rats in the initial group were given distilled water orally (*p.o.*) as a control.

Group II considered as Disease control and administered daily intramuscular injection of dexamethasone (1 mg/kg/day) (Brooks *et al.*, 2022). Standard group differ for all three activities as IIIa, IIIb and IIIc. Rats in group IIIa group were treated with standard drug glimepiride (1mg/kg/day, p.o) IIIb group with Morphine (6mg/kg *s.c*) IIIc group with donepezil (5 mg/kg *p.o*) and for all dexamethasone (1 mg/kg/day). Rats in experimental groups IV and Vas test I and test II respectively were treated orally with HbL-EAF at respective doses of 100 and 200 mg/kg/day along with daily injection of dexamethasone (1 mg/kg/day, p.o).

Measurement of Blood Glucose Levels and Body Weight

Blood glucose levels were recorded for each animal used in dexamethasone induced hyperglycemia on day zero (prior to the study) and day nine (the final day of the study). Blood samples were obtained via the tail flick method, and glucose concentrations were assessed using OneTouch glucometer. In addition to blood glucose measurements, the body weight of each animal in all groups were recorded at both time points (Kamboj *et al.*, 2013). This information allowed for a thorough examination of how the study circumstances affected body weight and glucose metabolism

Evaluation of Analgesic Activity

Eddy's Hot Plate Method

This is the common test used frequently to study the analgesic activity. In this test, the animals were placed on an Eddy's hot plate maintained at 55°C ± 1°C. The time taken for the animals to respond in a manner of paw licking or jumping to the thermal stimulus was recorded as the Basal reaction time in seconds. A reference time was used to reduce the tissue damage and animals which showed the response with in the reference time were selected for the study (Wani *et al.*, 2024). Hot plate test was conducted at 15, 30, 60, 90, 120 and 180 min after the administration of vehicle, drug and extract of different doses. For Group I animals was treated as normal control and the animals received normal saline. Group II was put on a hot plate kept at 55°C while being restrained, and the response time was noted. The third group (IIIb) received standard medication, morphine, and the response time was noted. Jumping or licking their paw was used to measure the response time, measured in seconds. The treatment groups IV and V were pre-treated with HbL-EAF at low (100 mg/kg) and high (200 mg/kg) doses. The basal reaction time was observed at the beginning (0 min) and at intervals of 15, 30, 60, 90, 120 and 180 min after the drug administration. Every animal in that specific group participated in the cycle, which was then repeated, and the response time was noted.

$$\%\ Increase\ in\ Basal\ Reaction\ Time = \frac{Basal\ reaction\ time\ in\ test\ group - Basal\ reaction\ time\ in\ control\ group}{Basal\ reaction\ time\ in\ test\ group} \times 100$$

Evaluation of Neuroprotective Activity

Behavioural Assessment

Morris Water Maze Test

Morris water maze test is commonly preferred to analyse the spatial learning and retention memory of animals. The water maze contains a circular pool with dimensions of 100 cm diameter and 50 cm height. The pool was filled up to 30 cm height with tinted opaque water which was due to the addition of titanium dioxide suspension. The circular pool divided into four equal quadrants as p, q, r and s to use as starting points. Within the pool a hidden platform was placed 2 cm below the water. Rats were practiced and trained for 5 days with 3 consecutive trials each day with an inter-trial period of 5 min. The time taken by the animal to find the maze hidden in water was noted separately for all the groups (Srinivasan *et al.*, 2021).

Ethical Statement

Experimental procedures involving the animals adhered to the guidelines set forth by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Furthermore, the Institutional Animal Ethical Committee (IAEC) has accepted the protocol for conducting animal studies. (1529/PO/Re/S/11/CPCSEA/CHIPS/IAEC11/PRO-01/2023-24).

Statistical Analysis

The Mean ± Standard Error of Mean (SEM) was used to express every result.

RESULTS

In the present study, HbL-EAF a plant leaf extract was screened for pharmacological actions through *in vitro* and *in vivo* methods. The natural extract HbL-EAF was subjected for *in vitro* antioxidant, anti-diabetic and *in vivo* anti-diabetic, analgesic and neuroprotective activities. After evaluation of various pharmacological actions, the obtained results were compared with that of the standard drug to know the efficacy of HbL-EAF.

