Effect of Fractions of Alcoholic Extract of Moringa oleifera lam. Bark on Dexamethasone induced Insulin Resistance in Rats

Hasanpasha N Sholapur*, Basanagouda M Patil†

*Department of Pharmacognosy, KLE University’s College of Pharmacy, Vidyanagar, Hubballi, Karnataka, INDIA.
†Department of Pharmacology, KLE University’s College of Pharmacy, Vidyanagar, Hubballi, Karnataka, INDIA.

ABSTRACT

Objective: Alcoholic extract of the bark of Moringa oleifera Lam. (MO), (Moringaceae), has been experimentally evaluated previously for its insulin sensitizing potentials. In the quest to explore the possibility of the class of phytochemical(s) responsible for this experimental claim, the alcoholic extract was fractionated and evaluated for insulin sensitizing effect in rat model for insulin resistance. Methods: Alcoholic extract of MO was fractionated into, non-polar (petroleum ether (PEF)), moderately non-polar [ethyl acetate (EAF)], and polar [aqueous (AQF)] fractions. The fractions obtained were investigated for their insulin sensitizing properties in dexamethasone induced insulin resistance in rats. The bioactive fraction was analysed by spectroscopy for further characterization of phytochemical(s) present. Results: Acute treatment for 4 h with dexamethasone (1 mg/kg i.p.) in rats led to the development of impaired oral glucose tolerance. Treatment with pioglitazone and EAF abolished dexamethasone induced oral glucose intolerance (OGT). Dexamethasone (1 mg/kg s.c., once daily for 11 d) administration led to the development of insulin resistance, characterised by fasting hyperglycemia, hyperinsulinemia, hypertriglyceridemia, impaired OGT and increased HOMA IR index. Treatments with EAF (140 mg/kg p.o.) and pioglitazone (PIO (10 mg/kg p.o)) significantly prevented dexamethasone induced metabolic changes. Similarly, treatment with AQF (95 mg/kg p.o) also significantly prevented metabolic changes due to dexamethasone except impaired OGT. In contrast PEF (15 mg/kg p.o.) failed to prevent these metabolic changes except hypertriglyceridemia. Conclusion: The present study reveals that triterpenoids and the polyphenols (procyanidin) class of phytochemicals detected in EAF of alcoholic extract of MO bark may be responsible for the prevention of dexamethasone-induced insulin resistance in rats.

Key words: Dexamethasone, insulin resistance, Moringa oleifera, oral glucose tolerance test, serum triglyceride.

Key message: This is the first report which confirms and speculates the presence of polyphenols and triterpenoids respectively in EAF of MO which may be responsible for preventing the development of insulin resistance in rat model.

Correspondence :
Dr. Hasanpasha N. Sholapur, Department of Pharmacognosy, KLE University’s College of Pharmacy, Vidyanagar, Hubballi-580 031, Karnataka, INDIA.
Phone: +91 836-2373174, Fax: +91 836-2371694
Email: hasanpashas@gmail.com; bmpatil59@hotmail.com
DOI: 10.5530/jyp.2017.9.81

INTRODUCTION

Diabetes, fourth leading causes of death worldwide is a cluster of hyperinsulinemia, dyslipidemia, glucose intolerance, obesity and hypertension.1 Insulin resistance, a prediabetic state of resistance towards the effects of insulin on its target tissues mainly in muscle, liver, and adipose tissue, is a central pathogenic feature of diabetes.2 Currently, PPARγ agonists are used as insulin sensitizers in the treatment of diabetes.3 Though, there are various techniques to manage diabetes and its complications, but still herbal formulations are the priority due to low treatment cost and lesser side effects. Around 80% of the world’s population depends on traditional medicines derived from plants. Among the 21,000 medicinal plants used, 800 of them have been reported to be used in the treatment of diabetes worldwide.4 Phytochemicals reported to have antidiabetic effect from these medicinal plants include alkaloids, triterpenoids, steroids, flavonoids, phenolics, iridoids, guaianolides etc., and these phytochemicals may also have potential beneficial effects on insulin resistance which may be investigated. Given that insulin resistance increases the risk of type 2 diabetes and cardiovascular diseases, modulatory effect on insulin resistance may be the important mechanism through which these phytoconstituents can offer protection. Hence identification of potentially effective class of phytochemicals for insulin resistance state represents an attractive and potentially effective approach to the problem.

Moringa oleifera Lam., (MO) syn., Moringa pterygosperma Gaertn., (Family-Moringaceae) commonly known as horseradish tree or drumstick tree.5 All parts of this plant are being employed for the treatment of various ailments in the indigenous system of medicine. Traditionally the bark is used as cardiac and circulatory stimulant.6 Experimental studies revealed, that the bark possess hypoglycaemic, antioxidant, cardioprotective effect and in our previous study we have reported the insulin sensitizing effect of the alcoholic extract of the bark.7 Various class of phytochemicals are reported in the bark which includes alkaloids (morinine and moringinine),8 glycosides (glucosinolates namely 4-(alpha-L-rhamnopyranosyloxy)-benzylglucosinolate),9 tannins, triterpenoids,10 and polyphenols (procyanidins).11 In order to explore the possibility of insulin sensitization by any of the reported class of phytochemical(s) present in the alcoholic extract of the bark,7 the present study was designed to test the various fractions of alcoholic extract in dexamethasone-induced chronic and acute rat model for insulin resistance.

MATERIALS AND METHODS

Chemicals

Dexamethasone (Dexona) injection was purchased from Zydus Cadila, Ahmedabad, India. Pioglitazone maleate was obtained as a gift sample from Torrent Pharmaceutical Ltd, Ahmedabad, India. Plasma biochemical estimation kits like glucose, triglyceride and total cholesterol were purchased.
form Erba Transasia Bio–Medicals Ltd, Baddi, Himachal Pradesh, India. Plasma insulin estimation kit was purchased form MP Biomedicals, LLC, Eschwege, Germany, (kit code no. CT Insulin, lot no. INK 1223) Instrument used was Gamma counter, ECIL.

**Plant material**

The fresh bark of MO was collected in the month of November & December, from the local areas of Hubli–Dharward, Karnataka, India. The bark was authenticated by botanist Dr.Ganesh R. Hegde, Professor, P.G. Department of Botany, Karnatak University, Dharward, Karnataka, India and a voucher specimen bearing no. DOUN09017 is maintained in the herbarium of department of pharmacognosy of KLES College of pharmacy, Hubli, Karnataka, India.

**Preparation of extract and fractions**

The collected fresh bark of MO was dried and powdered coarsely. The bark coarse powder of 250 g was macerated at room temperature with 3 × 1 L ethanol 80 %v/v. Following filtration, the combined alcoholic extract was concentrated in-vacuo below 40 °C and finally dried in desiccator.

The crude alcoholic extract was fractionated into non-polar [petroleum ether (PEF)], moderately non-polar [ethyl acetate (EAF)] and polar [aqueous (AQF)] fractions by reconstituting 10 g in 1000 ml water and the solution was fractionated successively in a separating funnel with petroleum ether (60–80) (3 × 500 ml) and ethyl acetate (3 × 500 ml) followed by evaporation of organic solvent layers. Finally, the remaining aqueous layer was evaporated to dryness under reduced pressure.

**Preliminary phytochemical investigation**

The fractions obtained were tested by qualitative chemical tests for the presence of phytoconstituents.

**HPTLC Analysis**

HPTLC analysis of the fractions were performed using precoated (silica gel 60 F 254, 0.25 mm thickness; size 20 x 20 cm; Merk, Darmstadt, Germany) TLC plates. Samples were prepared in methanol (10mg/ml) and were applied on the TLC plate by a Desaga auto sampler (AS 30 Desaga, Germany). The plates were developed in a CAMAG twin trough developing chamber (CAMAG, Muttenz, Switzerland) using chloroform and methanol (85:15) as mobile phase followed by air-drying twin trough developing chamber (CAMAG, Muttenz, Switzerland) using chloroform and methanol (85:15) as mobile phase followed by air-drying.

**Biochemical estimation**

The concentration of plasma biochemical levels like glucose, triglyceride, cholesterol and insulin were measured by glucose oxidation CHOD–POD enzymatic method,19 total cholesterol and plasma insulin were measured by glucose oxidation GPO–POD enzymatic method,20 and radioimmuno assay method21 respectively.

**Assessments of Insulin Resistance and Sensitivity**

The degree of insulin resistance was calculated by using Homeostasis Model Assessment (HOMA),20

\[ \text{HOMA} = \left( \frac{\text{fasting insulin} \times \text{fasting glucose}}{405} \right) \]
Statistical analysis
All the results are expressed as mean standard error mean (± S.E.M). Statistical analysis was carried out using Student’s t-test and One-way analysis of variance (ANOVA) followed by Dunnett’s test for multiple comparisons using GraphPad Prism 5 software. P values of less than 0.05 were regarded as significant (P<0.05).

Characterization of bioactive fraction
Fourier Transform Infrared (FT-IR) spectroscopy: A FT-IR spectrum was recorded on IR 200 Nicolet IR spectrophotometer (Thermo Electron Corporation, MA U.S.A.). Spectra were taken in KBr pellets. LC-MS analysis: EAF 100 mg was dissolved in 10 ml of methanol and was sonicated for 10 min at 30ºC in the ultrasonic bath. The sample was filtered through a 0.45-µm PVDF filter (Himedia labs. Pvt. Ltd., Mumbai, India) and was further used. Chromatography separation was performed using Surveyor Plus (Thermo Fisher Scientific, California, USA) HPLC system coupled to an MS analyser, LCQ Deca XP Max ion trap (Thermo finnigan, San Jose, CA, U.S.A.) with ESI interface using Xcalibur version 1.2 software for system control.

RESULTS
Preparation of extract and fractions
Three fractions were obtained viz., petroleum ether fraction (PEF) (yield 0.6 g, 6 %), ethyl acetate fraction (EAF) (yield 5.6 g, 56 %) and aqueous fraction (AQF) (yield 3.8g, 38 %).

Preliminary phytochemical investigation
Preliminary phytochemical studies exhibited the presence of steroids in PEF; triterpenoids and phenols in EAF; carbohydrates and glycosides in AQF respectively.

HPTLC Analysis
The HPTLC analysis of fractions revealed their finger print profiles. Petroleum ether fraction resolved 5 spots with Rf values 0.17, 0.20, 0.34, 0.56 and 0.67; ethyl acetate fraction resolved 7 spots with Rf values 0.25, 0.34, 0.41, 0.57, 0.65, 0.71 and 0.84; the aqueous fraction resolved 5 spots with Rf values 0.14, 0.39, 0.42, 0.54 and 0.59. Appearance of grey (with 10 % fecl3 reagent) or blue (LB reagent) spots was considered as positive evidence for the presence of phenols and triterpenes respectively (Figure 1).

Animal Experiment
Acute study
Glucose intolerance developed after acute treatment of rats with dexamethasone (Control AUC 9923±441.3 vs Dxa AUC 12650±90.78, P<0.001) was significantly prevented in EAF treated (Dxa+EAF AUC 10284±239.3, P<0.001) and PIO treated (Dxa+PIO AUC 9845±311.1, P<0.001) rats. In contrast, PEF treatment further aggravated the dexamethasone induced glucose intolerance (Dxa+PEF AUC 15788±527.1, P<0.001). AQF treatment had no effect on this parameter (Figure 2).

Figure 1: HPTLC chromatogram of PEF, EAF and AQF at UV 254nm.

Chronic study
As shown in Table 1, initial body weights of rats in all the groups were similar, dexamethasone treated rats lost the body weight significantly (P<0.001) compared to control rats. Treatment with PEF, EAF, AQF and PIO did not prevent the loss in body weight due to dexamethasone. Administration of dexamethasone showed significant increase in levels of fasting glucose (P<0.01), triglyceride (P<0.001), insulin (P<0.01) and HOMA IR (P<0.001). Treatment with EAF, AQF and PIO significantly (P<0.001) reduced fasting hyperglycemia, hypertriglyceridemia, hyper-insulinemia, HOMA IR and fasting hyperglycemia (EAF P<0.05; AQF P<0.05 and PIO P<0.001) induced by dexamethasone, whereas, treatment with PEF did not alter these metabolic changes. Dexamethasone, all the fractions and PIO tested did not show any effect on total cholesterol levels.
Administration of dexamethasone led to the development of impaired glucose tolerance as indicated by significant increase in the sum of glucose levels during the OGTT in this group compared to control (Control AUC 10310±590.2 vs Dexa AUC 13020±222.6, P<0.01). Treatment with PIO and EAF prevented dexamethasone-induced impaired glucose tolerance as indicated by significant reduction in sum of glucose levels during OGTT compared to dexamethasone group (Dexa+PIO AUC 10149±139.7, P<0.001; Dexa+EAF AUC 10529±314.5, P<0.001). Treatment with AQF had no effect on dexamethasone-induced glucose intolerance (Dexa+AQF AUC 12997±586.3). Further, treatment with PEF augmented significantly the effect of dexamethasone on the rise in sum of glucose levels during OGTT compared to dexamethasone group (Dexa+PEF AUC 16108±597.3, P<0.001) (Figure 3).

### Table 1: Effect of PEF, EAF, AQF and PIO on body weight, triglyceride, cholesterol, glucose and insulin levels in dexamethasone treated rats (Chronic study).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Body Weight</th>
<th>Triglyceride mg/dl</th>
<th>Cholesterol mg/dl</th>
<th>Glucose mg/dl</th>
<th>Insulin µU/ml</th>
<th>HOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>190.0±3.000</td>
<td>208.0±4.066</td>
<td>56.14±2.122</td>
<td>64.85±1.327</td>
<td>89.18±5.130</td>
<td>52.83±4.277</td>
</tr>
<tr>
<td>Dexa</td>
<td>198.7±0.843</td>
<td>157.2±2.725</td>
<td>297.8±12.29</td>
<td>53.34±9.602</td>
<td>109.6±0.680</td>
<td>78.00±3.890</td>
</tr>
<tr>
<td>Dexa+PEF</td>
<td>189.2±2.442</td>
<td>148.0±4.726</td>
<td>89.83±2.748</td>
<td>39.65±2.782</td>
<td>100.0±9.148</td>
<td>63.50±5.233</td>
</tr>
<tr>
<td>Dexa+EAF</td>
<td>189.0±3.588</td>
<td>152.0±4.606</td>
<td>74.98±5.596</td>
<td>39.61±3.586</td>
<td>92.83±7.441</td>
<td>42.67±6.020</td>
</tr>
<tr>
<td>Dexa+AQF</td>
<td>196.7±1.256</td>
<td>164.2±5.121</td>
<td>114.2±12.92</td>
<td>49.37±3.574</td>
<td>92.11±4.688</td>
<td>43.00±2.944</td>
</tr>
<tr>
<td>Dexa+PIO</td>
<td>196.7±1.202</td>
<td>152.0±2.955</td>
<td>70.62±3.433</td>
<td>40.16±3.448</td>
<td>89.79±2.909</td>
<td>40.33±3.007</td>
</tr>
</tbody>
</table>

Values are means ± SEM; Dexamethasone 1 mg/kg (Dexa); Dexamethasone+petroleum ether fraction 15 mg/kg (Dexa+PEF), Dexamethasone+ethyl acetate fraction 140 mg/kg (Dexa+EAF), Dexamethasone+aqueous fraction 95 mg/kg (Dexa+AQF), Dexamethasone+pioglitazone 10 mg/kg (Dexa+PIO).

*P< 0.01, *P< 0.001 Compared with Control rats.

*P< 0.05, *P< 0.001 Compared with Dexamethasone rats.

### Figure 2: (A) Total area under curve of OGTT values from zero to 120 min, from control, Dexa, Dexa+PEF, Dexa+EAF, Dexa+AQF, Dexa+PIO groups and (B) Glycemia levels after oral glucose charge in tolerance test (Acute study).

FT-IR spectroscopy was used to augment the findings of preliminary phytochemical investigations of bioactive EAF. The EAF spectra showed absorption at 3354.90 (O-H stretching 3400-3100), 22 2292.99 (asymmetric -C-H stretching 3000-2900), 22 1613.86 (-C=C- aromatic stretching), 22 1510.09 (-C=C-), 22 1266.01(C-O stretching 1300-1000), 22 807.24 and...
Figure 3: (A) Total area under curve of OGTT values from zero to 120 min, from control, Dexa, Dexa+PEF, Dexa+EAF, Dexa+AQF, Dexa+PIO groups and (B) Glycemia levels after oral glucose charge in tolerance test (Chronic study).

Values are means ± SEM; Area under curve (AUC); Dexamethasone 1 mg/kg (Dexa); Dexamethasone+Petroleum ether fraction 15 mg/kg (Dexa+PEF), Dexamethasone+Ethyl acetate fraction 140 mg/kg (Dexa+EAF), Dexamethasone+Aqueous fraction 95 mg/kg (Dexa+AQF), Dexamethasone+pioglitazone 10 mg/kg (Dexa+PIO).

bP ≤ 0.01 vs Control rats.

z P < 0.001 vs Dexamethasone rats.

Figure 4: FT IR Spectrum of EAF.

Figure 5: LC MS Spectrum of EAF.

615.33 which are characteristic for polyphenol (procyanidin type). The other absorptions at 1449.74 (C-H bending cyclic (CH2)n) and 1709.39 (C=O characteristic for esters 1710-1690) may be speculated due to the presence of triterpenes (Figure 4).

The LC MS and MS/MS (negative ion mode) analysis of bioactive EAF reveals that, some of the fragmentation values like m/z 576.98 and m/z 288.93 are in accordance with the reported fragmentation values for deprotonated molecule of (epi)catechin dimer and (epi)catechin monomer of a polyphenol (procyanidin type) indicating its presence as one of the phytochemical in the ethyl acetate fraction (Figure 5).

DISCUSSION

The results of the current study demonstrate that EAF of MO prevents whole body IR, whereas, AQF of MO prevents hepatic IR induced by dexamethasone.

Our previous study has demonstrated that alcoholic extract of MO bark prevented IR at the dose of 250mg/kg.7 The percentage yield of PEF, EAF
and AQF obtained from alcoholic extract in the present study were 6 %, 56 % and 38 % respectively. Based on these yield a quantity proportional to 250 mg was calculated for each fraction and employed as test dose. At the tissue level IR is observed in skeletal muscle, adipose tissue and liver. Skeletal muscles are generally considered as major peripheral tissue responsible for disposal of blood glucose and impaired glucose tolerance is characteristic of peripheral IR whereas hepatic IR is generally characterized by fasting hyperglycemia and hyperinsulinemia. Further, during IR antilipolytic effects of insulin is reduced which eventually leads to increased synthesis of TG from live. In the present study, rats treated for 11 days developed whole body IR characterized by fasting hyperglycemia, hyperinsulinemia, hypertriglyceridemia and impaired oral glucose tolerance. These results are consistent with previous report. In these rats EAF was effective in preventing both impaired oral glucose tolerance and fasting biochemical changes of IR, whereas, AQF was effective in abolishing only fasting biochemical changes of IR. These observations suggest that EAF was able to prevent dexamethasone induced IR in all target tissues of insulin, whereas, AQF was effective only at hepatic tissue. By employing euglycemic- hyperinsulinemia clamp study in rats it is reported that single dose of dexamethasone induces IR. Consistent with this report in the present study rats treated with single dose of dexamethasone developed IR as indicated by impaired oral glucose tolerance observed in these rats. Using this, model we tested the acute effect of different fractions on dexamethasone induced altered glucose uptake by peripheral tissues. Interestingly, only EAF could aboil dexamethasone induced impaired oral glucose tolerance, whereas PEF and AQF failed to modify this effect. These findings further support our observations made in chronic study that, EAF but not AQF of MO is effective in overcoming peripheral IR due to dexamethasone.

Qualitative, chemical identification tests and HPTLC analysis revealed the presence of polyphenols and triterpenoids in EAF of MO which may be responsible for abolishing only fasting biochemical changes of IR. These observations suggest that EAF was able to prevent dexamethasone induced IR in all target tissues of insulin, whereas, AQF was effective only at hepatic tissue. By employing euglycemic- hyperinsulinemia clamp study in rats it is reported that single dose of dexamethasone induces IR. Consistent with this report in the present study rats treated with single dose of dexamethasone developed IR as indicated by impaired oral glucose tolerance observed in these rats. Using this, model we tested the acute effect of different fractions on dexamethasone induced altered glucose uptake by peripheral tissues. Interestingly, only EAF could abol dexamethasone induced impaired oral glucose tolerance, whereas PEF and AQF failed to modify this effect. These findings further support our observations made in chronic study that, EAF but not AQF of MO is effective in overcoming peripheral IR due to dexamethasone.

CONCLUSION

This is the first report which confirms and speculates the presence of polyphenols and triterpenoids respectively in EAF of MO which may be responsible for preventing the development of IR in rat model. Isolation and pharmacological evaluation of phytoconstituents form these fractions may further confirm our finding.

ACKNOWLEDGEMENT

The authors are thankful to KLE University, Belgaum for providing the facilities to carry out the research.

CONFLICT OF INTEREST

Authors do not have any conflict of interest.

REFERENCES


