Antihyperglycemic potential of *Aloe vera* gel in experimental animal model

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder, characterized by absolute or relative deficiency in insulin secretion or insulin action. Currently available synthetic antidiabetic drugs used in clinical practice have characteristic profiles of adverse side effects. Plant based drugs are considered to be less toxic and free from adverse effects in comparison to modern allopathic medicines. *Aloe vera* L. (Syn.: Aloe barbadensis Miller; Hindi: Ghikanvar; AV) is a cactus-like perennial plant belonging to family Liliaceae (sub-family of the Asphodelaceae), native to North Africa and cultivated in warm climatic areas. The present work aimed to evaluate the antioxidant and antidiabetic potency of *Aloe vera* gel extract using alloxan induced experimental diabetic rats and its effect were compared with reference glibenclamide (GL). Inbreed adult male Charles-Foster (CF) albino rats were used in the experiment for hypoglycemic activity in oral glucose tolerance test (OGTT) and normoglycemic rats, and antidiabetic activity in alloxan induced rats. Preliminary phytochemical screening revealed that AV showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the AV showed total phenolic content (135.8 mg/g). Results revealed in the present experiment that the routine post-treatment for 21 days with the AV showed potential hypoglycemic activity in OGTT and normoglycemic rats and antidiabetic activity in alloxanized rats. In conclusion, isolation and establishment of exact mechanism of action of specific compound from AV is to be carried out in the future.

Keywords: Aloe vera, diabetes, hyperglycemia, dyslipidemia and oxidative stress.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder, characterized by absolute or relative deficiency in insulin secretion or insulin action. It has been estimated that Indian people are more
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genetically prone to diabetes accounting about 30 to 33 million and would go up to 40 million by the end of 2010 which further will reach to maximum 74 million by 2025 [1]. Due to high prevalence, morbidity and mortality of diabetes, it is becoming the third leading cause of death worldwide after cancer and cardiovascular diseases. Chronic hyperglycemia often leads to complications such as renal failure, coronary artery disorder, cerebro-vascular disease, neurological complications, blindness, limb amputation, long term damage, dysfunctions and failure of various organs and eventually premature death [2]. Disease management includes lifestyle modifications, diet, exercise, and long term use of oral hypoglycemic agents or insulin therapy [3]. Currently available synthetic antidiabetic drugs used in clinical practice have characteristic profiles of adverse side effects. Plant based drugs are considered to be less toxic and free from adverse effects in comparison to modern allopathic medicines [4]. Report of ethnobotany suggested that about 800 medicinal plants possess hypoglycemic or antidiabetic potential [5] and the bioactive compounds such as glycosides, alkaloids, terpenoids, carotenoids and flavonoids are effective drugs both in preclinical and clinical studies [6,7].

Aloe vera L. (Syn.: Aloe barbadensis Miller; Hindi: Ghikanvar; AV) is a cactus-like perennial plant belonging to family Liliaceae (sub-family of the Asphodelaceae), native to North Africa and cultivated in warm climatic areas [8-10]. The plant has elongated pointed fleshy leaves consisting of two parts viz. an outer skin (green rind) and an inner pulp (colourless mucilaginous gel) widely used as healing plants in the history of mankind [11,12]. AV consists of high content of phenolic compounds, glycosides (aloins), 1,8-dihydroxyanthraquinone derivatives (aloe emodin), beta-1,4 acetylated mannan, mannose-phosphate and alprogen glucoprotein [9,13,14]. This miraculous plant is as old as civilization has been used throughout history in folk medicine as valuable ingredient for the food, pharmaceutical and cosmetic industries [15]. Literature review revealed that leaf exudates and mucilaginous gel of Aloe possesses anti-inflammatory [16], antifungal [17], antibacterial [18], anticancer [19-22], antioxidant [23,24], cytoprotective, cardiac stimulatory and immunomodulatory activities [25]. It is used to protect against gastric ulceration [26-31], remedy against a variety of skin disorders [32,33], promotes wound healing [34-37] as well reducing edema and pain too [34]. It has also been shown to have antidiabetic and hypoglycaemic properties [9, 38-44] and cardiac stimulatory activity [45]. The present work aimed to evaluate the antioxidant and antidiabetic potency of Aloe vera gel extract using alloxan induced experimental diabetic rats and its effect were compared with reference glibenclamide (GL).