Phytochemical Screening

Several secondary metabolites were found in the crude hydroalcholic extract and its ethylacetate fraction after an initial phytochemical screening. Alkaloids were not found in the hydroalcholic extract, but carbohydrates, proteins, certain glycosides, steroids, flavonoids, phenols, terpenoids, and saponins occurred. The ethylacetate fraction showed the presence only two components those are flavonoids and phenols which are moderately polar and can be isolated specifically with ethylacetate (Tiwari *et al.*, 2011). The phytochemical screening results were given in Table 1.

In vitro Pharmacological Screening

Anti-Oxidant Activity

The reducing power of HbL-EAF was significant and the absorbance values were increased in response to the concentration of sample which is dose dependent. Each value is compared with the standard antioxidant ascorbic acid to assess the antioxidant potential of HbL-EAF. Effect of HbL-EAF on absorbance values of reducing power assay were given in Figure 1.

Anti-Diabetic Activity

Increase of Glucose Uptake in Yeast Cells

HbL-EAF effectively increased the percentage increase of glucose uptake at doses of 25 and 50 mg/ml which is almost closer to the standard drug glibenclamide. This might be due to the binding of HbL-EAF to glucose effectively and transporting it across the cell membrane for further metabolism. This proves the anti-diabetic effect of HbL-EAF. Analyzing the action of HbL-EAF, which might assist in the body's greater intake of glucose by muscle cells and adipose tissues, will certainly prove more significant. Figures 2 and 3 presented these outcomes.

In vivo Pharmacological Screening

Anti-Diabetic Activity

Effect of HbL-EAF on Blood Glucose Levels and Body Weights

Due to the hyperglycemic activity of dexamethasone, on day three the blood glucose levels were increased in Disease control, standard, test I and test II group animals. In control group, there is a mild change in both blood glucose and body weight from day 0 to 9. In disease control group significant increase in blood glucose levels and reduction in body weight were observed. In standard group, there is a reduction in blood glucose levels and slight increase in body weight on day 9. When compared with the test I, remarkable reduction was observed in blood glucose levels of test II group animals but showed similar effect in body weight improvement. Results were demonstrated in Table 2.

Analgesic Effect

Effect of HbL-EAF on Basal Reaction Time

Administration of HbL-EAF resulted in a significant, dose-dependent increase in basal reaction time compared to the control group, indicating analgesic potential. At the higher dose of 200 mg/kg/day orally, HbL-EAF produced a pronounced elevation in reaction time, with the maximum response observed at 90 minutes (14.73 ± 0.12 sec), corresponding to a 51.12% increase over the control group (7.20 ± 0.16 sec). The lower dose of 100 mg/kg/day also showed a noticeable increase in basal reaction time up to 90 min, demonstrating dose responsiveness. Notably, group IIIb animals, which received the reference analgesic morphine 6 mg/kg, showed the highest increase in reaction time (15.15 ± 0.11 sec, 52.48%), exceeding that of both HbL-EAF-treated and disease control groups. The disease control group initially displayed a slight decrease in reaction time during the first 60 min (7.18 ± 0.05 seconds, -0.27%), indicative of impaired nociceptive response, but showed a minimal recovery thereafter (7.21 ± 0.12 sec, 0.13%). These findings demonstrate that the high dose of HbL-EAF significantly prolongs reaction time on the hot plate, comparable to the reference analgesic, thus indicating potent analgesic properties likely mediated through

Table 1: Preliminary phytochemical screening for HbL-EAF.

Chemical tests	Reagent	Hydroalcoholic Extract	Ethylacetate Fraction
Carbohydrates	Molisch test Benedict's test Fehling's test	-	-
Proteins	Biuret test Ninhydrin test	-	-
Alkaloids	Dragendorff's test Mayer's test Hager's test	+	-
Glycosides	Keller killiani test	-	-
Steroids	Salkowski test	+	-
Flavonoids	Shinoda test Alkaline reagent test	+	+
Phenols and Tannins	Ferric chloride test Lead acetate test Folin-ciocalteu test	+	+
Terpenoids	Horizon test	-	-

+ indicates Presence - indicates absence.

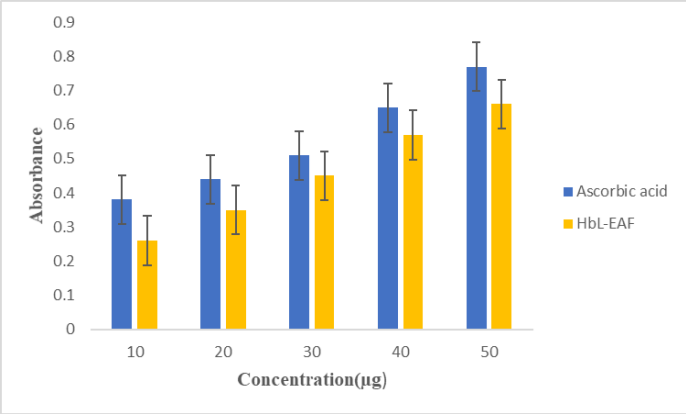

Figure 1: Effect of HbL-EAF on absorbance values of reducing power assay method.

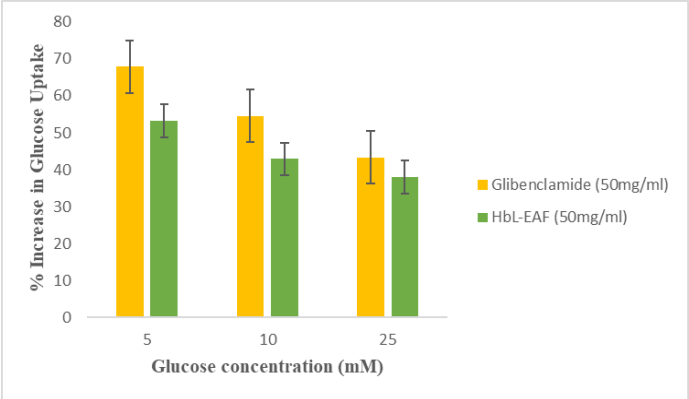

Figure 3: Effect of Glibenclamide and HbL-EAF on % increase in Glucose Uptake (50 mg/mL).

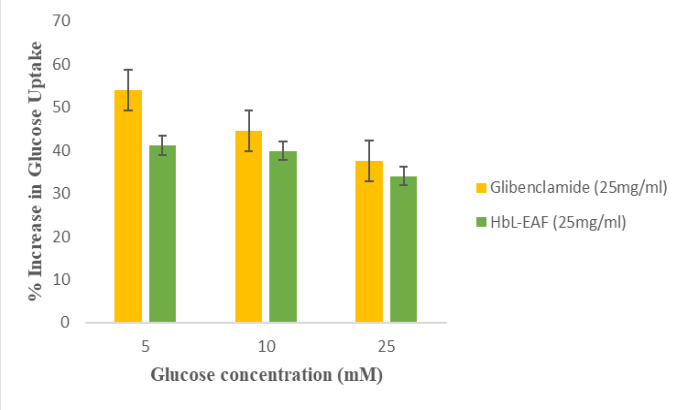

Figure 2: Effect of Glibenclamide and HbL-EAF on % increase in Glucose Uptake (25 mg/mL).

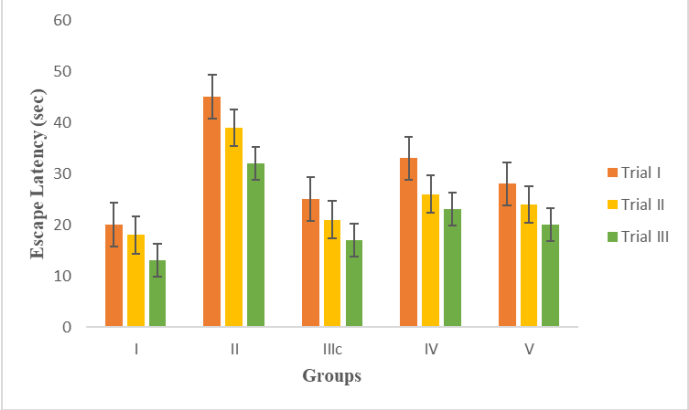

Figure 4: Effect of HbL-EAF on Escape Latency Time.

central nociceptive pathways. Results were demonstrated in Tables 3 and 4.

Neuroprotective Effect

Effect of HbL-EAF on Escape Latency Time

The variations in learning capacity were assessed by Morris water maze test. The learning capacities of the control, donepezil (5 mg/kg, *p.o.*), and HbL-EAF animals were evaluated on the 9th day using low and high doses of 100 and 200 mg/kg, *p.o* respectively. After training, on the 9th day the rats treated with control, donepezil, and HbL-EAF exhibited a substantial decrease in escape latencies. This finding indicates that dexamethasone caused significant cognitive impairment. The animals administered with HbL-EAF extracts exhibited a significant reduction in escape latencies on the 9th day, indicating an improvement in escape testing for locating the hidden platform in both groups in a concentration-dependent manner. These results were comparable to those of the standard reference group treated with donepezil in contrast with the dexamethasone-treated rats. Results were shown in Figure 4.

DISCUSSION

Phytochemical Screening

Previous studies revealed the presence of quinolones, mucilage, volatile oils, terpenoids, flavonoids, phenols and carbohydrates in petroleum ether, chloroform, acetone and ethanolic leaves extract of *H. binata* (Sharanabasappa *et al.*, 2007). A similar type of phytochemical study detected the absence of alkaloids, anthraquinones, emodins and resins (Pandey *et al.*, 2019). Various studies were conducted for the extracts of stem and root bark of *H. binata* and exhibit the presence of carbohydrates, glucosides, proteins, phenols, flavonoids, fixed oils and fats (Kocak and Kaysim, 2023). In the present work the ethylacetate fraction was used for the all *in vitro* and *in vivo* assessments to prove the activity of specific chemical constituents like phenols and flavonoids.

In vitro Pharmacological Screening

Anti-Oxidant Activity

A compound's capacity to reduce is typically dependent on the presence of reductants, which have demonstrated antioxidant capability by donating a hydrogen atom and breaking the chain

of free radicals (Bhalodia *et al.*, 2013). The presence of reductants in *H. binata* leaf ethylacetate fraction causes the reduction of free radicals that proves its antioxidant potential.

Previous studies reported that the *Hardwickia binata* plant extract contains the hydroxyl group that facilitates free radical scavenging, phenolic compounds have strong antioxidant action. Antioxidants that donate hydrogen can interact with reactive oxygen and nitrogen species in a termination reaction to interrupt the cycle of radical production (Manimegalai *et al.*, 2023).

Anti-Diabetic Activity

Increase of Glucose Uptake in Yeast Cells

In general, an accumulation of functional glucose transporter molecules in the cell membrane is the process that causes skeletal muscles to absorb glucose. Leptocytes and monocytes control the molecules that transport glucose in response to elevated insulin release in the blood, which has a hypoglycemic impact. However, research on how medications can lower postprandial hyperglycemia has been crucial to the management of diabetes mellitus, which is still a well-focused therapeutic approach (Yoshida *et al.*, 2012). In addition, yeast cells may absorb glucose differently than other eukaryotic or human body cells. Instead of using a phosphotransferase enzyme system or another unidentified mechanism, assisted diffusion may be used to transport glucose across the yeast membrane. Numerous factors, including the concentration of glucose within the cells or the subsequent metabolism of glucose, may influence the uptake of glucose by the yeast cells (Pulivarthi *et al.*, 2020).

In vivo Pharmacological Screening

Anti-Diabetic Activity

High blood glucose levels are a hallmark of diabetes mellitus, a chronic metabolic disease that over time seriously harms the heart, kidneys, blood vessels, and nerves. Nowadays, there are several methods for managing diabetes, including insulin injections, lifestyle modifications, and synthetic antidiabetic drugs. Synthetic antidiabetic medications include biguanides, glucosidase inhibitors, DPP-4, and sulfonylureas (Stottlemyer *et al.*, 2023). These synthetic diabetes drugs do, however, have serious adverse effects, including low blood sugar, weight gain, nausea, gastrointestinal distress, liver and heart failure, and diarrhoeal illness.

Because of the high expense of synthetic antidiabetic medications and the fact that diabetes constitutes a serious threat to world health, an affordable and environmentally friendly alternative way of managing the condition is required. Aromatic and medicinal plants can be used to create novel medications (Sunmonu and Afolayan, 2013). Therefore, in the present study I have selected *Hardwickia binata* for its well documented medicinal properties and potential antidiabetic activity. The evaluation of antidiabetic activity was carried out by observing the changes in blood glucose levels and body weight of experimental animals.

Table 2: Effect of HbL-EAF on Blood Glucose Levels and Body Weight.

Groups	Blood Glucose Levels (mg/dL) (Mean ± SEM) *			Body Weights (g) (Mean ± SEM) *	
	Day 0	Day 3	Day 9	Day 0	Day 9
I	94.43±2.33	95.64±3.66	98.16±2.51	250±5.93	245±6.00
II	96.53±2.56	123.28±4.01	195.68±5.73	275±6.88	225±6.51
IIIa	99.26±3.22	142.66±3.55	135.31±2.19	250±4.15	245±4.21
IV	95.27±3.66	134.73±3.44	124.40±3.58	285±6.30	290±7.22
V	98.76±2.66	151.24±3.20	128.63±3.23	230±4.28	225±3.87

*Mean ± Standard Error of Mean (SEM); N = 6.

Table 3: Effect of HbL-EAF on Basal Reaction Time (sec).

Groups	Basal Reaction Time (in sec) - Mean±SEM*						
	0 min	15 min	30 min	60 min	90 min	120 min	180 min
I	7.20±0.16	---	---	---	---	---	---
II	---	7.20±0.08	7.15±0.04	7.18±0.05	7.20±0.08	7.21±0.10	7.21±0.12
IIIb	---	7.73±0.17	9.40±0.12	12.23±0.09	15.15±0.11	8.22±0.10	7.33±0.06
IV	---	7.51±0.09	8.42±0.11	11.04±0.62	14.33±0.05	7.75±0.12	7.25±0.09
V	---	7.60±0.11	8.88±0.25	11.51±0.31	14.73±0.12	7.90±0.12	7.28±0.08

*Mean ± Standard Error of Mean (SEM); N = 6.

Table 4: Effect of HbL-EAF on % increase in basal reaction time.

Groups	% increase in basal reaction time						
	0 min	15 min	30 min	60 min	90 min	120 min	180 min
I	---	---	---	---	---	---	---
II	---	0	-0.69	-0.27	0	0.13	0.13
III	---	06.86	23.40	41.13	52.48	12.41	01.77
IV	---	04.13	14.18	34.78	49.76	07.09	0.68
V	---	05.26	18.18	37.44	51.12	08.86	1.09

Effect of HbL-EAF on Blood Glucose Levels and Body Weights

Dexamethasone has been reported to be a useful medication for generating diabetes (Kamani *et al.*, 2022). Hepatic hexokinase activity suppression, hepatic glucose oxidation, and hepatic gluconeogenesis promotion are some of the hypothesized mechanisms of dexamethasone-induced insulin resistance. According to earlier research, dexamethasone raised the levels of free fatty acids in rats, which may diminish the expression of the Glucose Transporter-4 (GLUT-4) in cell membranes, reducing the uptake of glucose and affecting the metabolism of glucose in organs involved in the disposal of glucose (Mahmoud *et al.*, 2022). Therefore, dexamethasone was selected as a diabetes-inducing drug in this study. The fraction HbL-EAF reduced elevated fasting blood glucose level.

When compared to the diabetic group in the current investigation, the HbL-EAF extract significantly increased body weight and significantly decreased blood glucose levels. The presence of well-known antioxidant phytochemicals including flavonoids and phenols, which function as free radical scavengers, may be the mechanism underlying the HbL-EAF's antidiabetic benefits (Maidadi *et al.*, 2023). These antioxidants were thought to work by inhibiting hepatic gluconeogenesis and glucose oxidation.

Analgesic Effect

Since pain is the most prevalent symptom of the majority of illnesses. Numerous synthetic Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), which frequently have negative side effects, have been utilized as the main painkiller. Consequently, it is advantageous to investigate other analgesic treatments. Centrally-acting analgesics change the body's normal physiological response to pain and reduce the pain threshold. On the other hand, analgesics that act peripherally stop pain chemoreceptors from generating impulses. Eddy's hot plate method was utilized to evaluate the analgesic activity of HbL-EAF by recording the basal reaction time to a thermal stimulus in *Wistar* rats (Pakale *et al.*, 2024).

Effect of HbL-EAF on Basal Reaction Time

An ethanolic leaf extract of *Hardwickia binata*, a member of the *leguminosae* family, was found to have analgesic properties in earlier research. When compared to normal analgin, it exhibited significant effects at a dose of 200 mg/kg body weight after 90 min and up to 120 min (Yimer *et al.*, 2020). According to earlier research, phytoconstituents extracted from medicinal plants, such as alkaloids, flavonoids, steroids, and tannin, have been shown to have a strong analgesic effect (Mishra *et al.*, 2022).

Neuroprotective Effect

Depending on which parts of the nervous system are impacted, neurodegenerative disorders can impact movement, language, perception, cognition, and memory. They are typified by the progressive degradation of nerve cells. According to reports, free radicals play a major role in neuronal death in a variety of neurodisorders, including Parkinson's disease, Alzheimer's disease, cerebral ischemia, schizophrenia, and seizure disorders. The treatment of memory impairment is significantly aided by medicinal plants (Ali *et al.*, 2025). The present study investigated the protective effect of HbL-EAF and its action against the Dexamethasone-induced cognitive impairment in rats.

Effect of HbL-EAF on Escape Latency Time

In comparison to LPS-induced neurotoxic control rats, the current study showed that treatment of *Hardwickia binata* leaf extract dramatically decreased escape latency time in the Morris water maze in a dose-dependent manner, indicating better spatial learning and memory (Rezvani-Kamran *et al.*, 2017). These results are consistent with other studies on neuroprotective plant extracts that improve cognitive impairment by lowering oxidative stress, adjusting neurotransmitter levels, and reducing neuroinflammation (Uabundit *et al.*, 2010). As evidenced by the behavioral changes noted, *Hardwickia binata's* neuroprotective efficiency is most likely due to its phytochemical richness, particularly its flavonoids and phenolics.

CONCLUSION

In conclusion, the ethyl acetate fraction of *Hardwickia binata* leaves exhibits pronounced antioxidant, antidiabetic, analgesic, and neuroprotective activities, particularly in dose dependent manner. These outcomes illustrate the therapeutic benefits of this plant extract in managing oxidative stress, diabetes, pain, and cognitive deficits. Additional study is recommended to isolate the active constituents and elucidate the underlying mechanisms responsible for these pharmacological effects.

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ABBREVIATIONS

HbL-EAF: *Hardwickia binata* leaves Ethylacetate Fraction; ***i.m.*:** Intramuscular; **WHO:** World Health Organization; **DPP-IV:** Dipeptidyl Peptidase IV; **STDs:** Sexually Transmitted Diseases; **DMSO:** Dimethyl Sulfoxide; ***p.o.*:** Per oral; ***s.c.***: Subcutaneous; **RPM:** Revolution Per Minute; **SEM:** Standard error of mean; **GLUT-4:** Glucose Transporter-4; **NSAIDs:** Non- Steroidal Anti Inflammatory Drugs; **LPS:** Lipopolysaccharide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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AUTHOR CONTRIBUTIONS

Ramineni Sai Reshma Performed the experimental work, administered the samples, handled data, and drafted the manuscript. Doppalapudi Sandeep Assisted in blood sample collection, provided necessary guidance, and reviewed the manuscript with suggestions for improvement. Suryadevara Vidyadhara Supervised the work and provided overall guidance throughout the research. All the authors provided approval for publishing the manuscript.

SUMMARY

The present study systematically evaluated the ethyl acetate fraction of *Hardwickia binata* leaves for a range of pharmacological activities. Phytochemical analysis confirmed the presence of bioactive compounds, steroids, flavonoids, phenols, terpenoids and saponins. and was found to possess marked antioxidant potential *in vitro*, as assessed by the reducing power assay. The HbL-EAF further exhibited significant *in vitro* antidiabetic potential via the glucose uptake inhibition method. *In vivo* studies in dexamethasone-induced diabetic rats revealed the high-dose 200 mg/kg HbL-EAF extract showed a significant rise in body weight and significant decline in blood glucose levels in compared to the diabetic group. Analgesic activity, evaluated by the hot plate method with morphine as the standard, was significant at higher doses of HbL-EAF. Additionally, the HbL-EAF showed substantial neuroprotective effects in dexamethasone-induced memory impairment using the Morris water maze, with donepezil as the reference drug.

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