A comparative study: Homoeopathic medicine and a medicinal plant *Withania somnifera* for antidiabetic activity

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ABSTRACT

Homeopathy has a distinct air of science involves books of rules, complex procedures, a proprietary lingo. Secondly, the method of carefully listening to the patient, meticulously recording every symptom experienced, lots of details from the patient’s background, is bound to make practitioners feel important and a lot of patients feel good. Homoeopathy is widely used, but specific effects of homoeopathic remedies seem implausible. In the present work pre-clinical trials have been performed to show that the Homeopathic Formulations have some Pharmacological effect. In the present work, ethanol extract of *Withania somnifera* shows significantly positive antidiabetic activity on rats when compared with Glibenclamide standard antidiabetic drug. Antidiabetic effect is thought to be due to increased hepatic metabolism, increased insulin release from pancreatic beta cells and/or insulin sparing effect. Homeopathic formulation of Potency Q, 30 and 200 shows significant pharmacological effect in animals (Rats) and shows the Pre-clinical effects. It shows Homeopathic formulations is not mere Placebo but have some Pharmacological/Therapeutic effect.

Keywords: Antidiabetic, Homoeopathy, *Withania Somnifera*

1. Introduction

Homeopathy is a form of alternative medicine, first proposed by German physician Dr. Samuel Hahnemann in 1976, in which practitioners use highly diluted preparations. Homeopathic remedies are prepared by serial dilution with shaking by forceful striking, which Homeopaths term *Succession*, after each dilution under the assumption that this increases the effect [1]. Homeopaths call this process *Potentialisation*, dilution often continues until none of the original substance remains. Apart from the symptoms, homeopaths use aspects of the patients physical and psychological state in recommending remedies [2]. Homeopathic reference books known as repertories are then consulted, and a remedy is selected is based on the totality of symptoms. Homeopathic remedies are with rare exceptions; considered safe through homeopathy has been criticized for putting patients at risk due to active against conventional medicine such as vaccinations and antimalarial drugs and antibiotics [3-4].

Depending on the dilution, Homeopathic remedies may not contain any pharmacologically active molecules and for such remedies to have Pharmacological effect would violet fundamental principles of sciences.
Modern homeopathists have proposed that water has a memory that allows homeopathic formulations to work without any of the original substance; however, there are neither verified observations nor scientifically plausible physical mechanisms for such a phenomenon [8].

The regulation and prevalence of homeopathy is highly variable from country to country. There are no specific legal regulations concerning its use in some countries. While in other licenses or degrees in conventional medicine from accredited universities are required. In several countries homeopathy is covered by the national insurance coverage to different extents, while in some it is fully integrated into the national health care system. In many countries, the laws that govern the regulation and testing the conventional drugs do not apply to homeopathic remedies [9].

1.1 Homeopathic remedies
Remedy is a technical term in homeopathy that refers to a substance to a particular procedure and intended for treating patients, it is not to be confused with the generally-accepted use of the word, which means “a medicine or therapy that cures disease or relieves pain”. A homeopathy materia medica is a collection of “drug pictures” organized alphabetically by remedy that describes the symptom patterns associated with individual remedies. Some modern homeopathists have considered more esoteric basis for remedies known as imponderable because they do not originate from a material but from electromagnetic energy presumed to have been “captured by alcohol or lactose example include X-rays and sunlight, recent ventures by homeopaths into even more esoteric substances include thunderstorms (prepared from collected rain water) today there are about 3000 different remedies commonly used in homeopathy. The principle of similar (or “Like cures like”) is a central homeopathic principle. The principle states that a disease can be cured by a substance that produces similar symptoms in a healthy person. This idea which can be traced back to Hippocrates, was further developed by Hahnemann after he repeatedly ingested Cinchona bark, a popular treatment for Malaria, and found that he developed the symptoms of the disease, Hahnemann theorized that if a substance could cause disease symptoms in a healthy person, small amounts could cure a sick person who had similar symptoms. The principle of Dilution (or Law of minimum doses) states that, “the lower the dose of medication, the greater its effectiveness”. Homeopathic formulations are so diluted that no molecules of the healing substance remain. However in homeopathy, it is believed that substance has left its imprint or essence which stimulates the body to heal itself (This theory is called the “Memory of water”) [10-11].