MATERIALS AND METHODS

2.1 Crude drugs, chemicals and reagents

Aloe vera (L.) N. Burman. leaves were harvested from local horticulture garden which was identified and authenticated by an expert Dr N Shiddamallayya of Regional Research Institute (Ay.), Bangalore (India), where Voucher Specimen (No. RRCBI/Mus/6) of the plant had been deposited. Tolbutamide (TBM) was supplied as gift sample by Hoechst Pharmaceuticals, Mumbai, India. Alloxan monohydrate was procured from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Folin-Ciocalteau reagents were from Sigma-Aldrich Inc. (St. Louis, MO, USA); One Touch Glucometer (Accu-chek Sensor) and Diagnostic-kits were purchased from

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Roche Diagnostics GmbH, Mannheim, Germany. All other reagents and chemicals used were of analytical/ pharmacopoieal grade purchased from E. Merck India Ltd and Ranbaxy respectively.

2.2 **Plant material and extraction**

Methods of preparation of AV gel were in simulation to previously reported by Rajasekaran et al., 2004 [46] with slight modification. Fresh and healthy *Aloe vera* leaves weighing between 550-650 g with approximate length 50-70 cm were collected from matured healthy plant and washed thoroughly with fresh running tap water. The leaves were dissected longitudinally and fleshy mucilaginous pulp (parenchymatous tissues) was selectively scraped out, leaving behind the thick epidermis layers. The scraped pulp was homogenized, centrifuged at 10000 g for 25 min to remove the fibers and immediately lyophilized were stored under controlled environment at 4±1°C until used further [47-49]. Thereafter, the known amount of lyophilized sample was subjected to methanolic solvent extraction. The recovered filtrates were evaporated till dryness under reduced pressure (250 mmHg) in a rotary evaporator.

2.3 **Preliminary phytochemical screening and standardization to total phenolic content**

Methanolic extract was subjected to identification tests for alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols [50]. Assaying method of Velioglu et al., 1998 [51] was followed for estimation of total phenolics content in methanolic extract using Folin-ciocalteu reagent with slight modification. Briefly, about 100 µl of plant extract (1 mg ml\(^{-1}\)) were prepared in a 100 ml Volumetric flask and added 0.75 ml of Folin-Ciocalteu reagent (previously diluted in 1:10 ratio with deionised water) was thoroughly mixed and allowed to incubate for 5-8 min at room temperature (25±1°C) condition. Thereafter, 0.75 ml of Na\(_2\)CO\(_3\) solution (60 g/l) was added to mixture and allowed it to stand at room temperature (25±1°C) for 2 h intervals. Absorbance was measured against blank using Double-Beam UV-Vis Spectrophotometer (Simadzu-1800) at \(\lambda_{\text{max}}\) 725 nm respectively. Gallic acid was used as a standard to construct calibration curve (0.02-0.1 mg ml\(^{-1}\)) and readings taken in triplicate to get accurate results. The total phenolic content was expressed as milligrams of gallic acid equivalent/ g extract.

2.4 **Animals**

Charles foster (CF) male albino strain rats weighing (160–200 g), aged 8-14 weeks older were obtained from School of Pharmacy, Chouksey Engineering College, Bilaspur and were used in the study. Animals were housed under standard conditions in polypropylene cages (34 × 47 × 18 cm\(^3\)) lined soft wood shavings as bedding (renewed every 24 h), 12/12 h light/dark cycles, relative humidity 50-60% RH and at temperature 22±3°C, were fed with standard rat pellet diet (Gold Moher, Lipton India Ltd) and water *ad libitum*. All procedures and techniques used in these studies were in accordance with the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India, approved by the institute animal ethics committee (Regd. No.1169/ac/08/CPCSEA).

2.5 **Oral toxicity studies**

Acute oral toxicity study was carried out as per prescribed Organization for Economic Co-operation and Development (OECD) guidelines. Prior to experimentation animals (n=6) were fasted overnight (but not water withheld for 3-4 h) and was oral administered with fixed extracts dose of 5, 50, 300 and 2000 mg kg\(^{-1}\) body weight respectively by gavage using intubation
canula. The dose was found tolerable as no death was found up to the maximum administered doses. Rats were observed individually after dosing for first 30 min periodically and daily thereafter, till 14 days for any toxicity sign of gross changes in skin and fur, eyes and mucous membranes, circulatory, respiratory, autonomic and central nervous systems, and behavior pattern if any. On the basis of earlier studies carried out by Noor et al., 2008 and Rajasekaran et al., 2004 [52,46] the effective dose 300 mg/kg was being selected for further studies.

2.6 Induction of diabetes in rats
Animals were induced diabetes by a single dose subcutaneous injection of freshly prepared alloxan monohydrate (120 mg/kg, Sigma chemicals, USA) dissolved in normal saline (0.9% w/v NaCl in distilled water) to overnight fasted male C F albino rats [53]. Blood glucose level was measured by using one-touch glucometer and diabetes was confirmed after 72 hr of alloxanisation. Rats shown marked hyperglycemia (FBG >250 mg/dl) after 72 h of injection was selected for further studies.

2.7 Experimental Design
2.7.1 Study on oral glucose tolerance test (OGTT) and normoglycemic studies
Initially, oral glucose tolerance test for hypoglycemic activity of plant extracts was carried out in overnight fasted normal rats, which were equally divided into three groups of six rats each. Animals belonging to normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and standard group received 1 ml of reference drug GL suspended in the vehicle (0.25 mg/kg, p.o.), while group third were administered with 1 ml of AV (300 mg/kg, p.o.) respectively. Following 30 min post extract administration all the animals were fed with glucose (2 g/kg). Thereafter, blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. Whereas, normoglycemic studies were carried out in overnight fasted normal rats, which were equally divided into three groups of six rats each. Normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and 1 ml of standard group received reference drug GL suspended in the vehicle (0.25 mg/kg, p.o.), while group third were administered with 1 ml of AV (300 mg/kg, p.o.) respectively. Thereafter, blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively and subjected to fasting blood glucose level. The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diagnostics, GmbH, Germany).

2.7.2 Study on Alloxan-induced diabetic rats
Rats selected were divided into four groups (n=6) as follows: Group-I: Normal control rats (non-alloxanized) that received vehicle (1 ml of 2.5% v/v Tween-80 in distilled water; p.o.) only; Group-II: Diabetic control rats (Untreated, alloxanized); Group-III: Diabetic rats that received GL (0.25 mg/kg; p.o.) as standard reference drug and Group-IV: Diabetic rats that received AV (300 mg/kg/day; p.o.) respectively. The treatment was continued for a period of 21 days respectively following oral administration by gastric intubation, using a force-feeding needle to the experimental animals. Plasma glucose was estimated on withdrawing blood samples were from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively all groups of animals. The body weight, food and fluid intake of all groups of animals were monitored on a daily basis for 21 days at regular time. Fixed amount of rat chow and fluid was given to each rat and
replenished the next day. At the end of 21st day, all the rats were euthanized by pentobarbitone sodium (60 mg/kg) and sacrificed by cervical dislocation. Blood sample was withdrawn from abdominal aorta into fresh centrifuge tubes and centrifuged at 2,500 rpm for 15 min to obtain serum and plasma. Serum samples were stored at -20°C until utilized for further biochemical estimation parameters.

2.7.3 Biochemical estimations
Serum was analyzed for lipid profiles viz. total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP), urea, creatinine, protein and albumin were estimated in a Hitachi Auto analyzer using commercial kit (Ecoline, E-Merck, India; Roche Diagnostics, GmbH, Germany) following standard procedures. Plasma insulin was assayed by using commercial enzyme-linked immuno sorbent assay kit (ELISA, Boehringer Mannheim, Germany). The level of hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) was estimated using Drabkin reagent.

2.7.4 Estimation of superoxide dismutase (SOD)
SOD activity was estimated by the method described by Ukeda et al., 1997 [54]. Into 2.6 ml of 50 mmol/L sodium citrate buffer (pH 9.4) were added 0.1 ml each of 30 mmol/l xanthine (dissolved in 1 mol/L NaOH), 3 mmol/l EDTA, 0.8 mmol/l XTT (3-[(phenylamino)carbonyl]-3,4-tetrazolium/- bis(4-methoxy-6-nitro)-benzenesulfonic acid hydrate) dissolved in buffer at 50°C and 0.05 ml of sample solution containing SOD or water. The reaction consists of the addition of 0.02 ml of xanthine oxidase solution (500 mU/ml) with the formation of a red formazan dye. Then, SOD activity was monitored for 30 s at 470 nm (25°C) by the degree of inhibition of this reaction.

2.7.5 Estimation of catalase (CAT)
Measurement for Catalase activity carried out by the method of Aebi, 1984 [55]. Homogenate was prepared with 50 mmol/l phosphate buffer, pH 7.0, with a drop of TritonX100 and centrifuged at 15,000×g for 15 min at 4°C. To 3.0 ml of phosphate buffer, 0.05 ml of 90 mmol/l hydrogen peroxide solutions and 0.02 ml of extract or water were added. Then absorbance was read at 240 nm for 30 s.

2.7.6 Estimation of lipid peroxidase
Estimation for plasma malondialdehyde (MDA) content was done by using the method of Uchiyama and Miura, 1978 [56] with minor modification by Sunderman et al., 1985 [57] based on the thiobarbituric acid reaction (TBAR) test. Blood plasma samples were added (1/10, v/v) to ice-cold 1.15% KCl solution and mixed with 0.1 ml of 8.1% sodium dodecyl sulfate (SDS), 0.75 ml of 20% acetic acid, and 0.75 ml of 0.8% TBA solution. Then mixture was made up to 2.0 ml with distilled water and heated at 95°C for 60 min. After cooling with tap water, 0.5 ml of distilled water and 2.5 ml of n-butanol/pyridine mixture (15:1, v/v) were added and the mixture shaken vigorously. The obtained mixture was centrifuged at 4000×g for 10 min and the absorbance of the upper organic layer was measured at 532 nm. Standardization of TBAR reaction was performed by the analysis of tetraethoxypropane standard solutions, which yield MDA, mole for mole, under the described reaction conditions.
2.8 Statistical Analysis

The results are expressed as mean±S.E.M. The statistical significance was determined by One-Way Analysis of Variance (ANOVA) followed by Post-hoc Student Newman Keuls test. P < 0.05 was considered to be statistically significant.

RESULTS

3.1. Effect of AV (300 mg/kg) on oral glucose tolerance test (OGTT) and plasma glucose level in normoglycemic and diabetic rats:

Table-1 illustrates the effect of AV (300 mg/kg) on OGTT at different time points. Statistical analysis by One-way ANOVA showed that there was no significant difference among the groups at 0 min [F (2, 15) = 0.18, P>0.05]. Similarly, statistical analysis at 30 min showed that there was significant difference among the groups [F (2, 15) = 4.27, P<0.05]. Post-hoc test revealed that GL (0.25 mg/kg) and AV (300 mg/kg) showed significant attenuation in the plasma sugar level compared to control. Further, statistical analysis at 60 min showed that there was significant difference among the groups [F (2, 15) = 8.31, P<0.05]. Post-hoc by Student Newmann Keuls test revealed that GL and AV (300 mg/kg) showed significant decrease in the plasma sugar level compared to control. Furthermore, AV (300 mg/kg) showed significant increase in the sugar level (P<0.05) compared to GL and this trend was similar at 90 min [F (2, 15) = 5.36, P<0.05] and 120 min [F (2, 15) = 57.13, P<0.05].

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>88.5±3.72</td>
<td>144.9±3.26</td>
<td>135.2±1.75</td>
<td>107.1±2.29</td>
<td>102.3±4.27</td>
</tr>
<tr>
<td>GL</td>
<td>84.7±1.52</td>
<td>112.8±3.64*</td>
<td>98.7±4.75*</td>
<td>80.7±1.79*</td>
<td>67.3±3.17*</td>
</tr>
<tr>
<td>AV-300</td>
<td>85.3±1.54</td>
<td>125.4±3.45a</td>
<td>115.1±2.66*</td>
<td>92.4±3.45*</td>
<td>77.3±1.76*</td>
</tr>
</tbody>
</table>

All values are Mean±SEM. *P<0.05 compared to CON, **P<0.05 compared to GL (One-way ANOVA followed by Student Newmann keuls test).

Time dependant effect of AV (300 mg/kg) on the level of plasma glucose level in fasted normoglycemic rats is depicted in Table-2. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups at 0 day [F (2, 15) = 0.19, P>0.05]. Similarly, statistical analysis at 7 day showed that there was significant difference among the groups [F (2, 15) = 3.29, P<0.05].

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>75.8±3.71</td>
<td>77.6±2.75</td>
<td>74.2±4.72</td>
<td>72.5±2.12</td>
</tr>
<tr>
<td>GL</td>
<td>72.7±1.65</td>
<td>55.8±3.48*</td>
<td>49.6±3.47*</td>
<td>51.4±1.42*</td>
</tr>
<tr>
<td>AV-300</td>
<td>73.2±2.71</td>
<td>59.7±5.39a</td>
<td>51.7±3.15a</td>
<td>51.6±3.05a</td>
</tr>
</tbody>
</table>

All values are Mean±SEM. *P<0.05 compared to CON, **P<0.05 compared to GL (One-way ANOVA followed by Student Newmann keuls test).
Post-hoc test revealed that GL and AV (300 mg/kg) showed significant decrease in the plasma sugar level compared to control. Further, AV (300 mg/kg) showed significant reduction in blood sugar levels compared to GL. Furthermore, the similar trend like at 7 day was observed at 14 day \([F (2, 15) = 12.33, P<0.05]\) and 21 day \([F (2, 15) = 8.92, P<0.05]\).

Table-3 showed time dependant effect of AV (300 mg/kg) on the level of plasma glucose level in alloxan treated rats. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups at 0 day \([F (3, 20) = 4.11, P>0.05]\). Further, statistical analysis at 7 day showed that there was significant difference among the groups \([F (3, 20) = 4.28, P<0.05]\). Post-hoc test revealed that DM, GL and AV (300 mg/kg) showed significant increase in the plasma sugar level compared to control. Further, GL and AV (300 mg/kg) groups showed significant decrease in the blood glucose level compared to DM. Furthermore, the similar trend like at 7 day was observed at 14 day \([F (3, 20) = 17.21, P<0.05]\) and 21 day \([F (3, 20) = 16.18, P<0.05]\).

### Table 3: Hypoglycemic effect of AV (300 mg/kg) in Alloxan induced animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>75.1±2.43</td>
<td>76.7±1.87</td>
<td>75.1±3.33</td>
<td>72.1±3.11</td>
</tr>
<tr>
<td>DM</td>
<td>343.2±2.75</td>
<td>422.6±4.06(^a)</td>
<td>396.3±9.45(^a)</td>
<td>363.3±9.23(^a)</td>
</tr>
<tr>
<td>GL</td>
<td>306.3±1.25</td>
<td>253.2±8.77(^{a,b})</td>
<td>195.6±11.52(^{a,b})</td>
<td>96.6±12.56(^{a,b})</td>
</tr>
<tr>
<td>AV-300</td>
<td>316.5±5.41</td>
<td>243.7±10.45(^{a,b})</td>
<td>216.6±10.73(^{a,b})</td>
<td>120.6±11.23(^{a,b})</td>
</tr>
</tbody>
</table>

All values are Mean±SEM. \(^a\)P<0.05 compared to CON, \(^b\)P<0.05 compared to DM (One-way ANOVA followed by Student Newmann keuls test).

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Fig-1. Effect of AV (300 mg/kg) on the body weight (A), food (B) and fluid (C) intake. All values are Mean±SEM. \(^a\)P<0.05 compared to CON, \(^b\)P<0.05 compared to DM, \(^c\)P<0.05 compared to GL (One-way ANOVA followed by Student Newmann keuls test).

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3.2. Effect of AV (300 mg/kg) on body weight, food and fluid intake of diabetic rats:
The effect of AV (300 mg/kg) on initial and final body weight is illustrated in figure-1(A). Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups during initial body weight estimation [F (3, 20) = 0.21, P>0.05]. Further, statistical analysis revealed that there was significant difference among the groups during final body weight estimation [F (3, 20) = 5.57, P<0.05]. Post-hoc test revealed that DM and AV (300 mg/kg) showed significant decrease in body weight compared to control. GL and AV (300 mg/kg) groups showed significant increase in body weight compared to DM.

The effects of AV (300 mg/kg) on food intake of diabetic rats are illustrated in figure-1 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 3.18, P<0.05]. Post-hoc test revealed that all the groups showed significant increase in food intake compared to control. However, GL and AV (300 mg/kg) showed significant reduction in food intake compared to DM group. Furthermore, the similar trend like food intake was observed in fluid intake [F (3, 20) = 3.18, P<0.05; figure-1 (C)].

3.3. Effect of AV (300 mg/kg) on plasma lipid profile:
The effect of AV (300 mg/kg) on TC, TG, LDL and HDL is depicted in figure-2. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 7.39, P<0.05]. Post-hoc test revealed that DM, GL and AV (300 mg/kg) showed significant increase in TC level compared to control. The TC levels were decreased significantly in GL and AV (300 mg/kg) compared to DM.

Fig. 2.

![Fig-2. Effect of AV (300 mg/kg) on plasma TC (A), TG (B), LDL (C) and HDL (D) levels. All values are Mean±SEM. aP<0.05 compared to CON, bP<0.05 compared to DM, cP<0.05 compared to GL (One-way ANOVA followed by Student Newmann keuls test).](image)

Furthermore, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 9.26, P<0.05]. Post-hoc test revealed that the TG levels were significantly elevated in DM and GL groups compared to control. Further, GL and AV (300 mg/kg) groups showed significant decrease in TG levels compared to DM.

Similarly, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 21.54, P<0.05]. Post-hoc test revealed that all the groups except...
GL showed significant increase in LDL levels compared to control. Further, GL and AV (300 mg/kg) groups showed significant decrease in LDL levels compared to DM.

Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups \[ F (3, 20) = 18.87, P<0.05 \]. Post-hoc test revealed that DM showed significant decrease in HDL levels, however there was no change in HDL levels of GL and AV (300 mg/kg) compared to control. Further, GL and AV (300 mg/kg) groups showed significant increase in HDL levels compared to DM.

3.4. Effect of AV (300 mg/kg) on liver function and plasma antioxidant profile:
The effect of AV (300 mg/kg) on SGOT is depicted in figure-3 (A). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups \[ F (3, 20) = 15.21, P<0.05 \]. Post-hoc test revealed that all the groups showed significant elevation in SGOT levels compared to control. GL and AV (300 mg/kg) showed significant decrease in SGOT levels compared to DM.

The effect of AV (300 mg/kg) on SGPT is depicted in figure-3 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups \[ F (3, 20) = 21.09, P<0.05 \]. Post-hoc test revealed that DM group showed significant increase in SGPT levels compared to control. GL and AV (300 mg/kg) showed significant decrease in SGPT levels compared to DM.

The effect of AV (300 mg/kg) on ALP is depicted in figure-3 (C). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups \[ F (3, 20) = 77.03, P<0.05 \]. Post-hoc test revealed that DM and AV (300 mg/kg) groups showed significant elevation in ALP levels compared to control. GL and AV (300 mg/kg) showed significant decrease in ALP levels compared to DM.
The effect on LPO of AV (300 mg/kg) is depicted in figure-3 (D). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups \([F (3, 20) = 11.07, P<0.05]\). Post-hoc test revealed that DM and AV (300 mg/kg) groups showed significant elevation in LPO levels compared to control. GL and AV (300 mg/kg) showed significant decrease in LPO levels compared to DM.

The effect of AV (300 mg/kg) on SOD is depicted in figure-3 (E). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups \([F (3, 20) = 21.05, P<0.05]\). Post-hoc test revealed that all the groups showed significant elevation in SGOT levels compared to control. GL and AV (300 mg/kg) showed significant decrease in SOD levels compared to DM.

The effect of AV (300 mg/kg) on CAT is depicted in figure-3 (F). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups \([F (3, 20) = 19.88, P<0.05]\). Post-hoc test revealed that DM group showed significant reduction in CAT levels compared to control. GL and AV (300 mg/kg) showed significant increase in CAT levels compared to DM.

3.5. Effect of AV (300 mg/kg) on Total Hb, HbA1c, plasma insulin, urea, creatinine and albumin:

The effect of AV (300 mg/kg) on total Hb is depicted in figure-4 (A). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups \([F (3, 20) = 0.67, P<0.05]\). Post-hoc test revealed that DM showed significant reduction and no other groups did not show any change in total Hb levels compared to control. The GL and AV (300 mg/kg) groups showed significant increase in total Hb levels compared to DM.

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All values are Mean±SEM. *(P<0.05 compared to CON, *P<0.05 compared to DM, **P<0.05 compared to GL (One-way ANOVA followed by Student Newmann keuls test)).

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The effect of AV (300 mg/kg) on HbA1c is depicted in figure-4 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 11.86, P<0.05]. Post-hoc test revealed that DM and AV (300 mg/kg) showed significant elevation in HbA1c levels compared to control. GL and AV (300 mg/kg) showed significant decrease in HbA1c levels compared to DM.

The effect of AV (300 mg/kg) on plasma insulin is depicted in figure-4 (C). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 144.28, P<0.05]. Post-hoc test revealed that DM showed significant reduction in plasma insulin levels compared to control. GL and AV (300 mg/kg) showed significant increase in plasma insulin levels compared to DM.

The effect of AV (300 mg/kg) on plasma urea levels is depicted in figure-4 (D). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 33.29, P<0.05]. Post-hoc test revealed that DM showed significant increase in plasma urea levels compared to control. The GL and AV (300 mg/kg) groups showed significant decrease in plasma urea levels compared to DM.

The effect of AV (300 mg/kg) on plasma creatinine is depicted in figure-4 (E). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 21.88, P<0.05]. Post-hoc test revealed that DM showed significant increase in plasma creatinine levels compared to control. GL and AV (300 mg/kg) showed significant decrease in plasma creatinine levels compared to DM.

The effect of AV (300 mg/kg) on plasma albumin is depicted in figure-4 (F). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (3, 20) = 11.06, P<0.05]. Post-hoc test revealed that DM showed significant decrease in plasma albumin levels compared to control. GL and AV (300 mg/kg) showed significant increase in plasma albumin levels compared to DM.

**DISCUSSION**

In the present experiment, the continuous post-treatment for 21 days with the AV showed potential hypoglycemic activity in OGTT and normoglycemic rats and antidiabetic activity in alloxanized rats.

Preliminary phytochemical screening revealed that AV showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the AV showed total phenolic content (135.8 mg/g). The decreased level of total hemoglobin in diabetic control group indicates the significant increased level of glycosylated hemoglobin (HbA1c). HbA1c is used as most reliable marker and standard diagnosis practices for estimating the degree of protein glycation during diabetes mellitus [58]. HbA1c is the product of non-enzymatic condensation reaction between excess glucose present in the blood and free amino groups on the globin component of hemoglobin [59]. Its concentration is abnormally high in chronic hyperglycemia which reflects long-term glycemic status and also correlates with risk for complications development such as retinopathy, nephropathy or neuropathy related to chronic diabetes stage.
However on oral administration of AV significantly decreased the HbA1c level possibly due to improved glycemic control mechanisms in experimental diabetic rats. The above results are in agreement with the previous carried out studies [61].

In our study, the alloxan induced diabetic rats showed lipid abnormalities with marked significant increase of serum TC, TG, LDL and reduced HDL level. Elevated serum lipids level contributing major risk factors for atherosclerosis and congestive heart diseases [62-64]. Disorders of lipid composition is due to impairment of insulin secretion at diabetic state which resulted into uninhibited actions of sensitive lipolytic hormones on the peripheral fat depots of which enhances mobilization of free fatty acids [65]. At normal condition insulin helps to hydrolyze triglycerides on activating the enzyme lipoprotein lipase but failure of which promotes to liver conversion of free fatty acids into phospholipids and cholesterol and discharged into blood [66-68]. The findings implies that after 21 days repeatedly treatment of AV may be helpful for lowering the TC, TG, and LDL levels as well as at the same time improved significantly (P<0.05) HDL level near to normal range that shows that it may possess insulin-like activity which would be helpful to reduce the incidence of lipid born complications. The significant control on serum lipids is the desirable biochemical factor to prevent from atherosclerosis and ischaemic risk factors. These results are in agreement with Ruzaidi et al. (2005) [69] that used Cocoa bean extract 1, 2 and 3% on streptozotocin induced diabetic rat models whom found significant lowering of TG, TC, LDL and while improved HDL levels in dose-dependent manners.

It has been well accepted that SOD and CAT are the markers for the measurement of total antioxidant profile which provides protection of membranes and biological structures against oxidative damage by Reactive Oxygen Species (ROS) [70-72]. SOD is a first metallo-protein enzyme involved in conversion of super oxide anion radical (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$) while CAT in association with GPx normally detoxifying H$_2$O$_2$ and decomposition into H$_2$O and O$_2$ [73,74]. Alloxan treated diabetic rats were observed with decreased serum concentration of total antioxidant enzymes indicating an imbalance between ROS production and antioxidant scavenging systems [74]. Living organisms consisting of reduced glutathione (GSH) as major non-protein thiol that acts as co-substrate for GPx and important key role in coordinating the body’s defense processes [75-77]. Oral administration with AV mitigated the antioxidant profile significantly.

Elevated level of sensitive qualitative biomarker enzymes viz. SGOT, SGPT and ALP was reported in circulation of diabetic rats when compared with normal control rats reflecting hepatocellular damage and/ or indicative of liver mitochondrial injury [78-81]. During diabetes the insulin deficiency contributes to increased serum level of transaminase enzymes due to increased easily availability of amino acids which leads to enhanced occurrence of gluconeogenesis and ketogenesis processes [82]. Treatment of experimental groups with AV significantly reversed the elevated concentration of marker enzymes suggesting that the extract might hepatoprotective properties and restoration of normal functioning of liver.

Oxidative stress plays a major role in the elevation of lipid peroxidation (LPO) which may leads to imbalanced in-vivo antioxidant system and several biochemical alterations in body due to
alloxan induced diabetes [83]. In our study the diabetic rats shown a significant (p<0.05) increase of LPO level in serum. The result suggested that the reduced oxidative stress may be due to presence of compounds like flavonoids phenolic acids and polyphenols of plant which helps to trap or scavenge oxidative free radicals such as peroxides, lipid peroxyld, hydroperoxides etc. thereby improves body’s glucose homeostasis system [70].

It has been found that AV is a potential antidiabetic candidate in alloxan-induced diabetic model through reducing oxidative damage and modulating antioxidant enzymes. Further, isolation and establishment of exact mechanism of action of specific compound from AV is to be carried out in the future.

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