Appendix
LIST OF PUBLICATIONS


ANTIHYPERTHYMIC ACTIVITY OF ANOGEISSUS LATIFOLIA IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present investigations explores the antihyperglycemic potential of Anogeissus latifolia bark and leaf extracts by using solvents viz. petroleum ether, chloroform and methanol in streptozotocin induced diabetic albino rats. Streptozotocin was administered intraperitoneally at a dose of 65 mg/kg body weight. The administration of oral dose at 200 mg/kg body weight and 300 mg/kg body weight of methanolic bark and leaf extracts, respectively produced significant lowering of blood glucose level in streptozotocin induced diabetic rats. Hypoglycemic effects were compared with control. The results of present study revealed that the plant leaf and bark extracts of A. latifolia possess potent antihyperglycemic activity.

Key words: Anogeissus latifolia, Antihyperglycemic, Streptozotocin, Induced diabetes.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosuria and disturbances in carbohydrate, protein and lipid metabolism exhibiting complications like retinopathy, microangiopathy and nephropathy\textsuperscript{1,2}. Diabetes mellitus along with its associated complications has become a growing problem causing morbidity and mortality in the contemporary world. The condition becomes more alarming, when one considers the complications associated with the disease, the substantial cost burden that the

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disease imposes to the society and in particular to those individuals with diabetes and their families and the various side effects that are associated with various drugs used for the management of the disease. In view of the side effects reported with the use of insulin and oral hypoglycemic agents, medicinal plants and some active constituents isolated from them are preferred and even recommended by WHO (1980) for the treatment of diabetes mellitus. According to the world health organization, at least 171 million people worldwide suffer from diabetes and its incidence is increasing rapidly. It is estimated that by the year 2030, this number will be doubled. *Anogeissus latifolia* (Roxb. ) Wall. ex. BEDD (Family Combretaceae) is a small or fairly large tree, commonly found in the forests of the sub-Himalayan region, Myanmar, Srilanka, Siwalik hills and throughout India upto 1200 m. It is an important timber and the leaves and bark are used for tanning. The bark was first examined by Reddy et. al, who isolated (+) leucocyanidin. Later ellagic acid and two new glycosides of ellagic and flavellagic acid were reported. Ethnobotanically, the bark has been reported to be used in the treatment of various disorders like skin diseases, snake and scorpion bite, stomach diseases, colic, cough and diarrhoea. Antioxidant activity has been evaluated in *A. latifolia*. However, the efficacy of the plant extract for antihyperglycemic activity has not been subjected to investigations. Hence, in the present study the sequential extracts, viz. petroleum ether, chloroform and methanol of leaf and bark of the plant *Anogeissus latifolia* were utilized to evaluate the antihyperglycemic property.

**EXPERIMENTAL**

The stem bark and leaf of *Anogeissus latifolia* were collected from Thyavarekoppa Forest Area of Shimoga District, Karnataka. The plant was authenticated by comparing with the herbarium voucher specimen deposited at Kuvempu University Herbaria Ku/sd/Tk/206, Shankarghatta. The materials were air dried under shade, powdered mechanically and stored in the airtight containers. About 1 kg of the powdered materials were refluxed successively with the solvents viz. petroleum ether, chloroform and methanol in a Soxhlet extractor for 48 hours in four batches of 250 g each. Every time before extracting with next solvent, the marc was dried at room temperature. The extracts were pooled together and concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland).

**Animal selection**

The experimental animals were procured from the Central Animal House, I. I. Sc, Bangalore. Healthy adult Wistar albino rats of either sex between 2-3 months old and
weighing 150-200 g were used for study. These animals were maintained at standard housing conditions (temperature 27 ± 1°C; relative humidity 60 ± 5%) and were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum*, during the experiment.

**Acute toxicity studies**

The acute toxicity studies were carried out as per stair case method$^{18}$. 50 Albino mice of either sex weighing 20-25 g and 90 days were used to determine LD$_{50}$ of various fractions. The mice were divided into 5 groups of 10 each and were administered with aliquot doses of the extracts orally (100, 150, 200, 250 and 300 mg/Kg body weight). Mortality was not noticed upto 300 mg/Kg for all the extracts except chloroform leaf and methanol bark, wherein 100% mortality was observed at the dose of 250 and 300 mg/Kg. Accordingly the LD$_{50}$ of the extracts was found to be 300 mg/Kg body weight for all the extracts except chloroform leaf and methanol bark, which was fixed to 200 mg/Kg body weight. 1/10$^{th}$ of this dose was selected as the therapeutic dose for the evaluation for the experiment. 1% Tween -80 was used as the vehicle to suspend various fractions and administered orally.

**Preliminary phytochemical screening**

Standard methods$^{19}$, were used for preliminary phytochemical screening of the individual fractions to know the nature of phytoconstituents present in the leaves viz. steroids, flavanoids, alkaloids, saponins, tannins, glycosides etc.

**Induction of experimental diabetes**

The albino rats were kept fasting for 24 hrs and thereafter diabetes was induced by intraperitoneal injection of streptozotocin (SISCO Research Laboratories, Hyderabad) freshly prepared in citrate buffer (pH = 4.5) immediately before use. Streptozotocin (STZ) was given at a dose of 65 mg/kg body weight$^{20}$.

**Determination of blood glucose**

The diabetic state of the animals was assessed by measuring blood glucose concentrations 72 h after streptozotocin treatment. Glucose determinations were made with One Touch Profile (Omnitest B/Braun, Mumbai). The rats with blood sugar level above 300 mg/dL as well as exhibiting polydipsia, polyurea and polyphagea were selected for the experiment.
Experimental design for antihyperglycemic study

The Experimental animals were divided into 8 groups, each of 6 animals. The groups were as follows;

**Group I** : This group consists of diabetic control and received 1% Tween 80

**Group II** : Diabetic animals received standard drug, glibenclamide (10 mg/kg body weight).

**Group III** : Diabetic animals received petroleum ether bark extract of (30 mg/kg body weight).

**Group IV** : Diabetic animals received chloroform bark extract of (30 mg/kg body weight).

**Group V** : Diabetic animals received methanolic bark extract of (20 mg/kg body weight).

**Group VI** : Diabetic animals received pet ether leaf extract of (30 mg/kg body weight).

**Group VII** : Diabetic animals received chloroform leaf extract of (20 mg/kg body weight).

**Group VIII** : Diabetic animal received methanol leaf extract of (30 mg/kg body weight).

The above formulations were given orally daily a single dose upto 7 days.

The blood samples were withdrawn from the rat by tail vein puncturing with hypodermic needles at 0, 1st, 3rd, 5th, 7th hour and 7th day after administration of the dose.

The data were expressed in Mean ± SE and was analyzed according to one way ANOVA.
RESULTS AND DISCUSSION

The data of the mean values of antihyperglycemic effect of A. latifolia extracts in STZ induced diabetic rats for three different extracts viz., petroleum ether, chloroform and methanol from bark and leaf at different time intervals is presented in Table 1. The results obtained revealed that diabetic control has showed increase in blood glucose level from 1st hour (332.83 ± 8.39) onwards in an increasing trend with maximum glucose level recorded on 7th day (405.50 ± 18.77). At 3rd hour of experiment, highest reduction in blood glucose level were recorded in both methanolic bark and leaf extracts exhibiting 315.83±5.29 and 310.17 ± 7.12, respectively as compared to other extracts. A similar trend of blood glucose lowering activity was observed at 5th, 7th hr and on 7th day, wherein highest reduction effect was observed with a mean values of 283.50 ± 5.40, 223.00 ± 7.15 and 180.17 ± 6.53 for bark and 292.33 ± 8.07, 252.50 ± 8.34 and 206.50 ± 6.29 for leaf, respectively. Further, among the bark extract highest blood glucose lowering activity was noticed in methanol followed by petroleum ether and chloroform while similar effect was noticed in leaf extracts in the order of methanol < chloroform < petroleum ether. From the data, it is also evident that among the two sources of extracts bark proved to be more effective when compared to extracts from leaf in terms of antihyperglycemic activity. The ANOVA analysis revealed significant differences between different extracts v/s control at 7th hr and 7th day for petroleum ether and chloroform of bark and leaf (p ≤ 0.05) and at 3rd hour onwards for methanolic extracts of both bark and leaf indicating the potent antihyperglycemic effect in the latter.

Table 1: Antihyperglycemic effect of Anogeissus latifolia extracts in streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 h</th>
<th>1st h</th>
<th>3rd h</th>
<th>5th h</th>
<th>7th h</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>332.83 ± 8.39</td>
<td>369.83 ± 5.33</td>
<td>345.83 ± 1.54</td>
<td>351.67 ± 6.30</td>
<td>372.33 ± 18.77</td>
<td>405.50 ± 108.67</td>
</tr>
<tr>
<td>Standard</td>
<td>332.17 ± 2.97</td>
<td>240.67 ± 3.14*</td>
<td>207.50 ± 3.45*</td>
<td>162.50 ± 3.97**</td>
<td>115.67 ± 2.62**</td>
<td>108.67 ± 1.76**</td>
</tr>
<tr>
<td>Petroleum ether bark</td>
<td>343.67 ± 11.83</td>
<td>361.00 ± 17.17</td>
<td>348.50 ± 13.13</td>
<td>320.17 ± 14.56</td>
<td>298.33 ± 17.71*</td>
<td>304.00 ± 15.24*</td>
</tr>
</tbody>
</table>

Cont...
### Blood glucose levels at different intervals (mg/dl)

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 h</th>
<th>1st h</th>
<th>3rd h</th>
<th>5th h</th>
<th>7th h</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>344.33±</td>
<td>377.50±</td>
<td>368.67±</td>
<td>340.50±</td>
<td>305.33±</td>
<td>308.17±</td>
</tr>
<tr>
<td>bark</td>
<td>10.01</td>
<td>9.72</td>
<td>12.51</td>
<td>14.20</td>
<td>17.82*</td>
<td>10.25*</td>
</tr>
<tr>
<td>Methanol</td>
<td>346.00±</td>
<td>333.33±</td>
<td>315.83±</td>
<td>283.50±</td>
<td>223.00±</td>
<td>180.17±</td>
</tr>
<tr>
<td>bark</td>
<td>13.68</td>
<td>15.67</td>
<td>5.29*</td>
<td>5.40**</td>
<td>7.15**</td>
<td>6.53**</td>
</tr>
<tr>
<td>Petroleum ether leaf</td>
<td>342.00±</td>
<td>378.83±</td>
<td>373.33±</td>
<td>352.00±</td>
<td>332.50±</td>
<td>321.00±</td>
</tr>
<tr>
<td>leaf</td>
<td>13.36</td>
<td>4.00</td>
<td>16.89</td>
<td>12.58</td>
<td>15.34*</td>
<td>13.97*</td>
</tr>
<tr>
<td>Chloroform leaf</td>
<td>345.67±</td>
<td>384.50±</td>
<td>372.67±</td>
<td>350.00±</td>
<td>336.83±</td>
<td>316.33±</td>
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<tr>
<td>leaf</td>
<td>11.75</td>
<td>12.23</td>
<td>12.4</td>
<td>13.10</td>
<td>10.30*</td>
<td>11.63*</td>
</tr>
<tr>
<td>Methanol</td>
<td>345.17±</td>
<td>349.83±</td>
<td>310.17±</td>
<td>292.33±</td>
<td>252.50±</td>
<td>206.50±</td>
</tr>
<tr>
<td>leaf</td>
<td>9.91</td>
<td>10.81</td>
<td>7.12*</td>
<td>8.07**</td>
<td>8.34**</td>
<td>6.29**</td>
</tr>
</tbody>
</table>

Values are mean ± SE of 6 replicates *p < 0.05 and **p < 0.01 v/s diabetic control.

In the present study, STZ deoxy-5-[(methyl-nitrosoamino) carbonyl]-amino)-D-glucopyranose molecule was utilized to induce diabetes, produces a selective toxic effect on β-cells of pancreas and induces diabetes in most laboratory animals\textsuperscript{21,22}. Insulin and sulfonylurea drugs (e.g., Glibencilamide) cause hypoglycemia and it is the most worrisome effect of these drugs\textsuperscript{23}. The present work has detected the antihyperglycemic effect of the extract of *A. latifolia* in three extracts viz., petroleum ether, chloroform and methanol in STZ induced diabetic rats. Among the two sources of extracts, the bark has been proved to be an ideal material than the leaf. Further the antihyperglycemic activity was more effective in methanolic extract in both; bark and leaf and has shown significant decrease in blood glucose level from 3rd hr onwards up to 7th day. This reduction of blood sugar may be because of the extract either protected the cell from the toxic effect of STZ or the cells recovered after the initial injury\textsuperscript{24}. It may be due to the presence of active principles in the plant, containing flavonoids and steroids. Similar observation was reported by several workers using plant extracts of different plants\textsuperscript{25-27}. Further flavonoids are also been reported to be effective in few diabetic complications such as heart disease, neuropathy and retinopathy\textsuperscript{28}. To conclude, the present investigation reveals the potent antihyperglycemic activity of methanolic leaf and bark extracts of *A. latifolia*. Therefore, a detailed investigation is warranted in order to identify and elucidate the specific compounds and to understand the exact mechanism of action so as to develop a potent antidiabetic drug.
ACKNOWLEDGEMENT

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REFERENCES


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Anthelmintic Activity of *Anogeissus Latifolia* Bark and Leaf Extracts

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Abstract: Present study reports the anthelmintic activity of various extracts viz., petroleum ether, chloroform and methanol obtained from the bark and leaf of *Anogeissus latifolia* against earthworms, *Pheritima posthuma*. Five concentrations (10, 20, 30, 40 and 50 mg/ml) of each extracts were studied in a bioassay which involved the determination of time of paralysis and time of death of the worm. All the extracts exhibited moderate to significant anthelmintic activity. Comparing all extracts, chloroform bark and petroleum ether leaf extracts showed potent anthelmintic activity. Results were compared with the reference drug albendazole.

Key words: Anthelmintic, *Anogeissus latifolia*

Introduction

Helminthiasis infections are prevalent in people all over the world, but most common in the tropical and subtropical regions. The World Health Assembly, in a number of resolutions has emphasized the need to the use of natural products with therapeutically proven efficacy particularly in patients residing in tribal areas who are very much prone to the attack of several infections due to lack of knowledge about proper sanitation (Ghosh et al., 2006). Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects. For example, plants that contain condensed tannins, a class of phenolic secondary metabolites, have these effects (Jalalpure et al., 2007). Search for anthelmintic factor in plants therefore remains a potential area of investigations.

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Anogeissus latifolia (Roxb) wall ex. B.E.D.D (Family: Combretaceae) is a smaller or fairly large tree, commonly found in the forests of the sub-himalayan region, Myanmar, Sri Lanka, Siwalik hills and throughout India up to 1200 m. It is an important timber and the leaves and bark are used for tanning. The bark was first examined by Reddy et al. in 1965 who isolated (+) leucoxyanidin. Later ellagic acid and two new glycosides of ellagic and flavelagic acid were reported (Deshpande et al., 1976) Ethnobotanically, the bark has been reported to be used in the treatment of various disorders like skin diseases such as sores, boils and itching (Roy et al., 1986.), Snake and Scorpion bite, Stomach diseases (Jain et al., 1970), Colic (Apparanantam et al., 1986), Cough (Ballia et al., 1982), diarrhoea (Ramachandran et al., 1981). Further, Antioxidant and wound healing (Govindarajan...
et al., 2004a and 2004b) activities have been evaluated in A. latifolia. However the efficacy of the plant extract for anthelmintic activity has not been subjected to investigations. Hence, in the present study, the sequential extracts, viz. petroleum ether, chloroform, and methanol of leaf and bark of the plant Anogeissus latifolia were utilized to evaluate the anthelmintic property.

Materials And Methods

Plant material

The bark and leaf of Anogeissus latifolia were collected from Thavarekoppa forest area of Shimoga District, Karnataka. The plant was authenticated by comparing with the herbarium voucher specimen deposited at Kuvempu University Herbaria, Ku/sd/Tk/206 Shankarghatta.

Preparation of extract

The bark and leaf material of Anogeissus latifolia were air dried under shade, powdered mechanically and stored in airtight containers. About 1 kg of the powdered materials was refluxed successively with the solvents petroleum ether, chloroform and methanol in a soxhlet extractor for 48 hours (approx 45cycles) in four batches of 250 g each. Every time before extracting with next solvent the marc was dried at room temperature. The extracts were pooled together and concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland).

Drug used

Albendazole was used as reference standard for study of anthelmintic activity.

Animals

Indian adult earthworms Pheritima posthuma collected from Earthworm Rearing Center, Dummalli, Shimoga (Karnataka) of 3-5 cm in length and 0.1-0.2 cm in width were used. The worms were washed with normal saline to remove all faecal matter before experimentation.

Preliminary Phytochemical Screening

Standard methods (Harborne, 1984, and Trease & Evans, 1989) were used for preliminary phytochemical screening of the extract to know the nature of phytoconstituents present in the bark and leaf extract of A. latifolia.

Evaluation of anthelmintic activity

The anthelmintic activity was evaluated on Indian adult earthworm Pheritima posthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being (Vidyarthi et al., 1977, Thorn et al., 1977 and Vigar et al., 1984). 32 groups each containing of 6 earthworms of approximately equal size were released into 25 ml of desired formulation. Each group was treated with one of the following, vehicle (1% Tween-20 in normal saline), albendazole (10 mg/ml) and petroleum ether, chloroform, methanolic extracts (10, 20, 30, 40 & 50 mg/ml each) of bark and leaf of Anogeissus latifolia containing 1% Tween-20 suspension was diluted in normal saline in order to study anthelmintic property. Observations were made for the time taken to cause paralysis and death time of individual worms. Paralysis was said to occur when the worms did not revive in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors. The experiment was carried out in triplicate for each group and the data was statistically analyzed for ANOVA.

Results and Discussion

Results of preliminary phytochemical tests suggest that extracts of Anogeissus latifolia bark and leaf possess tannins, terpenes, flavonoids, alkaloids and cardiac glycosides as shown in Table 1.
Acknowledgements

The authors express their gratitude to Sri. R. K. Baliga, Principal, S.R.N.M.N. College, Shivamogga, Secretary, N.E.S. Shivamogga, Prof. V. R. Padmanabhan, Head, Department of Zoology, and Prof. B.R. Siddaramappa, Principal, Sahyadri Science College (Autonomous), Shivamogga, for providing lab facility and encouragement.

References


The anthelmintic activity of various extracts of *A. latifolia* bark and leaf is presented in Table 2. It reveals that all the extracts of the bark and leaf possess dose dependent anthelmintic activity and comparable to standard drug albendazole on earthworms. For paralysis among bark extracts, chloroform has shown the maximum reduction in time followed by petroleum ether and methanol. Both chloroform and petroleum ether extracts at the 30mg/ml (3.75 ± 0.24 and 3.88 ± 0.24), 40 mg/ml (3.48 ± 0.15 and 3.66 ± 0.12) and 50 mg/ml (3.33 ± 0.20 and 3.59± 0.15) exhibited significant (P<0.05) reduction in mean values when compared to the standard. However, methanol bark has shown significant reduction only at 50mg/ml concentration (3.93 ± 0.20). Similarly among leaf extracts petroleum ether has exhibited maximum reduction in time (3.94 ± 0.09 at 30mg, 3.81 ±0.15 at 40mg and 3.66 ± 0.12 at 50mg/ml) followed by chloroform (3.91 ± 0.17 at 40mg and 3.75 ± 0.12 at 50mg/ml) and methanol (4.01 ± 0.09 at 50mg/ml) and the values are statically significant compared to the standard (P<0.05).

In regard to the time taken to death (minutes), both bark and leaf extracts have shown reduction in time required for majority of concentrations tested. Among bark extract, chloroform (6.20 ± 0.54 to 4.82 ± 0.18) and petroleum ether (7.80 ± 0.17 to 6.30 ± 0.27) proved to be better in terms of anthelmintic activity by exhibiting reduction in time at all the concentrations tested (P<0.05) followed by methanol which has shown lesser time (8.93 ± 0.26) than standard at 50mg/ml (P<0.05). Further, among leaf extract, petroleum ether (7.59 ± 0.21 to 6.40 ± 0.15) at all the concentrations exhibited reduction in time required than the standard (P<0.05) while, chloroform has shown significant reduction at all the concentrations (8.66±0.15 to 7.96±0.19) except at 10mg/ml. However, the methanol exhibited significant reduction (P<0.05) at two higher concentrations viz, 40 mg/ml (8.70±0.07) and 50 mg/ml (8.20±0.26). From the data it is evident that among the two sources of extracts bark proved to be more effective when compared to leaf in terms of anthelmintic activity for both the parameters studied.

The present study revealed that the various extracts of *Anogeissus latifolia* possess potent anthelmintic activity by exhibiting effectiveness for the parameters studied. It may be due to the presence of Flavanoids, Tannins, Triterpenoids, Phenolic compounds and cardiac glycosides present in the extracts (Pennazio *et al.*, 1997, Rahman *et al.*, 1997, Jayaprakasam *et al.*, 1999, and Walker *et al.*, 1999). Further, Reddy *et al.*, (1965) reported that the leaves of *A. latifolia* contained purely hydrolysable tannins and related compounds, where as the bark and wood extractives predominantly contains both flavanoid tannins and compounds related to

**Table 1**: Qualitative analysis of phytochemicals of *Anogeissus latifolia* bark and leaf.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Alkaloid</th>
<th>Flavanoid</th>
<th>Steroid</th>
<th>Saponin</th>
<th>Cardiac glycoside</th>
<th>Tannins</th>
<th>Terpene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether bark</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>Chloroform bark</td>
<td>-</td>
<td>+</td>
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<td>-</td>
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<td>+</td>
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<td>Methanol bark</td>
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<tr>
<td>Petroleum ether leaf</td>
<td>-</td>
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<td>+</td>
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<td>-</td>
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<td>Chloroform leaf</td>
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<td>+</td>
<td>-</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Methanol leaf</td>
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<td>+</td>
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Table 2: Anthelmintic activity of various extracts of *Anogeissus latifolia* bark and leaf

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration used (mg/ml)</th>
<th>Time taken for paralysis (min)</th>
<th>Time taken for death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>4.25 ± 0.11</td>
<td>10.35 ± 0.22</td>
</tr>
<tr>
<td>Albendazole</td>
<td>10</td>
<td>5.41 ± 0.27</td>
<td>7.80 ± 0.17*</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.90 ± 0.36</td>
<td>7.28 ± 0.14*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.88 ± 0.24*</td>
<td>6.82 ± 0.26*</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>3.66 ± 0.12*</td>
<td>6.71 ± 0.37*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.59 ± 0.15*</td>
<td>6.30 ± 0.27*</td>
</tr>
<tr>
<td>Petroleum ether bark</td>
<td>10</td>
<td>5.29 ± 0.32</td>
<td>6.20 ± 0.54*</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.57 ± 0.11</td>
<td>5.36 ± 0.23*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.75 ± 0.24*</td>
<td>5.37 ± 0.16*</td>
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<td>40</td>
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<td>5.15 ± 0.15*</td>
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<td></td>
<td>50</td>
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<td>4.82 ± 0.18*</td>
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<td>Chloroform bark</td>
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<td>6.47 ± 0.26</td>
<td>11.47 ± 0.55</td>
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<td>5.11 ± 0.54</td>
<td>10.98 ± 0.20</td>
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<td>9.20 ± 0.12</td>
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<td>3.93 ± 0.20*</td>
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<td>6.40 ± 0.15*</td>
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<td>Petroleum ether leaf</td>
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<td>6.41 ± 0.27</td>
<td>9.25 ± 0.19</td>
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<td></td>
<td>20</td>
<td>5.18 ± 0.36</td>
<td>8.66 ± 0.15*</td>
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<td>30</td>
<td>4.41 ± 0.15*</td>
<td>8.55 ± 0.53*</td>
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<td>40</td>
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<td>8.35 ± 0.47*</td>
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<td>40</td>
<td>4.90 ± 0.09</td>
<td>8.70 ± 0.07*</td>
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<tr>
<td></td>
<td>50</td>
<td>4.01 ± 0.09*</td>
<td>8.20 ± 0.26*</td>
</tr>
</tbody>
</table>

(Results expressed as Mean ± SEM from six observation; control worms were alive up to 24hrs of observation. Time taken for Paralysis: Standard Vs Test *P< 0.05; Time taken for Death, Standard Vs Test *P< 0.05)

hydrolysable and flavanoid tannins. The wormicidal activity of various extracts against earthworms suggests that it can be effective against parasitic infections of humans. In the light of the above, detailed investigation is warranted in order to identify the specific compound which is responsible for anthelmintic activity and the work is under progress.
Hypolipidemic activity of gum ghatti of Anogeissus latifolia.

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ABSTRACT

Cardiovascular diseases are becoming an increasing problem worldwide and hypercholesterolemia has been correlated for coronary heart diseases. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidemia poses no side effects and is relatively cheap and locally available. In view of this, the present study was carried out to investigate the effect of gum ghatti of Anogeissus latifolia on serum lipid levels of albino rats. Rats were made hyperlipidemic by the oral administration of cholesterol (400mg/kg body weight/day) along with cholic acid (50mg/kg) in coconut oil. The hypolipidemic effect was compared with control. The rats were divided into six groups of six animals each. In atherogenic diet induced hypolipidemic model, the rats receiving treatment with gum ghatti at 250 mg/kg dosage showed significant reduction in serum triglycerides (82.75±0.63) only and there was no significant changes either in serum total cholesterol or elevation in HDL. Whereas, at 500 and 750 mg/kg dosage showed significant reduction in serum total cholesterol (72.85±0.60, 68.17±0.95) and serum triglycerides (78.92±0.34, 75.93±1.05). Further, the 750 mg/kg dose also exhibited significant elevation in high density lipoprotein cholesterol (41.13±0.37).

KEYWORDS: Anogeissus latifolia, Hypolipidemic, Total cholesterol, Triglyceride, Gum-ghatti, HDL.

INTRODUCTION

Cardiovascular diseases with an incidence of approximately 50% are the main cause of death in most advanced countries and witnessing an increasing trend in the developing world also (1). The World Health Organization estimates that every year 12 million people worldwide die from cardiovascular diseases (2). The primary cause of cardiovascular disease is believed to be an atherosclerosis, a progressive multifactorial disease of the arterial wall (3, 4). Central to the pathogenesis of atherosclerosis is deposition of cholesterol in the arterial wall (5). Nearly all lipoproteins are involved in this process including cholesterol carried by very low density lipoproteins (VLDL), remnant lipoproteins and low density lipoproteins (LDL). Hypolipidemic therapy is highly effective in reducing the risk as has been demonstrated dramatically in the Scandinavian Simvastatin Survival Study (6).

In recent years different type of antihyperlipidemic agents have been developed and selected for such treatment, depending on symptoms and other condition of the subjects (7). The inhibitors of 3-hydroxy-3methylglicaryl coenzyme A reductase have high efficacy against hyperlipidemia (8, 9). These agents however, have serious adverse effects, including myopathy by destroying striate muscle in rare cases (9). Further, drug administration continues over a long period of time in this chronic disease and therefore adverse effects should be minim-
Hypolipidemic activity of gum ghatti of Anogeissus latifolia.

and as mild as possible. Hence other types of agents that are different in mechanism from the existing agents are also needed. Medicinal plants are part and parcel of human society to combat diseases from dawn of civilization (10). Pectin gums and soluble fiber have a serum cholesterol lowering effect. Mechanisms proposed to explain hypercholesterolemic effect of these include, 1) Altered intestinal absorption, metabolism and release of cholesterol through an influence on bile acids. 2) Altered hepatic metabolism and release of cholesterol with increased excretion of bile acids reducing the size of the bile acid pool and less cholesterol available for incorporation into lipoprotein and subsequent release into the circulation and 3) Altered peripheral metabolism of lipoproteins. Fibers may also alter the proportion of cholesterol incorporated into chylomicrons and lipoprotein (11). Plant gums also provide the soluble fiber in a healthy diet by absorbing water and adding bulk to the large intestine. Epidemiological studies have indicated that a diet low in fiber is associated with the incidence of the adult disease including coronary heart disease (12) and a colon cancer (13).

Gum Ghatti is the amorphous translucent exudates of the Anogeissus latifolia (Roxb) wall ex. BDEDD (Family: Combretaceae). It is a smaller or fairly large tree, commonly found in the forests of the sub-Himalayan region, Myanmar, Sri Lanka, Siwalik hills and throughout India up to 1200 m. It is an important timber and the leaves and bark are used for tanning. The bark was first examined by Reddy et al. in 1965 who isolated (+) leucocyanidin. Later ellagic acid and two new glycosides of ellagic and flavaglic acid were reported (14). Ethnobotanically, the bark has been reported to be used in the treatment of various disorders like skin diseases such as sores, boils and itching (15), snake and scorpion bite, stomach diseases (16), colic (17), cough (18), diarrhoea (19). Further, antioxidant and wound healing (20, 21) activities have been evaluated in A. latifolia.

The objectives of the study were to:

- Study the effect of gum ghatti on serum cholesterol, serum triglyceride and high density lipoproteins (HDL) in albino rats.
- Study the lipid lowering property of gum ghatti if any, along with standard atorvastatin.

**MATERIALS AND METHODS**

**Collection of Gum Ghatti**

Fresh gum ghatti of Anogeissus latifolia was collected from Thayaverekoppa village of Shivamogga District, Karnataka, India. The plant was authenticated by comparing with the herbarium voucher specimen deposited at Kuvempu University Herbarium, Department of Botany, Ku/sd/ Tk/206. The gum ghatti was completely dried and powdered in a pulverizer as and when required, sieved, labelled and stored in PET Bottles (22).

**Drug Formulation**

Various concentrations of gum ghatti of Anogeissus latifolia was dissolved in water for oral suspension.

**Animals**

Wistar albino rats of either sex weighing 150-200g were brought from Venkateshwara enterprises, Bangalore, were housed in polypropylene cages in a room where the optimum temperature was 27°C±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet (Hindustan Lever Limited, Bangalore) and water provided ad libitum. The experiment was approved by the institutional animal ethical committee (NCP/IAEC/CLEAR/01/06/2007-08).

**Evaluation of Hypolipidemic Activity**

Gum ghatti was used to study the effect on serum lipid profile. Rats were made hypolipidemic by the oral administration of cholesterol (400mg/kg) along with cholic acid (50mg/kg) in coconut oil for 20 days, once daily (23). The rats were then given drug treatment for 10 days. During these 10 days, all the groups also received cholesterol in the same dose as earlier. The animals were divided into following groups of six animals each.

- **Group I**: Normal Diet.
- **Group II**: Atherogenic control (400mg/kg body weight cholesterol p.o).
- **Group III**: Atherogenic treated + standard Atorvastatin (5.5 mg/kg body weight i.p).
- **Group IV**: Atherogenic treated + 250mg/kg body weight gum ghatti (p.o).
- **Group V**: Atherogenic treated + 500mg/kg body weight gum ghatti (p.o).
- **Group VI**: Atherogenic treated + 750mg/kg body weight gum ghatti (p.o).

**Collection of blood**

On the 30th day blood samples were withdrawn from rats, by retro orbital sinus puncture under mild ether anesthesia after an overnight fasting. The collected samples were centrifuged for 10 minutes. Then serum samples were
collected and used for the assaying of serum total cholesterol, triglyceride and HDL using commercially available enzymatic kits.

Statistical analysis

Values are expressed as mean ± SEM and the statistical analysis was done using one way ANOVA.

RESULTS

The changes in serum lipid levels in the gum ghatti treated groups are summarized in Table 1. Administration of 250mg/kg of gum ghatti has shown significant (P<0.05) decrease in serum triglyceride level only (82.75±0.63) where as in case of 500 mg/kg, significant decrease has occurred both in total cholesterol (72.85±0.60) and triglyceride (78.92±0.34). However, in case of 750mg/kg, in addition to significant decrease in the total cholesterol (68.17±0.95) and triglyceride (75.93±1.05) has also shown significant elevation in the HDL (41.13±0.37) level. Thus compared to all the three concentrations 750mg/kg has shown efficient lipid lowering activity.

DISCUSSION

The article by Dr. Elmehdavi R.R. entitled "Hypolipidemia: A word of caution" has shown the multifaceted properties of cholesterol which is the most highly decorated molecule in biology (24). Hyperlipidemia is classified into a primary and secondary type, which indicates the complexities associated with the disease. The primary disease may be treated by anti-lipidemic drugs but the secondary type originating from diabetes, renal nephrosis or hypothyroidism demands the treatment of the original disease rather than hyperlipidemia (25). Consumption of much fat may lead to the production of extra VLDL, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient causing blockages for the normal flow of blood. Therefore, improvement in human diet is highly recommended for disease prevention (26). Pancreatic lipase is a key enzyme for lipid breakdown and fatty acid absorption (27). Inhibitors of pancreatic lipase or HMG-CoA reductase are anti-hypercholesterolemia agents (27, 28, 29), such as orlistat and lovastatin which reduce the absorption of dietary triglycerides and inhibit cholesterol biosynthesis respectively. However, repeated use of these agents causes side effects (29, 30). Gum ghatti is a complex polysaccharide of high molecular weight. It occurs in nature as a mixed calcium, magnesium, potassium and sodium salt. Complete hydrolysis has shown that it is composed of L-arabinose, D-galactose, D-mannose, D-xylose and D-glucoronic acid in a molar ratio of 10:6:2:1:2 plus traces less than 1% of 6-deoxyhexose (31). Treatment with gum ghatti of Anogeissus latifolia at 750mg/kg produced a significant decrease in the serum cholesterol, triglyceride and elevation in HDL if atherogenic diet induced hyperlipidemia in rats. There was no significant effect on HDL level in 250 and 500mg kg gum ghatti concentrations. Atherogenic diet induce hyperlipidemic model has been successfully employed for the evaluation of hypcholesterolemic effect of protei (32). Hypolipidemic effect of the gums, saponins an beta sitosterol have also been reported by several author Ahluwalia and Amma (33) found that feeding of oleores of gum guggal (Commiphora mukul) lowered the total cholesterol and its fractions in lipoproteins. Kotaro, et al: (34) have reported cholesterol lowering effects of Ptilis seed associated with urea metabolism.

CONCLUSION

Authors conclude that, atherogenic animals treated with gum ghatti of Anogeissus latifolia have significantly improv the lipid profile and this effect might be an additive action with other cholesterol lowering regimes.
Hypolipidemic activity of gum ghatti of Anogeissus latifolia.

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1343 (2002).
643 (1976).
Antihyperglycemic Activity of Methanolic Extract of *Carthamus tinctorius* L., Annigere-2

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Abstract: In the present study, investigation was carried out to evaluate the effect of *Carthamus tinctorius* L. Annigere-2 leaves extract on Streptozotocin (SZT) induced hyperglycemic albino rats of Wistar strain. The methanolic extract of safflower leaves was extracted using Soxhlet extractor. The experiment was carried out using five groups of albino rats. Among them group I comprised normal rats and remaining four groups were diabetic induced where in II, served as diabetic control (untreated), group III was treated with standard drug (glibenclamide) while groups IV and V were treated with two different doses of extract viz. 20mg/kg body wt and 30mg/kg body wt. The results of present study revealed that, the plant leaves extract of safflower possess potent antidiabetic activity by exhibiting effectiveness for the parameters studied (antihyperglycemic and body weight). It showed that oral administration of methanolic extract of safflower at two different doses produced significant (P<0.01) decrease in blood glucose on 5th hour and 7th hour onwards for 30mg/kg and 20mg/kg respectively. Further, the body weight in the test extract treated animals showed the recovery in the body weight.

Key words: Anti diabetic activity, *Carthamus tinctorius* L.; Methanolic Extract.

Introduction

Diabetes is a global disease with a huge observes impact on health and mortality (Kannel and McGee, 1979). It occurs at any time of the life from infancy to old age. It is a metabolic disorder characterized by disturbances in carbohydrates, protein and lipid metabolism and manifests complications like retinopathy, microangiopathy and neuropathy (Akhtar and Ali, 1980). Currently many synthetic drugs are used for the diabetes, but which bears many side effects such as hypoglycemic coma and hepatorenal disturbances (Suba et al., 2004). Further, it is also not safe to inject antidiabetic drugs during pregnancy which may affect the baby. In response to WHO’s (1980) recommendation for research on the beneficial uses of medicinal and crop plants in the treatment of diabetes mellitus, several investigations have been carried out which resulted in identifying many plants exhibited positive activity (Shiralkar et al., 2004).

The ethnobotanicals study has been shown to have a long folkloric history for the treatment of blood sugar abnormalities.

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Extracts of various plant materials capable of decreasing blood sugar has been tested in experimental animal model and their effects was confirmed (Ajaonkar, 1984; Upadhyay and Pandey, 1984). The Carthamus tinctorius L., Annigere-2 is commonly known as 'safflower' is a member of the family Asteraceae. Plant is highly branched herbaceous, thistle like annual plant. The plant grows up to 150cm. Traditionally, this crop is also grown for its seeds which are used for the extraction of edible oil and also for its flower, which is used for coloring, flavoring of the food and medicinal uses. Many investigators have evaluated the pharmacological properties of safflower such as sedative, anti-tumor, anti-fertility, antioxidant, etc. (Li Dajue, 1996). However, an investigation of antidiabetic activity has not been reported and hence the present work was aimed to evaluate the antihyperglycemic activity of safflower leaves.

Materials and Methods

Plant material

The leaves of safflower Carthamus tinctorius, Annegeri-2 variety were collected from the agriculture research station, Annegeri, Gadag District (Karnataka). Fresh leaves are washed in running tap water followed by rinsing in distilled water and then leaves are shade dried. Later it was milled in to coarse powder by mechanical grinder. The powder was passed through sieve and used for extraction.

Preparation of extract

The powdered leaves were extracted with methanol using soxhlet apparatus for 48hrs (approximately 48cycles) in batches of 350gm each (Kuppasta and Vasudeva Nayak, 2004). The extract was concentrated in vacuum using rotary evaporator (Buchi, Flawil, Switzerland). The residual solvent was completely removed over the water bath and finally dried by desiccator, which yielded sticky residues. The dried extract was suspended in 1% Tween-80 and used as vehicle to screen antihyperglycemic activity.

Preliminary phytochemical Screening

Standard methods (Trease and Evans, 1989) were used for preliminary phytochemical screening of the extract to know the nature of Phyto-constituents present in the leaves. (Table-1)

Animal selection

The experimental animals were procured from the Central Animal House, I.I.Sc, Bangalore. Healthy adult Wistar albino rats of either sex between 2-3 month old and weighing 150-200gm were used for study. These animals were maintained at standard housing conditions (temperature 27°±1°C; relative humidity 60±5%) and were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water ad libitum, during the experiment. The Institutional Animal Ethical Committee (Reg. No. 144/1999/CPCSEA/Col 3/SMG) permitted the study.

Acute toxicity studies

The acute toxicity studies were carried out as per stair case method (Ghosh, 1984). Accordingly, the LD₅₀ of Methanolic leaf extract was found to be 300 mg/Kg. One tenth of the LD₅₀ dose was selected as the therapeutic dose for the evaluation of

| Table 1: Preliminary Phytochemical Screening of Methanol Extracts of Safflower |
|-----------------|---------------------------------|
| Extract         | Phytochemical constituents present |
| Methanol Extract | Lipids, Alkaloids, Triterpenoids| Tannins, Phenolic compounds, Flavanoids, Cardiac Glycosides, Steroids, Anthraquinone Glycosides, Organic acid. |
Antihyperglycemic activity of C. tinctorius

Induction and screening of anti diabetic property using Streptozotocin (SZT)

The antihyperglycemic effect was evaluated in diabetic induced rats. Before induction of diabetes, the rats were fasted for 18hrs (allowed free access to water). The albino Wistar rats were induced for diabetes by intraperitoneal injection of Streptozotocin (SZT, SISCO research laboratories PVT Ltd Mumbai), which is freshly dissolved in citrate buffer (pH-4.3) immediately before use. Streptozotocin was given at a dose of 65mg/kg body weight (Pellegrino et al., 1998). The Streptozotocin induced diabetic animal models were divided into four groups of six animals each. In addition, one group kept as non diabetes control and it is categorized as group I. The animals of group II, received vehicle (1% Tween 80 in water) only and served as untreated diabetic control where as, group III received Standard drug (Glibenclamide) @ 100mg/kg body weight while, IV and V group received Methanolic extract of Carthamus tinctorius, Annegeri-2 leaf at two different doses viz. 20 and 30 mg/kg body weight respectively. The diabetic state of the animals was assessed by measuring glucose concentration after 72 hours of Streptozotocin treatment. All the experimental formulations were given a single oral dose up to 7 days consistently. The blood samples were analyzed for blood glucose levels by one touch profile method using Gluco- Meter (Omnitest B/ BRUAN, Mumbai). The rats with a blood sugar 300- 310 mg/dl as well as exhibiting polydipsia and polyphagia were selected for the experiment and the body weight of all the rats were determined before the start of the experiment. The blood samples were withdrawn from the rats by tail vein puncturing with hypodermic needles. The blood sugar level determined at 0, 1, 3, 5 and 7th hour and 7th day after administration of the dose. On the seventh day of treatment the body weight of rats were noted. In order to determine the changes in body weight, the difference between 0 hour and 7th day were recorded.

Statistical analysis

The results are statistically evaluated using one way ANOVA. The values were considered significant when P<0.05.

Results

The effect of methanolic leaf extract of Carthamus tinctorius L., for two parameters viz. antihyperglycemic activity and body weight is presented in Table-2. The mean values of the anti hyperglycemic activity revealed that, among the five groups, the diabetic control (group II) animals showed increase in blood sugar level since 1st hour (335.67±7.43mg/dl) onwards and an increasing trend with maximum sugar level recorded on 7th day (400.17 ±2.23). In case of group III animals (Glibenclamide), the serum glucose level was found in a decreasing order from 1st hour (281.50 ±6.63) onwards and lowest values were recorded on 7th day (105.83±1.89). Similar trend was also noticed in experimental batches of group IV (20mg/kg) and group V (30mg/kg) animals which has shown 309.33±9.38 and 302.83±10.50, and 145.83±3.85 and 129.33±3.80 at 1st hour and 7th day respectively even though, the level of reduction was slightly less when compared to the standard drug, among the two concentrations of extract experimented, 30mg/kg was found to be more effective than that of 20mg/kg in terms of antihyperglycemic activity. Further, the one way ANOVA results analyzed between diabetic control Vs. glibenclamide treated and test extracts revealed that the standard drug showed highly significant differences (P<0.01) from 3rd hour onwards. However, the test extracts exhibited significant (P<0.01) differences from 7th hour and 5th hour.
Table 2: Antihyperglycemic activity of Methanolic Extract of Safflower

<table>
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<th>Change in Body</th>
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<td>0hrs</td>
<td>1hrs</td>
<td>3rd hrs</td>
</tr>
<tr>
<td>Normal</td>
<td>102.33</td>
<td>±2.67</td>
<td>104.5</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>304.67</td>
<td>±3.76</td>
<td>346.5</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>308.33</td>
<td>±4.20</td>
<td>276.17</td>
</tr>
<tr>
<td>20mg/kg</td>
<td>307.33</td>
<td>±6.63</td>
<td>295.83</td>
</tr>
<tr>
<td>30mg/kg</td>
<td>307.33</td>
<td>±5.92</td>
<td>7.33</td>
</tr>
</tbody>
</table>

**P<0.01, # Difference in body weight between zero hour and 7th day.

The result of present study reveals that, the plant leaves extract of safflower possess potent antidiabetic activity by exhibiting effectiveness for the parameters studied. It showed that oral administration of methanolic extract of safflower at two different concentration, 30mg/kg body wt. and 20mg/kg body wt. produced significant decrease in blood glucose on 5th hour and 7th hour onwards respectively. This reduction of blood sugar may be because of the extract either protected the cell from the toxic effect of STZ or the cells recovered after the initial injury (Sharma and Mamta Chaturvedi, 2007). It may be due to the presence of active principles in the plant, containing flavonoids and steroids. Similar observation was reported by several workers using plant extracts of different plants (Chakravarty et al., 1980; Rajnarayan et al., 2001 and Ray et al., 2006). Further, flavonoids are also been reported to be effective in few diabetic complications such as heart disease, neuropathy and retinopathy (Leland et al., 2006).

Induction of diabetes with SZT is associated with characteristic loss of body weight, which is due to increased muscle

onwards respectively for 20mg/kg and 30mg/kg body weight.

The effect of the extract was also seen on the body weight of the diabetic rats. The body weight was slightly increased (36.7gms) in the normal animals (Group I), where as, in diabetic control there was a decrease in the body weight of animals to the extent of -17.33gms. But the standard drug, glibenclamide treated rats showed slight increase in body weight (8.67gms). Similarly, rats treated with extract @ 20mg/kg and 30mg/kg showed a slight raise in body weight to the i.e. 4.17gms and 7.33gms respectively and this showed that extract has prevented the reduction in body weight.

Discussion

In the present study SZT deoxy-5'[(methyl-nitrosoamino) carbonyl]-amino]-D glucopyranose molecule was utilized to induce diabetes, produces a selective toxic effect on β-cells of pancreas and induces diabetes in most laboratory animals (Lawn et al., 1979 and Doux et al., 1986). Insulin and sulfonylurea drugs (e.g. Glibenclamide) cause hypoglycemia is the most worrisome effect of these drugs (Chakrabarti and Rajagopalan, 2002).
wasting and loss of tissue proteins (Swanson-Flat et al., 1990; Chatterjea and Shinde, 2002). Diabetic rats treated with the extract showed an increase in body weight compared to the diabetic control, which may be due to its effect in controlling muscle wasting i.e. by reversal of antagonizing effect (Whitton and Hems, 1975). The present investigation has demonstrated that, methanolic extract of the leaves of safflower has antidiabetic activity and also showed increase in body weight of diabetic rats. Therefore, a detailed investigation is warranted in order to identify and elucidate the specific compounds and to understand the exact mechanism of action so as to develop a potent antidiabetic drug.

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