IN VITRO AND IN VIVO STUDIES ON THE HYPOGLYCAEMIC POTENTIAL OF ASHWAGANDHA (*WITHANIA SOMNIFERA*) ROOT

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**ABSTRACT**

Hypoglycaemic effects of the herb ashwagandha, *Withania somnifera*, using *in vitro* assays and its potential in preventing stress induced hyperglycaemia in rat (*in vivo*) were studied. *W. somnifera* roots were subjected to extraction using different solvents with increasing polarity. All the extracts were tested for hypoglycaemic effect using *in vitro* methods viz., glucose uptake by yeast cells, α-amylase inhibitory activity, glucose adsorption and diffusion. Chloroform and ethanolic extracts were more effective in increasing glucose uptake by yeast cells, inhibition of α-amylase activity, increasing glucose adsorption and decreasing diffusion compared to other extracts in *in vitro* study. *In vivo* hypoglycaemic action was investigated by treating adult male rats with different doses (5, 10, 20 mg/kg body weight) of ethanol and chloroform extracts prior to exposing them to a stress regime consisting of restraint (1h) followed by forced swimming exercise (15 minutes) after a gap of 4h. Chloroform and ethanolic extracts were potent in attenuating stress induced hyperglycaemia with a minimum effective dose of 10 mg/kg body weight. The study reveals that *W. somnifera* utilise different mechanisms to exert hypoglycaemic effect and has phytocomponent which can prevent stress induced hyperglycaemia. Hence phytocomponent present in these extracts may be a potential anti stress drug.

**KEYWORDS:** α-amylase, hypoglycaemia, forced swimming, glucose uptake, restraint, *Withania somnifera*.

**INTRODUCTION**

More than 400 plants worldwide have been documented as beneficial in the treatment of diabetes [1]. The majority of traditional anti diabetic plants await proper scientific and medical evaluation for their ability to improve glycemic status [2]. One such plant which is known to possess various biological properties is *Withania somnifera* (WS) Dunal (Family, Solanaceae), known as ashwagandha in ayurveda or Indian ginseng [3]. A number of earlier investigations have revealed multiple properties of WS such as antioxidant, adaptogenic, aphrodisiac, astringent and antiulcer [4, 5], anti-inflammatory [6], antitumor and radiosensitizing [7], and cyclophosphamide toxicity suppressor [8]. There is also report on the attenuation of stress induced glucose intolerance by ashwagandha in rats [9]. Hypoglycaemic effects of ashwagandha in NIDDM patients [10] and alloxan induced diabetic rats [11] have been reported. However, it is not
known whether ashwagandha exerts hypoglycaemic effect by enhancing glucose uptake by cells, inhibition of glucose diffusion and adsorption of glucose in GI tract and inhibition of α-amylase activity. The present study tests these actions using *in vitro* methods.

Stress results in the activation of HPA axis resulting in the increased secretion of glucocorticoids \([^{12}\] which enhances gluconeogenesis leading to hyperglycaemia. Many earlier studies have reported hyperglycaemia in experimental animals following exposure to different kinds of stressors for instance, restraint for 1 hour \([^{13}\] or 2.5 hours \([^{14}\]; random foot shock \([^{9}\], hypokinesia \([^{14}\] and water immersion \([^{15}\] in rats, snare restraint for 5 minutes in pigs \([^{16}\] and water spray bath for 15 minutes in cats \([^{17}\). Similarly water immersion stress resulted in hyperglycaemia in humans \([^{18}\). Hyperglycaemia for a prolonged period leads to insulin resistance \([^{19, 20}\; which is a major causative factor of metabolic syndrome and type 2 diabetes. Hence there is a need to prevent stress induced hyperglycaemia. Therefore in the present study we have investigated the efficacy of the most potent extract of ashwagandha, as revealed by *in vitro* studies, in attenuating the stress induced hyperglycaemia. The stress protocol used in the present study was standardized in our laboratory which induces hyperglycaemia for a prolonged period (8 hours) \([^{21}\). Hence the objective of the work was to demonstrate the various mechanisms of hypoglycaemic action of ashwagandha and to investigate whether or not the most potent extract can alleviate the acute stress induced hyperglycaemia.

**MATERIALS AND METHODS**

**Experimental animals:** Male Wistar rats weighing 200-230g were used for the study. The rats were kept in clean and dry cages containing bedding material and were maintained under 12 h light- 12 h dark cycle. The animals were provided standard rat chow and water ad *libitum*. Animal maintenance, handling, care and treatment were according to guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA). The experimental protocols were approved by the Institutional animal ethics committee, Central animal facility, University of Mysore, Mysore-06. The certification number of approval is MGZ/3772/2008-09 dated 18.03.2009.

**Plant and preparation of plant extracts:** *W. somnifera* (WS) were collected from Mysore Ayurvedic medical college park, Mysore, Karnataka and were authenticated by a Botanist. The herbarium number is 2063, Department of Botany, University of Mysore, Mysore, India. The roots of WS were shade dried and a coarse powder was prepared. The powder was subjected to successive extractions using solvents with increasing polarity viz. petroleum ether, benzene, chloroform and ethanol. Each extract was concentrated by distilling off the solvent in flash.
evaporator and dried in desiccator. The residue obtained after soxhlet extraction was dissolved in cold water and kept in refrigerator at 4º C overnight. It was filtered and dried in oven. Hot water extract was prepared by dissolving the residue in water and boiled at 80º C for 24 hours. It was filtered and dried in oven. The residue was dissolved in 0.2 N sodium hydroxide (NaOH) and kept overnight and then filtered. It was dried in oven. The aqueous crude extract was prepared by boiling 100 g of root powder with 1600 ml of distilled water and it is condensed to 1/16th of its original volume. It was filtered and evaporated to dryness. The concentrated extract was dissolved in rectified spirit.

**In vitro assays:**

**Glucose uptake/transport by yeast cells**

Yeast cell suspension (1%) was prepared in distilled water and kept overnight at room temperature. Different concentrations of WS extracts (0.3, 0.6, 0.9, 1.2 and 1.5mg) were individually added to 1ml of 25mM glucose solution and incubated for 10 minutes at 37°C. After incubation, reaction was initiated by adding 100μL of yeast suspension to each tube, vortexed and further incubated at 37°C for 60 minutes. After incubation, glucose left in each tube was estimated by dinitro salicylic acid method (DNS). A control tube containing all the reagents except plant extract was simultaneously prepared. The percentage increase in the glucose uptake/transport (activity) by yeast cells was calculated using the formula:

\[
\% \text{ activity} = \left( \frac{\text{Abs. control} - \text{Abs. of sample}}{\text{Abs. of control}} \right) \times 100
\]

**Glucose adsorption**

Increasing concentrations of glucose (10, 20, 50 and 100 mM L⁻¹) were taken in different test tubes and 0.6mg extract of WS was added to each tube and the mixture was stirred well, incubated in shaker bath for 37°C for 30 minutes and glucose content was determined using DNS method. Only the free glucose will react with DNS. Hence glucose bound was calculated using the formula:

\[
\text{Glucose} = \frac{\text{Glucose concentration in original solution} - \text{Glucose concentration after 30 minutes incubation}}{\text{Vol. of solution}} \times \text{Vol. of solution} \times \text{Weight of the sample}
\]

**α- amylase inhibition:** The α- amylase inhibitory activity of each extract was determined by modifying the method described by Nickavar. Salivary amylase was used for the study.
Saliva was diluted 1:50. Different concentrations of WS extracts (0.3, 0.6, 0.9, 1.2 and 1.5 mg/ml) were individually added to test tubes containing 1 ml of enzyme solution and incubated at 37°C for 30 minutes. To this, 1 ml of 1% starch solution in 6.7 mM sodium chloride was added and incubated at 25°C for 10 minutes. After incubation, 0.5 ml of DNS reagent was added and kept in boiling water bath, cooled and 2.5 ml of water was added to each tube and OD was read at 540nm. Controls were incubated without plant extract. A blank tube was also prepared using distilled water for correcting the background absorbance. The inhibition percentage of α-amylase (Iα-amylase %) was assessed using the formula,

\[ I_{\alpha\text{-amylase}}\% = 100 \times \left( \Delta A_{\text{control}} - \Delta A_{\text{sample}} \right) / \Delta A_{\text{control}} \]

Whereas, \( \Delta A_{\text{control}} = A_{\text{test}} - A_{\text{blank}} \)

\( \Delta A_{\text{sample}} = A_{\text{test}} - A_{\text{blank}} \)

**Glucose diffusion** [25]: The method used in the present experiment consisted of a dialysis bag into which 1 ml of 50 g/L plant extract in suitable solvent and 1ml of 0.22 M glucose in 0.15 M sodium chloride were added. The dialysis bag was sealed at both the ends and placed in a beaker containing 45 ml of 0.15 M sodium chloride. The beaker was placed on the orbital shaker and kept at 37°C temperature. The movement of glucose into the external solution was monitored at regular time intervals by estimating glucose concentration in the external medium by DNS method.

**In vivo hypoglycaemic action of plant extracts:** This experiment was conducted to test whether chloroform and ethanolic extracts of WS exert inhibitory action on acute stress induced hyperglycaemia. Adult male rats were randomly segregated into 8 groups (n=5 rats / group). The rats in first group served as controls and those in second group were exposed to restraint for 1 hour and after 4 hours interval, they were forced to swim for 15 minutes. Procedure of Grissom [26] was followed for exposing rats to restraint and forced swimming exercise. Third, 4th and 5th group of rats were administered with 5, 10 and 20 mg/ kg body weight chloroform extract respectively whereas those in 6th, 7th and 8th group received same doses (5, 10 and 20mg/kg body weight) of ethanolic extract of WS prior to stress regime similar to rats in group 2. The rats in all the groups were fasted overnight before the commencement of the experiment. The blood samples were collected before commencement of experiment for estimation of fasting blood glucose levels. The blood samples were collected 2 hours after restraint and 4 hours after forced swimming exercise in stressed, stress + plant extract treated and control rats. The blood samples were collected by tail snipping. The blood glucose levels were estimated by GOD- POD method.
**Statistical analysis:** Each parameter was expressed as mean ± SEM. The mean values of different groups were compared by one way analysis of variance (ANOVA) followed by Duncan’s multiple test and judged significant if $p < 0.05$.

**RESULTS**

**In vitro assays:**

**Glucose uptake/ transport by yeast cells**

Consistent changes in glucose transport in yeast cells were observed with chloroform, alcohol, cold and aqueous crude extracts and there was a significant increase in the glucose uptake by the yeast cells with chloroform and ethanolic extracts at all concentrations compared to other extracts (Table I). Minimum effective concentration to cause the maximum glucose uptake for chloroform as well as ethanolic extract was 0.9mg/ml.

Table I: Effect of different solvent extracts and crude aqueous extract of ashwagandha on *in vitro* glucose uptake/ transport in yeast cells.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>% increase in glucose uptake by yeast cells at different concentration (mg/ml) of plant extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3mg</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>11.2 ± 3.35$^a$</td>
</tr>
<tr>
<td>Chloroform</td>
<td>26.58 ± 8.56$^b$</td>
</tr>
<tr>
<td>Alcohol</td>
<td>33.56 ± 5.89$^b$</td>
</tr>
<tr>
<td>Cold water</td>
<td>39.30 ± 2.86$^b$</td>
</tr>
<tr>
<td>Hot water</td>
<td>8.39 ± 1.96$^a$</td>
</tr>
<tr>
<td>NaOH</td>
<td>10.54 ± 1.33$^a$</td>
</tr>
<tr>
<td>Aq. Crude</td>
<td>28.93 ± 4.21$^b$</td>
</tr>
<tr>
<td>F value</td>
<td>7.08</td>
</tr>
<tr>
<td>df-7,4</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

- All values are mean of five assays ± SEM; n=5
- Values with same superscript letters are not significantly different whereas, those with different superscript letters are significantly (P < 0.01) different as judged by Duncan’s multiple test.
In vitro glucose adsorption

The chloroform and ethanolic extract showed significantly higher adsorption at all concentrations of the glucose tested. Maximum adsorption was found with 10mM glucose concentration and the adsorption decreased with increase in glucose concentration added to the medium (Table II). Other extracts had poor adsorbing capacity (Table II).

Table II: Effect of different solvent extracts and crude aqueous extract of ashwagandha on in vitro glucose adsorption at different glucose concentrations

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Bound glucose concentration (mM)</th>
<th>10</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td></td>
<td>1.11 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.46 ± 1.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzene</td>
<td></td>
<td>1.24 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.38 ± 0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.04 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chloroform</td>
<td></td>
<td>3.91 ± 1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.03 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.60 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.19 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td>3.04 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.06 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.81 ± 0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.57 ± 1.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cold water</td>
<td></td>
<td>1.11 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.51 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.03 ± 0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.99 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hot water</td>
<td></td>
<td>1.14 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.85 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.05 ± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NaOH</td>
<td></td>
<td>1.23 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.17 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.64 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.85 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aqueous Crude</td>
<td></td>
<td>1.03 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.39 ± 0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.53 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.74 ± 1.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-value

<table>
<thead>
<tr>
<th>df 7, 4</th>
<th>4.19</th>
<th>12.82</th>
<th>9.48</th>
<th>7.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

- All values are mean of five assays ± SEM; n=5
- Values with same superscript letters are not significantly different whereas, those with different superscript letters are significantly (P < 0.01) different as judged by Duncan’s multiple test.

Glucose diffusion

The petroleum ether and cold water extracts significantly inhibited the diffusion of glucose across the dialysis membrane whereas the other extracts were not so effective in inhibiting the glucose diffusion (Table III).

α- Amylase inhibitory activity

The hot water, cold water, NaOH and aqueous crude extracts did not show α- amylase inhibitory activity. Chloroform showed the significant α- amylase inhibitory activity at all concentrations compared to petroleum ether, benzene and ethanol extracts. Ethanol showed significant activity at 0.9mg concentration whereas petroleum ether and benzene showed significant inhibitory activity only at 0.3mg and 0.6mg concentrations respectively (Table IV).
Table III: Effect of different solvent extracts (50g/L, each) of ashwagandha on movement of glucose out of dialysis bag over 5 h incubation period.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Glucose concentration (mM) transported out of dialysis bag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Control (in the absence of extract)</td>
<td>72.04 ± 0.73(^a)</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>56.36 ± 9.08(^b)</td>
</tr>
<tr>
<td>Benzene</td>
<td>61.42 ± 3.23(^c)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>54.18 ± 3.52(^d)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>71.75 ± 2.39(^e)</td>
</tr>
<tr>
<td>Cold water</td>
<td>61.53 ± 1.40(^f)</td>
</tr>
<tr>
<td>Hot water</td>
<td>60.18 ± 0.69(^g)</td>
</tr>
<tr>
<td>NaOH</td>
<td>69.31 ± 1.25(^h)</td>
</tr>
<tr>
<td>Aq. Crude</td>
<td>70.62 ± 1.20(^i)</td>
</tr>
<tr>
<td>F-value</td>
<td>8.34 (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

- All values are mean of five assays ± SEM; n=5
- Values with same superscript letters are not significantly different whereas, those with different superscript letters are significantly (P < 0.01) different as judged by Duncan’s multiple test.

Table IV: Effect of different solvent extracts and crude aqueous extract of WS on *in vitro* α-amylase activity

<table>
<thead>
<tr>
<th>Extract</th>
<th>% inhibitory activity at different concentration (mg/ml) of plant extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3mg</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>23.41 ± 1.37(^a)</td>
</tr>
<tr>
<td>Benzene</td>
<td>13.01 ± 0.55(^b)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>28.14 ± 2.79(^a)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>13.09 ± 1.15(^b)</td>
</tr>
<tr>
<td>F-value</td>
<td>20.49 (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

- All values are mean of five assays ± SEM; n=5
- Values with same superscript letters are not significantly different whereas, those with different superscript letters are significantly (P < 0.01) different as judged by Duncan’s multiple test.
In vivo hypoglycaemic activity

The fasting blood glucose concentration among different groups prior to commencement of experiments did not significantly differ. There was a significant increase in blood glucose levels after restraint and further significant increase after forced swimming exercise in stress group rats (Figure 1). The stressed rats treated with 5, 10 and 20mg/kg body weight (bw) ethanolic extract showed significant decrease in blood glucose concentration compared to stress group rats and did not significantly differ from controls after RS or FS (Figure 1). The suppression of hyperglycaemia was similar in 10 or 20mg/kg bw treated rats whereas it was lower in 5mg/kg bw treated rats after FS. A similar pattern i.e. suppression of stress induced hyperglycaemia was observed in chloroform extract treated stressed rats either after RS or FS compared to controls excepting that 5mg/kg bw chloroform extract treated rats did not result in suppression of hyperglycaemia after FS (Figure 1).

DISCUSSION

Many plants have been documented in the Indian folk medicine (Ayurveda) as anti-hyperglycaemic or anti-diabetic [1, 27]. Further many mechanisms have been proposed to illustrate the anti-hyperglycaemic action of herbs viz. glucose uptake/transport, glucose adsorption and diffusion [28, 29], inhibition of α-amylase [30], and glucosidase activity [31], insulin secretion [32, 33].
and proliferation of β-cells of islets. In the present study mechanism of hypoglycaemic effect of *Withania somnifera* (WS) commonly known as ashwagandha or winter cherry are elucidated using some of the *in vitro* methods.

The mechanism of glucose transport across yeast cell membrane is very complex and is mediated by several membrane carriers. In the present study, an increase in glucose uptake by yeast cells in the presence of WS plant extract indicated the hypoglycaemic effect of WS. The maximum uptake was found with chloroform and ethanolic extracts. Similarly other plants *Ficus racemosa* and *Butea monosperma* have been shown to enhance the glucose uptake by yeast cells. However these studies have utilized high concentration of crude extracts whereas the present study shows that lower concentration is effective.

Another mechanism to reduce blood glucose would be to prevent or reduce absorption from the intestinal lumen by adsorbing the glucose molecules. The chloroform or alcoholic extracts of WS was effective in adsorbing glucose and thereby reducing the glucose concentration in the medium. Glucose adsorption was relatively higher in chloroform and ethanolic extracts compared to all other extracts in the present study. There was 78% adsorption in chloroform and ethanolic extracts at 10mM glucose concentration and the percentage adsorption decreased as the concentration of glucose increased suggesting that the plant was effective only at lower concentration of glucose. The adsorption capacity might be due to high concentration of fibre in the extract. Hence it is suggested that these extracts could help to retain the glucose in the intestinal lumen and thus reduce increase in postprandial blood glucose concentration. Similar observations were also reported with other plants viz. *Averrhoa carambola* and *Ficus racemosa*. In addition to these, in the present study the different solvent extracts of ashwagandha were effective in reducing glucose diffusion across the dialysis membrane. However petroleum ether and cold water extracts were more potent. This retardation might be due to entrapment of glucose molecules within the network formed by fibres as suggested by Gallagher. Hence WS can also reduce the glucose absorption in the GI tract. Similar observations were also reported using plants viz. *Agrimonia eupatoria*, *Persea Americana*, *Agaricus campestris*, *Coriandrum sativum*, *Eucalyptus globules*, *Juniperus communis*, *Medicago sativa*, *Viscum album*, *Urtica dioica*, *Sambucus nigra*.

Another possibility to reduce postprandial hyperglycaemia is to retard carbohydrate digestion by inhibiting the activity of enzyme so that high concentration of glucose in circulating blood could be prevented. In the present study, chloroform extract showed high percentage of α-amylase inhibition (50%) at a concentration of 1.2 mg/ml compared to petroleum ether, benzene.
and alcohol extracts. However cold water, hot water, NaOH and aqueous crude extracts did not show α-amylase inhibitory activity. Similarly other anti diabetic plants viz. Allium akaka, Allium ampeloprasum, Allium sativum and Allium cepa[^37], are also known to inhibit α-amylase activity. This property of extract may be utilized to reduce postprandial hyperglycaemia by inhibiting/retarding initial carbohydrate digestion. The present study clearly demonstrated that WS act via different routes viz. suppressing α-amylase activity, reducing glucose diffusion, adsorption of glucose and increasing the glucose uptake by yeast cells. Of the 8 different extracts of ashwagandha tested for their in vitro hypoglycaemic effect, chloroform and ethanol extracts were more promising. Hence these 2 extracts may be subjected to further fractionation and isolation of compound(s) that may be helpful in treating diabetes.

Stress is known to induce hyperglycaemia by altering the regulation of carbohydrate metabolism[^38]. Various stressors viz. Restraint[^13, 14], forced swimming[^15] etc. are known to induce elevation in blood glucose levels. Earlier workers have used different stressors for different durations ranging from few days to several weeks and found hyperglycaemia in rats[^13, 38]. In the present study, a stress regime which induces hyperglycaemia for nearly 4h was used[^21]. In the present study, 2h after restraint there was a significant elevation in blood glucose levels which was further significantly increased 4h after forced swimming exercise in stressed rats compared to controls. This model, which develops hyperglycaemia due to repeated stress experiences, mimics the human life situations i.e. a person is subjected to acute stressors several times in a day due to life situations. Oral administration of ethanolic and chloroform extracts prevented stress induced elevation in the blood glucose levels in dose dependent manner. A dose of 10 mg/kg body weight was minimum effective dose. It is interesting to note that both chloroform and ethanol extracts were effective in preventing hyperglycaemia after the exposure to the second stressor (forced swimming) which was applied 5 hours after administration of plant extracts thereby indicating that ashwagandha effects prevail for a long period.

It is reported that the hyperglycaemia induced by stress is mainly contributed by endogenous mobilization of glucose (glycogenolysis/gluconeogenesis) mediated by stress related hormones especially glucocorticoids[^39]. It is known that hypothalamo-pituitary-adrenal axis activation under stress results in increased glucocorticoid secretion[^40, 41], and glucocorticoids induce hyperglycaemia. The present experimental results provide a clue that ashwagandha can suppress and maintain normoglycaemia despite exposure to stress. The present study combining in vitro and in vivo methods demonstrated a remarkable property of chloroform and alcoholic extracts of ashwagandha as it is shown that ashwagandha prevents either postprandial or stress
induced hyperglycaemia by utilizing different mechanisms. Hence ashwagandha proves to be an ideal herb for preventing stress related metabolic disorders and it has a potential to be used as a preventive drug in anticipation of stressful conditions.

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