2. Materials and Methods

2.1 Plant material and Homeopathic medicines
Leaves of plant Withania somnifera Linn were collected in month of July, from the campus of SGVU (Gyan Vihar University Jaipur), Rajasthan. Withania somnifera was authenticated and confirmed by Botany Department, Rajasthan University Campus Jaipur. A voucher specimen has No- RUBL20872 is deposited in the department of Botany, Rajasthan University, Jaipur (Rajasthan).

2.2 Extraction
2.2.1 Ethanol Soluble Extractive
The leaves (5 gm) were shade dried and coarsely powdered, taken in a stoppered conical flask and matured with 100 ml. of ethanol (90%) for 24 hours. It was shaken frequently during the first 6 hours and allowed to stand for 18 hours. There after it was filtered rapidly taking precaution against loss of ethanol and then 25 ml of the filtrate was evaporated in tared flat bottom shallow dish, dried at 105 °C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the shade dried drug [12-13].

2.2.2 Preliminary Phytochemical Screening
In order to determine the presence of phytocomponents, a preliminary phytochemical study (colour reaction) with MEAV was performed. It was identified that Withania somnifera contain Alkaloids, Phytosterols and Tannins, Flavonoids and Saponins. Thin layer chromatographic investigations of Ethanolic extract revealed the presence of alkaloids and Phytosterols.

2.3 Animals
Throughout the experiment, experimental rats were processed in accordance experiments on animals (CPCSEA). Albino wistar male rats weighing 150-200 gm was used for the present study. They were maintained in the animal house for experimental purpose. The animals were maintained under controlled conditions of temperature (23±2 °C), humidity (50±5 %) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. We selected male animals for all our studies, since females are shown to be protected from changes in lipid–induced insulin activity.

2.3.1 Pain threshold: The evaluation of pain threshold was done to evaluate sensory functions. The hot plate test was carried out. Animals were placed on the hot plate maintained at 55±1 °C and the reaction time was recorded as response latency. The response latencies were measured before treatment and after treatment. The cut off time for hot plate latency was set at 10 seconds.

2.3.2 Biochemical Estimations: The blood samples were collected from rat-tail vein on day before injecting the alloxan and on subsequent 7and 21 days after injection of alloxan for estimation of blood glucose level [14-16]. The blood glucose estimation was done weekly after administration of test compounds, with the help of glucometer.

2.4 Induction of Diabetes
Healthy Wistar strain albino rats of either sex weighing about 150-200 grams were taken. Animals were deprived to food for 16 hours but allowed free access to water after that blood sample was collected from tail of rats and measure blood glucose level by using digital display glucometer. Then they were injected with alloxan dissolved in 0.1 M sodium citrate and citric acid at a dose of 60 mg/kg body weight intraperitoneally then animals were kept for 21 days during which food and water was allowed. After 7 and 21 days of alloxan administration blood glucose level, and pain sensation measurements were taken. The animals showed fasting blood glucose level above 250 mg/dl considered diabetic after that they were divided into seven groups in which each group contain six animals. After that the administered control, standard and test drug
oraly. The blood glucose level and pain sensation measurements of each rat were taken. Weekly after oral administration of drug/extracts. The mean values of these parameters were taken for each group and compared with the value of standard drug [17-18].

2.4.1 Experimental Design
Treatments were selected on the basis of survey of literature, hence dose of 1 ml/rat of OF N1 to N4 was selected for the screening of antidiabetic activity.
- N1-Lab extract
- N2-Homeopathic Formulation(HF) Q Potency
- N3-HF 30 Potency
- N4-HF 200 Potency

Hypoglycemic activity of N1 to N4 was studied both in normal and alloxan induced diabetic rats. Thirty six albino rats weighing 150-200 g were fasted for 18h and were divided into six groups of six animals in each. The groups included:

1) GROUP I (Vehicle): Diabetic rats received 5% gum acacia in normal saline (1ml/200g b.w.)
2) GROUP II (N 1): Diabetic rats received alcoholic extract of leaves (1 ml/rat b.w. i.p.)
3) GROUP III (N 2): Received 1 ml/rat p.o.
4) GROUP IV (N 3): Received 1 ml/rat p.o.
5) GROUP V (N 4): Received 1 ml/rat p.o.
6) GROUP IV (Standard): Received Glibenclamide (0.5 mg/kg p.o. 10% w/v, 1 ml/200 g rat).

2.4.2 Methods
One milliliter of blood from the tail of each rat was collected at ‘0’ day. Than at 2nd day, 5th day and 10th day subsequently, blood samples were collected again from the treated animals and blood glucose was estimated by digital display glucometer.

2.4.3 Statistical Analysis
Results are expressed as mean ± SD. The differences between experimental groups were compared by one-way Analysis of Variance (ANOVA) followed by Bonferroni’s test. The results were considered statistically significant when P<0.05.

<table>
<thead>
<tr>
<th>Treatment and Dose (mg/kg p. o)</th>
<th>Serum glucose level (mg/dl)</th>
<th>0th day</th>
<th>2nd day</th>
<th>5th day</th>
<th>10th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>266.48±3.73</td>
<td>269.16±3.15</td>
<td>265.33±3.76</td>
<td>268.29±2.41</td>
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<tr>
<td>Reference Group* (Glibenclamide)</td>
<td>263.06±3.58</td>
<td>224.00±2.91</td>
<td>210.76±4.59</td>
<td>182.66±1.72</td>
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<tr>
<td>N1 *</td>
<td>269.73±3.34</td>
<td>244.22±3.10</td>
<td>213.17±1.25</td>
<td>192.36±4.45</td>
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</tr>
<tr>
<td>N2*</td>
<td>276.55±3.68</td>
<td>240.70±3.22</td>
<td>224.58±2.14</td>
<td>198.33±1.68</td>
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<tr>
<td>N3 *</td>
<td>269.44±2.90</td>
<td>241.53±1.26</td>
<td>220.66±4.58</td>
<td>201.11±4.28</td>
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<tr>
<td>N4 *</td>
<td>271.17±2.32</td>
<td>255.22±2.22</td>
<td>232.41±2.28</td>
<td>212.32±3.64</td>
<td></td>
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</tbody>
</table>

** Extremely significant (P<0.01), * Significant (p<0.05), ns- Not significant (P> 0.05)

3. Result
The work was carried out on the leaves of *Withania somnifera*. Pharmacognostic investigation emphasized on authentication of plant and then macroscopic and microscopic analysis of leaves of *Withania somnifera*. Macroscopic observation shows that the leaf is Greenish grey in color with not distinct odour and taste. The shape of *Withania somnifera* leaves is elliptical at margin, and length and width varying from 1.2-2 cm and 0.5-1 cm respectively. Microscopic analysis revealed the presence of unicellular stomata and anomocytic stomata. The stomatal index 10.12, vein islet number 8, vein termination 5, and palisade ratio is 9.7. Phytochemical investigation was focused on preparation of Aqueous and Ethanolic extract of leaves of *Withania somnifera*. Both extracts and *Withania somnifera* powder were studied to identify the presence of specific phytoconstituents in them. It was identified that *Withania somnifera* contain Alkaloids, Phytosterols, Tannis, Flavonoids and Saponins. Thin layer chromatographic investigations of Ethanolic extract revealed the presence of alkaloids and Phytosterols. Ethanol extract and Homeopathic formulation of different potency Q, 30 in Streptozotocin induced diabetes model also.

4. Conclusion
Homeopathy is a form of alternative medicine in which highly diluted preparations are used. Homeopathic remedies are prepared by serial dilution with shaking by forceful striking, which homeopaths term sucussion, after each dilution under the assumption that this increases the effect. Homeopaths call this process potentization. Dilution often continues until none of the original substance remains. As none of the original substance remains in the Formulation, these are considered as Placebo. Various Pre-Clinical trials have been performed to show that the Homeopathic Formulations have some Pharmacological effect. In the present work *Withania somnifera* shows significantly positive antidiabetic activity when compared with Glibenclamide and Glimepiride as standard antidiabetic drug. Antidiabetic effect is thought to be due to increased hepatic metabolism, increased insulin release from pancreatic beta cells and/or insulin sparing effect. It can be concluded from the present research that Homeopathic formulation of Potency Q, 30 and 200 shows significant pharmacological effect in animals (Rats) and shows the Pre-clinical effects. From this we conclude that the “Homeopathic formulations are not mere placebo but have some Pharmacological/Therapeutic effect.”
4.1 Future prospects
As LANCET challenge the Homeopathic formulations Placebo, more Pre-clinical and clinical study can be done to show the Pharmacological/Therapeutic effect of Homeopathic formulations. More toxicity study can be done to calculate the LD₅₀ and ED₅₀ of homeopathic formulations. More study can be done to find the Dose Relationship and to find the Mechanism of Action of Homeopathic formulations.

5. Reference: