ASSESSMENT OF INULA CAPPA LEAVES EXTRACTS FOR ANTIDIABETIC POTENTIAL IN EXPERIMENTAL ANIMALS

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ABSTRACT

Diabetes mellitus (DM) is a long-term disease that can be caused by a lack of insulin production by the pancreas, or by the ineffectiveness of the insulin produced. For the study, the antidiabetic effects of methanolic and 50% v/v hydroalcoholic methanolic and extracts of the leaves of *Inula cappa* were used as oral doses at 100, 200, and 400 mg/kg and Metformine (50 mg/kg) were administered continuously for 21 days as a single morning dose. 18 hours of fasting was used to assess the drug's anti-diabetic effects in normoglycemic rats. On the 0th, 10th, and 21st days of the experiment, glucose oxidase method readings were collected using an autoanalyzer to assess fasting blood glucose levels. Anti-oxidant parameters were determined including, total phenolic content, total flavonoid concentration, DPPH free radical scavenging activity, Hydroxyl radical scavenging activity, Scavenging of Hydrogen Peroxide, Superoxide radical scavenging assay, Nitric oxide scavenging activity and Reducing power assay. The methanolic (ICME) and hydroalcoholic (ICHE) extracts of *Inula cappa* leaves (100, 200, and 400 mg/kg, p.o.) passes the Oral Glucose Tolerance Test. Both extracts had a greater impact in lowering blood glucose levels. Out of which ICHE at all doses showed significant effect (P<0.05-P<0.001) when compared with control. ICME in terms of glucose tolerance at all doses had significant effects.

*Keywords:* Diabetes mellitus, insulin, metformine, scavenging, *Inula cappa.*
INTRODUCTION

As a diabetic, you may have a variety of metabolic issues due to a lack of insulin or a lack of insulin resistance. Patients with hyperglycemia are all affected by the body's inability to maintain appropriate blood glucose levels (1). Diabetes has long been recognised as a serious health problem, even before insulin treatment became widely accessible (2). Despite the fact that insulin and anti-diabetic medications can now be used to treat all types of diabetes, the long-term risks are still rather high. Among those ages 20 to 79, diabetes mellitus is expected to be the sixth leading cause of mortality in 2021, accounting for one out of every eight fatalities (International Diabetes Federation, 2021).

Two of the most frequent forms of diabetes are Type 1 and Type 2. One and two are referred to as insulin-dependent diabetic mellitus (IDDM) and Non-insulin dependent diabetic mellitus (NIDDM). Non-insulin dependent diabetic mellitus is one of the most common type with 99.4% of the cases. Approximately 87.3 percent of all diabetes mellitus cases in Scotland are caused by type 2 diabetes. Diabetes Type 1 (T1DM) affects 5 to 15% of the world's population, with Scotland accounting for 13% of those numbers. Less than 2% of the world's diabetic population and 0.6% of the Scottish population have one of the several types of diabetes mellitus.

In diabetics, their tissues are more susceptible to metabolic alterations, which may lead to tissue damage and loss of function. The kidneys, eyes, and nerves, as well as large and tiny blood arteries, seem to be the most severely affected bodily components. Most tissue damage is assumed to be caused by pro-inflammatory cytokines and adipokines, although other variables may also be involved. These include the inclusion of non-enzymatically glycated proteins, which alter the structure and antigenicity of structural proteins, as well as the accumulation of oxygen free radicals as a result of metabolic mis-regulation in glucose metabolism.

Inula cappa is a shrub that may reach a height of 1.8 metres (6ft). Hermaphrodites are flowers (have both male and female organs). It is locally known as "Sheep Ear". *Inula cappa* contains Stigmasterol, Cleomiscosin C (or D), Luteolin, 3, 4-dihydroxy-Benzoi acid, Apigenin, and Fortuneletin. It also consist of phenolic chemicals such as coniferaldehyde, isovanillin, 6-deoxyjacareubin, scopoletin, syring aldehyde, and 6-deoxyjacareubin. *Inula cappa* is used for a variety of purposes, including as an analgesic, antiphlogistic, carminative, depurative, expectorant, and clot-busting agent. As a remedy for clotting, the root's juice is added to bath water, which reduces muscle and joint discomfort caused by hard work. Poultices of pounded root are applied to the forehead with a towel or gauze for headache relief. The bark juice of Ficus semicordata and Myrica esculenta is used in equal parts to treat menstrual disorders.
MATERIALS AND METHODS

Chemicals and Drugs –

All chemicals which were used are of analytical grade, and procured in India from Loba Chemicals Ltd. in Ahmedabad and SD-Fine Chemicals Ltd. in Mumbai. Diagnostic kits for assessment of biochemical parameters were obtained from Erba diagnostic, India.

Plant Collection and Authentication: *Inula cappa* leaves were gathered in March from rural regions of Ghaziabad. The plant material was authenticated by Prof. (Dr.) Vijai Malik (Department of Botany) from CCS University of Meerut, India. A voucher specimen of *Inula cappa* (CCSU/BOT/HRB/15/08) was deposited in the institute for further reference.

Extract Preparation for Animal Study: Extract Preparation for Animal Study: The freshly collected leaves of *Inula cappa* were washed with distilled water to remove dirt and soil and shade dried in a ventilated place at room temperature. Dried leaves were cut into small pieces and reduced to coarse powder by mechanical grinder and further the extraction process was carried out using the Soxhlet equipment in which 50g of weighed drug material was packed properly. The cycle of soxhlet was run by methanol and hydroalcoholic solvent in ratio of 1:1. After 12-14 complete cycles, the extract was filtered and concentrated under reduced pressure below 40±1°C using roteva vacuum rotary evaporator (Model no- UDOIAB-2391 Medica instrument) to dryness to get a constant weight. The % yield was found to be 18.11 % w/w. The extract was stored in −20 °C freezer and used for Pharmacological investigation.

Animal Experiments:

Adult Wistar rats weighing 150 ± 20 g were obtained for the study from National Institute of Biologicals, Noida, Uttar Pradesh-201309. They were kept in Animal House of Sunder Deep Pharmacy College, Ghaziabad. The animals were separately housed in polypropylene cages for acclimitization at a temperature of (23 ± 2 °C) and 50–60% relative humidity, with a 12 hr light/dark cycle one week before and during the commencement of the experiment. Animal were kept on standard pellet diet (Dayal animal feed, Unnao, India) and drinking water *ad libitum* throughout the housing period.

All experimental procedures involving animals were conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The study protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Sunder Deep Pharmacy College, Ghaziabad, India (Reg. no. 1673/PO/Re/S/12/CPCSEA).
Experimental Protocol:

a) Induction of diabetes

STZ (45 mg/kg bw) in citrate buffer 0.1 M with ph 4.5 was injected intraperitoneally into overnight starved rats. After three days, blood samples were taken from the tail veins of fasting animals, at least for 12 hours. Tubes containing fluoride were used to hold blood samples. A clear serum was obtained after 10 minutes of centrifugation at 4000 rpm. After one week of STZ injection, glucose levels were calculated using the Glucose Oxidase Method, and weight was monitored on a regular basis until stable hyperglycemia was reached. The research only included animals with severe hyperglycemia (fasting blood glucose >250 mg/dL). All of the animals were maintained in the lab on a regular diet after the administration of a diabetes-inducing drug.

b) Study design:

Totally 48 experimental animals (wistar rats) randomly divided into eight groups consisting of six rats (n=6) per group were used in this study as detailed in following Table 1:

Table 1: Animals and dosage of drug in each group

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group No.</th>
<th>Treatments</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group-I</td>
<td>distilled water</td>
<td>1 ml (p.o)</td>
</tr>
<tr>
<td>2.</td>
<td>Group-II</td>
<td>distilled water</td>
<td>1 ml (p.o)</td>
</tr>
<tr>
<td>3.</td>
<td>Group-III</td>
<td>metformin</td>
<td>50 mg/kg, orally</td>
</tr>
<tr>
<td>4.</td>
<td>Group-IV</td>
<td>methanolic leaf extracts of <em>Inula cappa</em></td>
<td>100mg/kg (p.o)</td>
</tr>
<tr>
<td>5.</td>
<td>Group-V</td>
<td>methanolic leaf extracts of <em>Inula cappa</em></td>
<td>200mg/kg (p.o)</td>
</tr>
<tr>
<td>6.</td>
<td>Group-VI</td>
<td>methanolic leaf extracts of <em>Inula cappa</em></td>
<td>400mg/kg (p.o)</td>
</tr>
<tr>
<td>7.</td>
<td>Group-VII</td>
<td>Hydroalcholic leaf extracts of <em>Inula cappa</em></td>
<td>200mg/kg (p.o)</td>
</tr>
<tr>
<td>8.</td>
<td>Group-VIII</td>
<td>Hydroalcholic leaf extracts of <em>Inula cappa</em></td>
<td>400mg/kg (p.o)</td>
</tr>
</tbody>
</table>

Serum Collection: Blood was drawn from a tail vein at regular intervals throughout the study. The serum was separated from the other components by centrifugation at 4000 rpm for ten
minutes in a microcentrifuge. On the 0th, 10th, and 21st days of the experiment, glucose oxidase method readings were collected using an autoanalyzer to assess fasting blood glucose levels and estimation of various biochemical parameters including serum biomarkers and anti-oxidant parameters. The weights of all the animals included in the study were determined using a gravimetric scale.

**Preliminary phytochemical analysis of extracts:** The extract was assessed for various phytochemical elements such as alkaloids, saponins, reducing sugars, sterols, tannins, and terpenoids.

**Antioxidant assay:** Anti-oxidant parameters were determined including, total phenolic content, total flavonoid concentration, DPPH free radical scavenging activity, Hydroxyl radical scavenging activity, Scavenging of Hydrogen Peroxide, Superoxide radical scavenging assay, Nitric oxide scavenging activity and Reducing power assay. All estimations were carried out using UV spectrophotometer (Shimadzu, India) as per standard kit methods.

**Statistical Analysis:** An analysis of variance using a post hoc test Dunnett's t-test was used to analyse the mean and standard deviation (SEM) values of body weight, fasting blood sugar, and biochemical estimates. A p-value of 0.05 was considered significant in the comparison of the groups.

**RESULTS:**

**Preliminary phytochemical analysis of extracts:** Alkaloids, saponic acids, reducing sugars, steroids, terpenoids, carbohydrate, steroids, Carbohydrates, flavanoids, and tannins were found in methanolic and hydroalcoholic leaves extracts of *Inula cappa*.

**Effect of *I. cappa* Leaves Extracts on Oral Glucose Tolerance Test:** Methanolic (ICME) and hydroalcoholic (ICHE) extracts of *Inula cappa* leaves (100, 200, and 400 mg/kg, p.o.) pass the Oral Glucose Tolerance Test. Both extracts had a greater impact in lowering blood glucose levels. Overall, the ICHE extract 100, 200 and 400 mg/kg p.o., b.wt. showed the best results. In terms of glucose tolerance, ICME 100, 200, and 400 all had significant effects.

**Table 2: Effect of *Inula cappa* Leaves Extracts on Oral Glucose Tolerance Test**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood Glucose Level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>NormalControl</td>
<td>99.43±1.05</td>
</tr>
<tr>
<td>Metformine (50mg/kg)</td>
<td>96.72±2.32</td>
</tr>
<tr>
<td>ICME(100mg/kg)</td>
<td>95.05±2.12</td>
</tr>
</tbody>
</table>
Effect of *Inula cappa* leaves extracts on fasting blood glucose in STZ induced diabetic rats

The fasting blood glucose level basal values were almost the same and statistically no significant difference was observed while conducting the animal experimentation. Fasting blood glucose level of control group ranged from 85±2.14 to 92±2.19 mg/dL in 21 days of study, while there was a significant increase in fasting blood glucose level in STZ treated animals 246±2.4 mg/dL as compared to control group (Figure 2). Fasting blood glucose level was measured on the 7th, 14th, and 21st days. Different doses of ICME and ICHE (100, 200, and 400 mg/kg) were administered continuously for 21 days and significant reduction in the level of blood glucose was observed in the STZ treated diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>0 day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICME(200mg/kg)</td>
<td>96.72±1.04</td>
<td>117±2.01*</td>
<td>116.20±2.01*</td>
<td>105.44±2.21*</td>
<td></td>
</tr>
<tr>
<td>ICME(400mg/kg)</td>
<td>98.45±1.85</td>
<td>123.03±0.81*</td>
<td>113.28±1.56*</td>
<td>99.05±1.15*</td>
<td></td>
</tr>
<tr>
<td>ICHE(100mg/kg)</td>
<td>99.80±2.15</td>
<td>118.04±2.63*</td>
<td>113.54±0.95*</td>
<td>122.94±1.81*</td>
<td></td>
</tr>
<tr>
<td>ICHE (200mg/kg)</td>
<td>97.23±2.12</td>
<td>117.05±2.12*</td>
<td>115.20±2.15*</td>
<td>112.30±2.12*</td>
<td></td>
</tr>
<tr>
<td>ICHE (400mg/kg)</td>
<td>98.31±2.51</td>
<td>115.86±0.92*</td>
<td>106.24±1.20*</td>
<td>99.86±2.46*</td>
<td></td>
</tr>
</tbody>
</table>

All values expressed as mean±SEM. * = p < 0.001, when compared to control.

**Figure 2: Effect of *Inula cappa* Leaves Extracts on fasting blood glucose level**
Effect of ICME and ICHE extracts of *Inula cappa* leaves on fasting blood glucose level (mg/dL) in type-2 diabetic Wistar rats. Each group \((n=6)\) represents mean ± standard error of means. Data was analyzed by using Two way ANOVA followed by Tukey’s multiple test; a versus control, b versus Diabetic control, c versus Metformin treated, d versus 200 mg/kg of ICME and ICHE, e versus 400 mg/kg of ICME and ICHE. *\(P< 0.0001\), #\(P<0.001\), \(\varpi P<0.01\), \(\textsf{Ж}P<0.05\).

**Effect of *Inula cappa* leaves extracts on average body weight in STZ induced diabetic rat:**

Rats of the same weight range were used in the experiment. During the study, the body weight of control group was naturally increased, while the body weight was significantly found to be decreased in STZ-induced diabetic group when compared with the control group (Figure 2). After 21 days of continuous administration of metformin, a significant increase in body weight was seen as compared to diabetic control group. Oral administration of ICME and ICHE extracts to diabetic rats also increased significantly the body weight at the 14th day of intervention which thereafter reversed the STZ effect comparable to metformin treated animals. Moreover, on 14th and 21st days of treatment, no significant difference in body weight was seen between metformin, 100, 200, and 400 mg/kg of ICME and ICHE treated groups and the effect of ICME and ICHE administration produced dose-independent effect on body weight in diabetic rats.

**Fig 2:** ICME and ICHE extracts of *Inula cappa* leaves on body weight (gms) in type-2 diabetic Wistar rats. Each group \((n=6)\) represents mean ± standard error of means. Data was analyzed by using Two way ANOVA followed by Tukey’s multiple test; a versus control, b versus Diabetic control, c versus Metformin treated, d versus 200 mg/kg of ICME and ICHE, e versus 400 mg/kg of ICME and ICHE. *\(P< 0.0001\), #\(P<0.001\), \(\varpi P<0.01\), \(\textsf{Ж}P<0.05\)
Anti oxidant assay
Assays for free radical scavenging were conducted using a variety of in vitro methods. Extracts were tested for their ability to scavenge free radicals using the DPPH radical as a substrate. At a concentration of 2500 g/ml, *Inula cappa* methanolic extract scavenged 86.42 percent of the DPPH radical, while *Inula cappa* hydroalcoholic extract scavenged 89.15 percent. Extractions have a considerable impact in scavenging free radicals, according to these findings. Using *Inula cappa* leaves extract as a DPPH radical scavenger is shown in Figure 3.4 to significantly reduce DPPH radical levels. BHA, ascorbic acid, and α-tocopherol were used as benchmarks for the study.

**Free radical scavenging activity of ICME, ICHE**

![Graph showing free radical scavenging activity of ICME, ICHE, BHA, and α-tocopherol](image)

▲ Ascorbic acid, ■ ICME extract, ▲ ICHE extract, □ BHA and ■ α-tocopherol

**Fig.3.** Free radical scavenging activity of ICME, ICHE, BHA, Ascorbic acid, and α-tocopherol by 2,2-diphenyl-1-picryl hydrazyl radicals. Results are mean ± SD of three parallel measurements. (Concentrations of ICME and ICHE are expressed in mg/ml whereas concentrations of BHA, Ascorbic acid and α-tocopherol are expressed in 20μg/ml).

**Superoxide radical scavenging assay:** Using the PMS-NADH method, extracts were tested for their ability to neutralise superoxide anion radicals. Figure 3.5 compares extracts with ascorbic acid, BHA, and α-tocopherol in inhibiting superoxide radical formation by a certain percentage. It was discovered that ICME and ICHE had 75.24 and 79.71 percent suppression of superoxide production when used at a dose of 2500 g/ml. Superoxide radicals may be inhibited by ascorbic acid, BHA, and α-tocopherol at a dosage of 50 micrograms per litre.
Fig. 3.5 Comparison of percentage inhibition of Superoxide radicals generation by 50 μg/ml concentration of ascorbic acid, BHA, α-tocopherol and 2500 μg/ml of ICME and ICHE using PMS-NADH-NBT Method.

Hydrogen Peroxide scavenging assay: The capacity of H2O2 to enter cellular membranes is critical. Although H2O2 itself does not pose a threat to cells, the hydroxyl radicals it produces may. For this reason, removing H2O2 from food systems is critical. At 2500 /ml, ICME and ICHE are capable of removing H2O2 from the environment. This is compared to BHA and -tocopherol concentrations of 50 /ml. H2O2 scavenging activity was determined to be 74.23% for ICME, 80.37 for ICHE, 89.25 for BHA, and 94.54 for -tocopherol.

Fig. 3.6 Comparison of percentage inhibition of Hydrogen Peroxide by ICME, ICHE, BHA and α-Tocopherol
**Hydroxyl radical scavenging activity:** Lipid oxidation and significant cellular damage are mostly caused by hydroxyl radicals, the most active oxygen species [54]. As extract concentrations rose, so did the fraction of hydroxyl radical scavengers (Fig. 3.7). In vitro techniques were used to compare the antioxidant activity of extracts and reference compounds.

![Hydroxyl Radical Scavenging Activity](image)

**Fig. 3.7** Comparison of percentage inhibition of Hydroxyl radical by ICME, ICHE and BHA.

**Nitric Oxide scavenging activity:** Smooth muscle relaxation, neuronal signalling, platelet aggregation inhibition, and cell-mediated toxicity regulation are all mediated in part by nitric oxide (NO), a powerful pleiotropic mediator. As an effector molecule in a variety of biological systems, it serves as a diffusible free radical that is involved in neural communication, vasodilation, and antibacterial and anticancer activity [55]. Inhibition of nitric oxide synthesis by ICME and ICHE is seen in Fig. 3.8. As a standard, ascorbic acid was used.

![Nitric Oxide Scavenging Activity](image)

**Fig. 3.8** Comparison of percentage inhibition of Nitric Oxide scavenging activity by ICME, ICHE, BHA
Reducing power of ICME, ICHE: Fe$^{3+}$ to Fe$^{2+}$ transition in the presence of ICME and ICHE was discovered in the measurements of the reducing ability. The ability of a chemical to reduce oxidative stress may be a good predictor of its antioxidant capability. Extracts were compared to -Tocopherol in terms of their ability to reduce free radicals. ICME and ICHE's reducing abilities were observed to grow as concentrations increased. (Fig. 3.9).

![Graph showing absorbance vs concentration for ICME, ICHE, and α-tocopherol](image)

**Fig. 3.9.** Reducing power of ICME, ICHE and α-tocopherol. Results are mean ± SD of three parallel measurements. (Concentrations of ICME and ICHE are expressed in mg/ml whereas concentration α-Tocopherol is expressed in 20 xμg/ml)

**CONCLUSION**

Concluding from the above results it indicates that anti-diabetic activity was found in both methanolic and hydroalcoholic extracts of the leaves of Inula cappa. Because of these characteristics, traditional Indian healers utilise Inula cappa for treating diabetes in their patients. However, result of this investigation support the conventional wisdom that treating diabetic rats with methanol and hydroalcoholic extract may lower blood sugar levels. Based on in vitro testing, both extracts show significant antioxidant activity when compared to other well-known and well-characterized systems of antioxidant protection. These plants may also contain phenolic compounds that contribute to their antioxidant capabilities. However, the antioxidative activity of ICME and ICHE is still unknown due to the lack of clarity on the components responsible for this activity. The antioxidant components of Inula cappa should thus be isolated and identified in further detail.

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CONFLICT OF INTEREST: The authors declare that there are no conflicts of interest.

REFERENCES:


