The methanolic fraction of *Centratherum anthelminticum* seed downregulates pro-inflammatory cytokines, oxidative stress, and hyperglycemia in STZ-nicotinamide-induced type 2 diabetic rats

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**A B S T R A C T**

This study aimed to ascertain the potential of *Centratherum anthelminticum* plant seeds (CAMFs) for the management of type 2 diabetes and its associated complications, CAMFs was initially tested on β-TC6 cells for H2O2-induced nuclear factor-κB (NF-κB) translocation effects. The result displayed that CAMFs significantly inhibited NF-κB translocation from cytoplasm into the nucleus, dose-dependently. Furthermore, a 12-week sub-chronic CAMFs study was carried out on streptozotocin (STZ)-nicotinamide–induced type 2 diabetic rats to evaluate glycemia, essential biochemical parameters, lipid levels, oxidative stress markers, and pro-inflammatory cytokine levels. Our study revealed that CAMFs reduced hyperglycemia by significantly increasing serum insulin, C-peptide, total protein, and albumin levels. In contrast, elevated blood glucose, glycated hemoglobin, lipids and enzyme activities were restored to near normal. CAMFs confirmed antioxidant potential by elevating glutathione (GSH) and reducing malondialdehyde (MDA) levels in diabetic rats. Interestingly, CAMFs also down-regulated elevated tumor necrosis factor α (TNF-α), interleukin (IL)-1β and IL-6 in the tissues and serum of the diabetic treated rats. We conclude that CAMFs exerted apparent anti-diabetic effects and demonstrated as a valuable candidate nutraceutical for insulin-resistant type 2 diabetes and its associated complications such as dyslipidemia, oxidative stress, and inflammation.

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promotes low-grade inflammation via activation of nuclear factor-κB (NF-κB) [Arkan et al., 2005] and provokes an inflammatory process accompanied by local production and secretion of pro-inflammatory cytokines and chemokines (Hotamisligil et al., 1995; Jager et al., 2007). It has been hypothesized that DM2 is a manifestation of an ongoing acute-phase response that is primarily characterized by alterations of the so-called acute-phase proteins, such as C-reactive protein (CRP) [Pickup and Crook, 1998; Pickup et al., 1997], with other cytokines that are central mediators of inflammatory reactions, such as interleukin (IL) 6, IL-1α, or tumor necrosis factor α (TNF-α). It is well established that cytokines operate as a network in stimulating the production of acute-phase proteins. For example, the effects of IL-6 on CRP synthesis largely depend on its interaction with IL-1β [Joachim, 2003]. The pro-inflammatory cytokine TNF-α reduces insulin sensitivity in muscle tissue and stimulates hepatic lipogenesis and hyperlipemia [Franckhauser et al., 2008]. Therefore, it appears that treatments aimed at reducing the degree of oxidative stress and the production of pro-inflammatory cytokines in DM2 is warranted.

Centratherum anthelminticum (L) kuntze (bitter cumin) is a member of the Asteraceae family and is an important plant of great significance and usage in Ayurvedic medicine. The records kept by traditional healers and ethno-botanists state that it is useful in alleviating diabetes. Experimental studies have proven the pharmacological potential of this plant in diverse biological activities, some of which are anti-cancer, anti-diabetic, and anti-inflammatory (Ani and Naidu, 2008; Arya et al., 2012a,b; Fatima et al., 2010). Nevertheless, researchers have yet to investigate the hypoglycemic action of sub-chronic administration of C. anthelminticum seed crude methanolic fractions (CAMFs) or the plant’s other healing properties, some of which might act against other inflammatory processes and oxidative stress associated with DM2.

In the present study, we attempted to gain a better understanding of the effect of CAMFs on ROS-associated insulin resistance signaling pathways by investigating the role of CAMFs in H2O2-induced NF-κB activation in mouse pancreatic β-TG cells. Subsequently, we carried out in vivo studies to determine whether long-term (sub-chronic) administration of CAMFs exerts anti-hyperglycemic, anti-hyperlipidemic, anti-oxidant, and inflammatory cytokines inhibitory effects in streptozotocin (STZ)-nicotinamide–induced type 2 diabetic rats.

2. Materials and methods

2.1. Preparation of CAMFs

2.1.1. Collection of plant material

Dried C. anthelminticum seeds were procured from the medicinal plant cultivation zone of Arumicht Bio-Botanica Herbs Research Laboratory Pvt. Ltd., (Madhya Pradesh, India) in April 2008. The seeds were botanically classified and authenticated by the company’s quality control department. Voucher specimens (CA-9) were deposited with the company and with the Department of Pharmacology in the Faculty of Medicine at the University of Malaya.

2.1.2. Extraction and fractionation

Two kilograms of seeds were coarsely powdered and first extracted with 100% n-hexane using hot extraction with a Soxhlet extractor for 24 h. Further fractionation of the obtained defatted residue was carried out using 100% chloroform, and lastly with 100% (absolute) methanol. The solvents from each crude fractions were dried by rotary evaporation under reduced pressure at a maximal temperature of 40 °C. The final fraction was then freeze-dried to yield a crude methanolic fraction (CAMFs), that was stored at −20 °C until further use. Thereafter, CAMFs was subjected to mass spectrometry analysis by using LCMS-MS, for the qualitative analysis of major compounds, as well as evaluated for the total phenolic and flavonoid contents. The mobile phase consists of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (ACN) by gradient elution at a flow rate of 0.4 mL/min with an injection volume of 20 μL. Separation of the compounds was performed using a Luna 3-μm RP C18 column (100 × 2.00 mm; Phenomenex). The turbo ion source settings were as follows: capillary voltage, −4000 V; dry gas flow (N2), 9 L/min; nebulizer pressure, 35 psi; and capillary temperature, 365 °C. A full scan of the mass spectra was recorded from m/z 20 to m/z 1000. The acquisition data was processed with Analyst Software version 1.5.1. Compounds were characterized based on their UV spectra and MS2 and MS3 fragmentations spectra data by correlation with previous reports (Table 1). Compounds F, G, H, I and J in the table are unknown compounds that will be subjected to further purification and confirmation by nuclear magnetic resonance (NMR) analysis in a subsequent study.

2.1.4. Determination of total phenolic content

The total phenolic content in CAMFs was determined by adapting the method as published in our previous article (Arya et al., 2012a,b). In brief, CAMFs was prepared in a concentration of 10 mg/mL in methanol. Five microliters of this solution were transferred to 96-well microplate (TPP, USA). To this, 80 μL of Folin–Ciocalteu reagent (1:10) were added and mixed thoroughly. After 5 min, 160 μL of sodium bicarbonate solution (NaHCO3 7.5%) were added and the mixture was allowed to stand for 30 min with intermittent shaking. Absorbance was measured at 765 nm using microplate reader (Molecular Devices, Sunnyvale, USA). The TPC was expressed as gallic acid equivalent (GAE) in mg/g fraction, obtained from the standard curve of gallic acid.

The gallic acid standard curve was established by plotting concentration (mg/mL) versus absorbance (nm) (y = 0.001x + 0.045; R2 = 0.9975), where y is absorbance and x is concentration in GAE (n = 3).

2.1.5. Determination of total flavonoid content

The total flavonoid content in CAMFs was determined by following the method published in our previous article (Arya et al., 2012a,b). In brief, 5 mL of 2% aluminum trichloride was mixed with the same volume of all the plant extracts. Absorbance readings at 415 nm were taken after 10 min against a blank sample consisting of 5 mL of sample solution and 5 mL of methanol without aluminum trichloride. The total flavonoid content was determined using a standard curve of mg Quercetin (Q) equivalent to10 mg. The average of three readings was used and then expressed as quercetin equivalents (QE) on a dry weight (DW) basis.

2.2. In vitro assay

2.2.1. Cell culture

Mouse pancreas β-TG cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in 15% fetal bovine serum (FBS) in Dulbecco’s Modified Eagle Medium (DMEM). Cultures were maintained at 37 °C in 5% CO2 in a humidified incubator. The growth medium was changed every 3 days.

2.2.2. NF-κB translocation assay

We seeded 1.5 × 105 cells/mL onto a 96-well plate. The cells were pre-treated for 1 h with 6.25, 12.5, or 25 μg/mL CAMFs, or were left untreated. The cells were then stimulated for NF-κB translocation with 50 μM of H2O2 for 30 min. NF-κB staining was performed according to the manufacturer’s instructions with an NF-κB activation kit (Cellomics Inc., Pittsburgh, PA, USA). We used the Ariza (growth and content screening (HCS) system (Cellomics Inc., Pittsburgh, PA, USA) to quantify the difference between the intensity of nuclear and cytoplasmic NF-κB-associated fluorescence.

2.3. Preclinical studies

2.3.1. Experimental animals

We obtained Sprague–Dawley rats weighing 180–200 g from the Animal Care Unit of the University Malay University Medical Centre (Kuala Lumpur, Malaysia) and maintained them under pathogen-free conditions in the animal housing unit in a temperature (23 ± 2 °C) and light-controlled (12 h light/dark cycle) room with 35–60% humidity. The animals were acclimatized for 10 days prior to the experiments and were provided rodent chow and water ad libitum.

The animal experiments were performed in accordance with the guidelines for animal experimentation issued by the Animal Care and Use Committee at the University of Malaya (Ethics Number: FAR/10/11/2008/AA[R]) and was conducted in accordance with internationally accepted principles for laboratory animal use and care.

2.3.2. Oral acute toxicity studies

CAMFs oral acute toxicity tests were carried out according to the guidelines of the Organization for Economic Co-operation and Development (OECD). For these test, we used healthy adult Sprague Dawley rats of either sex (180-200 g). These rats were fasted overnight, divided into 6 groups (n = 6), and orally fed with CAMFs in doses of 10, 20, 50, 100 and 500 mg/kg: the control group was given distilled water. We observed the rats for 1 h continuously and then hourly for 4 h for any
changes in the blood glucose levels and finally after every 24 h up to 14 days for any physical signs of toxicity, such as writhing, gasping, palpitation and decreased respiratory rate or for any lethality.

### 3. Results

#### 3.1. Total phenolic and flavonoid contents in CAMFs

The total phenolic and flavonoid contents in CAMFs was determined to be (665.3 ± 188.8 mg GAE/g, and 98.2 ± 27.6 mg Quercetin/g).

#### 3.2. CAMFs analysis by LC–MS–MS

The LC–MS–MS phytochemical analysis detected quercetin glycoside, 3,4–0-dicaffeoylquinic acid, caffeic acid, naringenin–7–O–glucoside and kaempferol as the major compounds in CAMFs, as well as with other unknown compounds (Table 1 and Fig. 1). The fragmentation patterns of known compounds were in agreement with those documented in the literatures.

#### 3.3. Effect of CAMFs on NF-κB activation

We tested CAMFs for its in vitro inhibitory effects against H₂O₂-induced NF-κB translocation. NF-κB was detected in the cytoplasm, but not in the nucleus of non-H₂O₂–induced cells. There was a significant increase in NF-κB staining in the nucleus area when cells were stimulated with H₂O₂ alone, suggesting that NF-κB translocated from the cytoplasm into the nucleus. However, treatment with 25 μg/mL CAMFs significantly inhibited H₂O₂–induced NF-κB translocation (Fig. 2). In addition, CAMFs had no cytotoxic effects on β-Tc6 cell proliferation as compared to untreated control cells (data not shown).

#### 3.4. Acute toxicity study

No lethality or toxic reactions were observed at any graded doses of CAMFs up to a dosage of 500 mg/kg. Hence, produced no alterations in the blood glucose levels, and on the general behavior of the rats during the whole experimental period. Based on these findings, the concentration was fixed as 50, 25 and 10 mg/kg; which were used as the maximum doses for

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### Table 1

Characterization of phenolic compounds in CAMFs by LC–MS/MS.

<table>
<thead>
<tr>
<th>Tentative compounds</th>
<th>[M+H]+ (m/z)</th>
<th>Major fragment ions (m/z)</th>
<th>UV λ_{max} (nm)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Quercetin glycoside</td>
<td>463</td>
<td>301, 287, 271</td>
<td>220, 300</td>
<td>Lin and Harrny (2007)</td>
</tr>
<tr>
<td>B 3,4–0-Dicaffeoylquinic acid</td>
<td>515</td>
<td>353, 191, 179, 135</td>
<td>240, 300</td>
<td>Gouveia and Castilho (2011) and Lin and Harrny (2008)</td>
</tr>
<tr>
<td>C Caffeic acid</td>
<td>179</td>
<td>135</td>
<td>220, 325</td>
<td>Luo et al. (2003), Lin and Harrny (2008) and Plazonic et al., 2011</td>
</tr>
<tr>
<td>D Naringenin–7–O–glucoside</td>
<td>433</td>
<td>271, 153</td>
<td>200, 300</td>
<td>Charrouf et al. (2007)</td>
</tr>
<tr>
<td>E Kaempferol</td>
<td>287</td>
<td>287, 151</td>
<td>220, 340</td>
<td>Sun et al. (2007)</td>
</tr>
<tr>
<td>F Unknown</td>
<td>377</td>
<td>253, 215, 135</td>
<td>200, 300</td>
<td>–</td>
</tr>
<tr>
<td>G Unknown</td>
<td>303</td>
<td>181, 167, 135</td>
<td>220,300</td>
<td>–</td>
</tr>
<tr>
<td>H Unknown</td>
<td>341</td>
<td>303, 167, 135</td>
<td>200, 300</td>
<td>–</td>
</tr>
<tr>
<td>I Unknown</td>
<td>539</td>
<td>377, 221</td>
<td>200, 300</td>
<td>–</td>
</tr>
</tbody>
</table>
3.5. Effect of CAMFs on blood glucose levels

From the first day up until the end of the 12-week treatment period, monitoring of the rats' fasting blood glucose levels revealed a significant reduction in the elevated blood glucose levels of diabetic rats treated with CAMFs compared to that of untreated diabetic rats (Table 2). At weeks 3 and 6, the inhibition percentage of blood glucose levels in CAMFs-treated diabetic rats was lower compared to that of glibenclamide-treated diabetic rats. At weeks 9 and 12, the percentage inhibition in rats treated with a higher concentration of CAMFs was greater than that in glibenclamide-treated rats. At the end of the treatment period, the percentage inhibition effected by 50, 25, and 10 mg/kg bw CAMFs was...
75.86%, 69.82%, and 65.08%, respectively, compared to that of glibenclamide (68.53%). The blood glucose levels of normal rats were not greatly altered after week 12 when compared to that of week 0, whereas that of the untreated diabetic rats increased to 23.2 mmol/L after 12 weeks as compared to 12.3 mmol/L at week 0.

3.6. Effect of CAMFs on serum biochemical parameters

After the 12-week treatment period, serum insulin, C-peptide, total protein, and albumin levels in diabetic untreated rats were significantly reduced, while their HbA1c levels were significantly elevated compared to that of the normal control group. Upon treatment with CAMFs or glibenclamide, serum insulin, C-peptide, total protein, and albumin levels in diabetic rats were significantly elevated, and CAMFs significantly inhibited HbA1c levels in a dose-dependent manner compared to that of the diabetic control rats (Fig. 3), indicating significant improvement in glycemic control by CAMFs in diabetic rats.

In addition, there was a significant improvement in the overall body weights of CAMFs-treated diabetic rats compared to untreated diabetic rats at the end of the treatment period, and we
3.7. Effect of CAMFs on hyperlipidemia

Fig. 5 depicts the effect of CAMFs and glibenclamide on serum TG, TC, HDL-C, LDL-C, and FFA activity in treated and untreated diabetic rats and in normal control rats. Serum TG, TC, LDL-C, and FFA levels in untreated diabetic rats were significantly elevated when compared to that of normal control rats, while the HDL-C levels in untreated diabetic rats were significantly decreased compared to those in normal rats.

Serum TG, TC, LDL-C, and FFA levels were significantly reduced after treatment with glibenclamide and CAMFs; CAMFs effected a dose-dependent reduction, and HDL-C levels were significantly elevated compared to that of untreated diabetic rats after the 12-week treatment period. Evidently, continuous treatment with CAMFs decreased these lipid parameters in diabetic rats to near normal levels.

3.8. Effect of CAMFs on enzyme markers

Table 3 illustrates the effects of CAMFs on AST, ALT, ALP, and \( \gamma \)-GT enzyme activities in the serum, liver, and kidney of treated and untreated diabetic rats and in that of normal rats. The enzyme activities in the serum and liver of the untreated diabetic rats were significantly increased; ALP and \( \gamma \)-GT activities in the kidney were increased, while AST and ALT activities were not affected. Near-normalization of the AST, ALT, ALP, and \( \gamma \)-GT activities in the serum, liver, and kidney in diabetic rats was achieved after 12-week administration of glibenclamide and CAMFs; CAMFs effected normalization dose-dependently.

3.9. Effect of CAMFs on oxidative stress markers

GSH levels in untreated diabetic rats were reduced while MDA levels were significantly elevated compared to that of normal control rats (Fig. 6). Compared to untreated diabetic rats, GSH levels in the liver and pancreas of diabetic rats were significantly elevated upon administration of glibenclamide and CAMFs; CAMFs effected elevation dose-dependently, although not much change was observed in kidney GSH levels. There was maximum reduction of MDA levels in the liver of diabetic rats treated with glibenclamide and CAMFs compared to that of untreated diabetic control rats; reduction was effected dose-dependently by CAMFs, while there was a significant reduction of MDA in the pancreas following administration of glibenclamide and 50 mg/kg bw CAMFs. In the kidney, only glibenclamide suppressed MDA levels significantly, with CAMFs effecting a slight reduction in a dose-dependent manner.

3.10. Effect of CAMFs on pro-inflammatory cytokines level

The levels of the inflammatory cytokines TNF-\( \alpha \), IL-1\( \beta \), and IL-6 in the pancreas, kidney, and serum of diabetic untreated rats were significantly elevated compared to that of normal control rats (Fig. 7). Compared to untreated diabetic rats, the levels of TNF-\( \alpha \) in the pancreas, kidney and serum of diabetic treated rats were significantly down-regulated by daily administration of glibenclamide and CAMFs after the 12-week period; CAMFs down-regulated these parameters dose-dependently. In the same manner, glibenclamide and CAMFs significantly reduced the IL-1\( \beta \) and IL-6 levels in the pancreas, with non-significant reduction being observed in the kidney and serum after the 12-week study period.

4. Discussion

In an effort to uncover novel and effective treatment from plants traditionally used in India for the management of diabetes mellitus and its associated complications, we evaluated the hypoglycemic effects of a number of plants in an in vivo experiment and selected the most potent plant, namely, C. anthelminticum, whose seed yields a fraction that exerts maximum glycemic control. To the best of our knowledge, this is the first in vitro cell-based and...
in vivo preclinical study on the crude methanolic fraction of the seed (CAMFs), which demonstrated great therapeutic utility in the management of elevated complications associated with type 2 diabetic conditions.


Fig. 2. Stained β-TC6 cells were treated with different concentrations of CAMFs for 1 h and stimulated for 30 min with 50 μM H2O2 (NF-κB activation). (A) Dose–response histogram of CAMFs-treated β-TC6 cells for quantitative image analysis of NF-κB translocation. (B) Representative images of control, H2O2, and H2O2 pre-treated cells treated with 25.0 μg/mL CAMFs. *Significant difference compared to non-H2O2–treated cells (P < 0.05). †Significant difference compared to H2O2-treated cells (P < 0.05).

Table 2
Effects of CAMFs and glibenclamide on blood glucose levels of normal, diabetic control, and diabetic treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting blood glucose level (mmol/L)</th>
<th>Pretreatment period</th>
<th>Treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 0</td>
<td>Week 3</td>
</tr>
<tr>
<td>Normal control</td>
<td>3.9 ± 0.23 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>12.3 ± 0.44 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glibenclamide (50 mg/kg)</td>
<td>12.2 ± 0.65</td>
<td>6.9 ± 0.33 a</td>
<td>6.6 ± 0.48 a</td>
</tr>
<tr>
<td>CAMFs (50 mg/kg)</td>
<td>11.9 ± 0.53</td>
<td>7.8 ± 0.34 a</td>
<td>6.7 ± 0.32 a</td>
</tr>
<tr>
<td>CAMFs (25 mg/kg)</td>
<td>12.1 ± 0.46</td>
<td>8.8 ± 0.65 a</td>
<td>7.5 ± 0.47 a</td>
</tr>
<tr>
<td>CAMFs (10 mg/kg)</td>
<td>12.3 ± 0.51</td>
<td>9.8 ± 0.43 a</td>
<td>8.7 ± 0.77 a</td>
</tr>
<tr>
<td>Glibenclamide (50 mg/kg)</td>
<td></td>
<td>(53.69)</td>
<td>(62.92)</td>
</tr>
<tr>
<td>CAMFs (50 mg/kg)</td>
<td></td>
<td>(19.9 ± 1.09 a)</td>
<td>(19.9 ± 1.09 a)</td>
</tr>
<tr>
<td>CAMFs (25 mg/kg)</td>
<td></td>
<td>(17.8 ± 0.89 a)</td>
<td>(17.8 ± 0.89 a)</td>
</tr>
<tr>
<td>CAMFs (10 mg/kg)</td>
<td></td>
<td>(14.9 ± 0.64 a)</td>
<td>(14.9 ± 0.64 a)</td>
</tr>
</tbody>
</table>

Values denote mean ± SD, n = 9–10. Values in parentheses denote that group's percentage decrease in blood glucose level when compared to the diabetic control group in that week.

* Mean values that are significantly different from each other as revealed by the Tukey–Kramer multiple comparisons test (P < 0.05).
CAMFs to diabetic rats for 12 weeks significantly reduced blood glucose levels, thereby increased insulin and C-peptide levels. Elevated insulin levels in diabetics usually normalize the serum and tissue proteins by increasing protein synthesis, decreasing protein degradation or protein glycosylation, supporting our study (Almdal and Vilstrup, 1988). In addition, we observed a reduction in HbA1c level. High HbA1c level has been linked to micro- and macrovascular diabetes complications (Selvin et al., 2004), while decline in HbA1c level reduced morbidity and mortality (Wagner et al., 2001). The recovery of body and organ weights observed in the CAMFs-treated diabetic rats could be due to an improvement in insulin secretion and glycemic control produced by CAMFs. In line with the normalized serum lipid levels, CAMFs restored elevated levels of the enzyme markers AST, ALT, ALP, and c-GT to normal in the serum, liver, and kidney of diabetic rats. This suggests that CAMFs can be helpful in preventing hepatocellular damage and tissue necrosis through suppression of gluconeogenesis.

Furthermore, we studied the role of ROS in the development of DM2 by activating stress signaling pathways known to participate in insulin signaling pathways, such as the ROS-induced NF-kB activation pathway (Barnes and Karin, 1997; Bierhaus et al., 2001; Mohamed et al., 1999; Schreck et al., 1992). Our result demonstrated that CAMFs inhibited the H$_2$O$_2$-induced NF-kB translocation from the cytoplasm into the nucleus in β-TG cells (Fig. 2). Moreover, in line with the inhibitory effects of NF-kB translocation, we confirmed antioxidant effects by investigating oxidative stress markers, i.e., GSH and MDA levels in CAMFs treated and untreated diabetic rats, which showed that CAMFs dose-dependently augmented GSH production in the pancreas, kidney, and liver of diabetic rats, with reduction caused in MDA levels, suggesting CAMFs antioxidant ability. It is well established that GSH is an intracellular antioxidant with several biological functions, such as cellular protection against oxidation, which is one of the more important GSH functions because its sulphydryl (SH) group is a strong nucleophile that confers antioxidant protection and protects DNA, proteins, and other biomolecules from ROS (Fang et al., 2002). In this regard, an increased level of GSH implicates augmentation of the antioxidant capacity and reduced peroxidation of membrane lipids, whose principal end product is MDA, which is a marker of damage caused by oxidative stress (Johansen et al., 2005; Pastore et al., 2003). However, we should not disregard the fact that the antioxidant property of CAMFs may be, in part, a result of the reduction in hyperglycemia and elevated pro-inflammatory cytokines levels as observed in the diabetic rats.

As we know, DM2 is linked with oxidative stress resulting from free radicals/ROS. ROS act as intercellular second messengers downstream of many signaling molecules, including transcription factors (NF-kB), which mediate vascular smooth muscle cell (SMc) growth/migration and the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (Reuter et al., 2010; Touyz, 2004). These elevated pro-inflammatory cytokines possess antagonistic properties to insulin because of their ability to augment insulin receptor substrate (IRS) phosphorylation, leading to insulin resistance (Emanuelli et al., 2000; Senn et al., 2003; Steinberg, 2007; Tataranni and Ortega, 2005). Therefore, inhibition of H$_2$O$_2$-induced NF-kB translocation in β-TG cells and ameliorating oxidative stress in diabetic rats provides a possible explanation why the levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) were...
reduced in serum and in the pancreas of CAMFs treated diabetic rats, suggesting beneficial anti-inflammatory effect of CAMFs on insulin resistance in DM2.

Taken together, our data indicate that 50 mg/kg bw of CAMFs possessed effects that were highly similar to the same dose of glibenclamide. Interestingly, this might be due to the presence of...
Fig. 6. Antioxidant effect of CAMFs on the pancreas, kidney, and liver of STZ-nicotinamide–induced diabetic rats in comparison with normal and diabetic control rats after the 12-week treatment period. At the end of the treatment, the organs were removed, homogenized, and centrifuged to collect the tissue supernatant. Panels denote (A) pancreas, (B) kidney, and (C) liver, respectively, and GSH and MDA levels. Data are presented as means ± SD (n = 9–10). *Significant difference compared to the normal control group (P < 0.05).

Table 3
Effect of CAMFs and glibenclamide on enzymes marker in the serum, liver, and kidney of STZ-nicotinamide–induced diabetic rats in comparison with normal and diabetic control rats after the 12-week treatment period.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST a</th>
<th>ALT b</th>
<th>ALP c</th>
<th>γ-GT d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>49.9 ± 3.66 e</td>
<td>43.7 ± 2.64 e</td>
<td>67.1 ± 3.14 e</td>
<td>19.7 ± 1.63 e</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>87.5 ± 3.73 e</td>
<td>72.2 ± 3.05 e</td>
<td>107.3 ± 4.49 e</td>
<td>33.4 ± 1.05 e</td>
</tr>
<tr>
<td>Glibenclamide (50 mg/kg)</td>
<td>55.4 ± 2.92 e</td>
<td>48.6 ± 2.03 e</td>
<td>69.4 ± 4.23 e</td>
<td>21.1 ± 1.01 e</td>
</tr>
<tr>
<td>CAMFs (25 mg/kg)</td>
<td>62.2 ± 2.33 e</td>
<td>44.2 ± 1.36 e</td>
<td>57.6 ± 2.07 e</td>
<td>19.8 ± 1.75 e</td>
</tr>
<tr>
<td>CAMFs (10 mg/kg)</td>
<td>73.7 ± 3.12</td>
<td>63.7 ± 2.47</td>
<td>81.2 ± 6.41 e</td>
<td>30.1 ± 2.73</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>723.7 ± 13.6 e</td>
<td>883.8 ± 17.25 e</td>
<td>0.19 ± 0.52 e</td>
<td>3.11 ± 0.26 e</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>937.2 ± 20.18 e</td>
<td>1259.6 ± 26.31 e</td>
<td>0.36 ± 0.21 e</td>
<td>5.53 ± 1.21 e</td>
</tr>
<tr>
<td>Glibenclamide (50 mg/kg)</td>
<td>754.3 ± 17.38 e</td>
<td>905.2 ± 16.42 e</td>
<td>0.22 ± 0.23</td>
<td>3.31 ± 0.87</td>
</tr>
<tr>
<td>CAMFs (50 mg/kg)</td>
<td>757.5 ± 18.23 e</td>
<td>876.8 ± 22.43 e</td>
<td>0.20 ± 0.13</td>
<td>3.01 ± 0.13 e</td>
</tr>
<tr>
<td>CAMFs (25 mg/kg)</td>
<td>807.2 ± 19.36</td>
<td>923.4 ± 21.41</td>
<td>0.29 ± 0.76</td>
<td>3.82 ± 0.17</td>
</tr>
<tr>
<td>CAMFs (10 mg/kg)</td>
<td>893.4 ± 22.46</td>
<td>1035.5 ± 21.32</td>
<td>0.33 ± 0.26</td>
<td>4.23 ± 0.72</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>848.2 ± 19.31</td>
<td>935.7 ± 21.67</td>
<td>0.43 ± 0.16 e</td>
<td>2.96 ± 0.87 e</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>839.4 ± 18.36</td>
<td>929.4 ± 21.61</td>
<td>0.98 ± 0.56 e</td>
<td>4.21 ± 0.74 e</td>
</tr>
<tr>
<td>Glibenclamide (50 mg/kg)</td>
<td>852.4 ± 24.26</td>
<td>927.3 ± 24.18</td>
<td>0.50 ± 0.32 e</td>
<td>3.02 ± 0.21</td>
</tr>
<tr>
<td>CAMFs (50 mg/kg)</td>
<td>846.3 ± 22.43</td>
<td>944.5 ± 20.78</td>
<td>0.39 ± 0.13 e</td>
<td>2.70 ± 0.32 e</td>
</tr>
<tr>
<td>CAMFs (25 mg/kg)</td>
<td>826.1 ± 18.21</td>
<td>903.2 ± 20.13</td>
<td>0.58 ± 0.34</td>
<td>3.16 ± 0.56</td>
</tr>
<tr>
<td>CAMFs (10 mg/kg)</td>
<td>818.9 ± 21.23</td>
<td>896.5 ± 22.79</td>
<td>0.68 ± 0.23</td>
<td>3.53 ± 0.19</td>
</tr>
</tbody>
</table>

Values denote mean ± SD, n = 9–10.

Units of measurement (per L) for AST and ALT: µmol of pyruvate liberated/h; ALP: µmol of phenol liberated/min; γ-GT: mol of p-nitroaniline liberated/min.

a Aspartate transaminase.
b Alanine transaminase.
c Alkaline phosphatase.
d γ-Glutamyltransferase.

* Mean values that are significantly different from each other as revealed by the Tukey–Kramer multiple comparisons test (P < 0.05).
the phytochemicals in CAMFs, such as quercetin glycoside, 3,4-0–
dicaffeoylquinic acid, caffeic acid, naringenin-7–0-glucoside and
kaempferol. Several studies on these compounds have demon-
strated antidiabetic, antioxidant and anti-inflammatory properties
(Jung et al., 2006; Ortiz-Andrade et al., 2008; Shih et al., 2012).
Thus, the combination of these compounds in CAMFs may be
responsible for the synergistic effects observed in STZ-nicotin-
amide induced type 2 diabetic rats.

5. Conclusion

The crude methanolic fraction of C. anthelminticum seeds (CAM-
Fs) displayed inhibitory effects on NF-κB translocation in H2O2-
stimulated β-TC6 cells. This observation was corroborated with
the beneficial effects of CAMFs in attenuating hyperglycemia in
an animal model of type 2 diabetes, by down-regulating elevated
levels of pro-inflammatory cytokines and oxidative stress markers.
Thus, the traditional basis of C. anthelminticum use has been sci-
centifically verified using a subchronic study model. These findings
pave the way for a novel approach to potential treatments of insu-
lin-resistant type 2 diabetes and propose CAMFs as a valuable can-
didate nutraceutical for the type 2 diabetic complications.

Conflict of Interest

The authors disclose no conflicts of interest.

Role of the funding source

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sign; in the collection, analysis, and interpretation of data; in the
writing of the report; and in the decision to submit the article for
publication.

8. Uncited references

Bjornholm and Zierath (2005), Kraegen et al. (1993), Slot et al.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in
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