ISSN: 2714-4674 (Online)
ISSN: 2714-4666 (Print)

Annals of Clinical and Experimental Medicine

(ACEMedicine)

This Journal Is A Publication of
ASSOCIATION OF SPECIALIST MEDICAL DOCTORS IN ACADEMICS SOKOTO STATE CHAPTER

Volume 2, No. 1, January - June 2021

In this issue

www.acemedicine.asmeda.org

Crude Stem Bark Extract of *Vitellaria paradoxa* Improves Diabetic Symptoms and Modulates Some Plasma Cytokine Activities in Alloxan Induced Diabetic Rats

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ACCESS TO THIS ARTICLE ONLINE

DOI: 10.47838/acem.26011977.127122021.asmeda.1.3

Website
https://www.asmeda.org/acemedicine

Abstract

Background: *Vitellaria paradoxa* tree is found in most parts of Nigeria with a variety of ethnomedicinal importance. Many bioactive components with good anti-bacterial, anti-fungal, anti-viral and anti-arthritic activities have been documented in this plant. This study investigates the immunomodulatory effect of crude stem bark extract of *Vitellaria paradoxa* in alloxan-induced diabetic rats by serum cytokine quantification.

Methods: Thirty (30) Wistar rats of both sexes were allotted into five groups of 6 rats each: Group I: Non diabetic control, Group II: diabetic control, Group III: diabetic treated with 100 mg/kg crude stem bark extract of *Vitellaria paradoxa,* Group IV diabetic treated with 200 mg/kg crude stem bark extract of *Vitellaria paradoxa* and Group V: diabetic treated with 200 mg/kg Metformin. A single dose of Alloxan, 150 mg/kg was used to induce diabetes in rats. Tumour Necrosis Factor Alpha (TNF -α), Interleukin (IL)-6, and Interleukin (IL) -10 were assayed.

Results: Treated diabetic groups were compared with controls. Induced hyperglycaemia increased the concentration of TNF-α and IL-6 but decreased the concentration of IL-10. Significant reduction in the levels of TNF-α and IL-6 was observed in diabetic treated rats. Significant increase in the levels of IL-10 and Neutrophil to Lymphocyte Ratio were observed in diabetic treated rats.

Conclusion: This study has validated the folkloric claim of the anti-hyperglycaemic, anti-diabetic and immunomodulatory properties of the crude stem bark extract of *Vitellaria paradoxa* in experimental diabetic rats.

Keywords: Alloxan, Cytokines, Immunomodulation, Pro-Inflammatory, Phytotherapy

Introduction

Diabetes is a common, unbearable metabolic disease which is typically managed with medications like insulin injection, glibenclamide and Metformin drugs. However, the cost of management therapy and accompanying side effects of these drugs remain a major concern (1). Therefore, there is the need for an alternate, cost–effective way of managing the disease. Phytotherapy could be an alternative means to improve health care globally (2). Medicinal plants are locally available and therefore easily accessible regardless of social status of individuals (3). Phytochemicals offer a remarkable hope for the discovery of new varieties of therapeutics. As a result, efforts are being geared globally towards the use of these medicinal plants which possess significant number of phytochemicals exhibiting diverse beneficial effects in tackling diabetes and associated complications (4).

Cytokines are implicated in diabetic microvascular complications (5-8), therefore, the development of desirable therapeutic strategies in order to prevent this complication become necessary. Interestingly, however natural products possess bioactive components that have been confirmed to inhibit cytokines over expression, elicit antibodies production or contain agents that block cytokines receptors to improve insulin resistance through suppression of inflammatory signalling pathways with less or no side effects (9-11).

Vitellaria paradoxa, commonly known as shea butter tree in English language and "Kade" in Hausa language belongs to *Sapotaceae* family and is largely distributed in semi-arid zone of sub-Saharan Africa (12). In Nigeria, it is found in most parts of the country with a variety of ethnomedicinal importance (13). Studies have found that *Vitellaria paradoxa* stem bark extract (VPSBE) is rich in bioactive components with good anti-bacterial (12), anti-arthritic (14, 15), anti-fungal and anti-viral (16) activities. Flavonoids, alkaloids, phenols, tannins and cardiac glycosides are common phytochemicals found in the crude stem bark extract of *Vitellaria paradoxa* (12, 13, 17, 18). Crude stem bark extract of *Vitellaria paradoxa* has also been claimed to have

Materials and Methods

Study Location

Vitellaria paradoxa stem bark was obtained from Yargeda village, Talata Mafara plantation, Zamfara State, Northwest Nigeria during the rainy season (19). The stem bark was authenticated at the Pharmacognosy Department, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria with a verification number PCG/UDUS/SAPO/0001. The extraction and phytochemical screening of VPSBE was carried out in the pharmacognosy laboratory at the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto while the laboratory analysis was carried out at the Veterinary Research Laboratory Centre in the faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

Chemicals and Reagents

All the chemicals and reagents used were of analytical grade. Alloxan monohydrate, methanol, chloroform and metformin were purchased (Sigma-Aldrich Co., St Louis, USA). Kits for glucose assay were obtained (Lifescan Inc Milpitas, USA), cytokines reagent (TNF-α, IL-6, IL-10 with catalogue no.: E-EL-R0019, E-EL-R0015 and E-EL-R0013 respectively were purchased (E-Lab Science Technology, USA). The rotary evaporator and freeze drier were obtained from (Eins-sci laboratory equipment, South Africa).

Experimental Animals

Thirty (30) healthy adult Wistar rats of both sexes (selected females were nulliparous and non-pregnant; weight 150–200g; age: 10-12 weeks) were purchased from the Faculty of Pharmaceutical Sciences of the Ahmadu Bello University, Zaria, Nigeria, transported to and allowed to acclimatize at the animal house of the Faculty of Pharmaceutical Sciences of the Usmanu Danfodiyo University, Sokoto, Nigeria for a week before commencement of the experiment. Rats were kept in clean cages (male rats were separated from female rats) at room temperature with free access to food and water. These conditions were maintained constant throughout the experiments.

Ethical consideration

This study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine (Ethics certificate No. Animal Research UDUS/FAREC/AUP-R3/2019), Usmanu Danfodiyo University Sokoto, Nigeria. All protocols for animal handling were according to the principles of laboratory animal care of the National Academy of Science and published by the National Institute of Health (Publication no. 80-23, revised 1978).

Methanol Preparation of extract

The stem bark of *Vitellaria paradoxa* was dried under the shade and crushed to powder. Three hundred (300g) of the powder was macerated in 1500ml methanol for 24hours with continuous shaking, kept at room temperature. The supernatant was filtered using Whatman no. 1 filter paper and the filtrate (crude extracts) was evaporated in a vacuum rotary evaporator at 48^{0}C and then dried in a dryer and stored (20).

Phytochemical Screening

Qualitative method for the phytochemical screening was done on crude stem bark extract of *Vitellaria paradoxa* (21). The Phytochemicals screened for include saponins, glycosides, alkaloids, steroid, volatile oil, flavonoids, tannins, saponins-glycoside, balsams, cardiac glycosides and anthraquines.

Test for Flavonoids

Three millilitres (3ml) of crude stem bark extract of *Vitellaria paradoxa* were treated with 1ml of 10% sodium hydroxide solution. An intense yellow colour was formed which became colourless on addition of dilute hydrochloric acid, thus indicating the presence of flavonoids (22).

Test for Tannins

Five percent (5%) ferric chloride solution was added drop by drop to a 2ml of crude stem bark extract of *Vitellaria paradoxa*. A white precipitate was formed which indicated the presence of tannins (23).

Test for Saponins

A mixture of 5ml of crude stem bark extract of *Vitellaria paradoxa* and 5ml of water in a test tube was shaken vigorously. The whole tube was filled up with foam. The persistence of foam for several minutes indicated the presence of saponins (23).

Test for glycosides

Two and half millilitre (2.5ml) of 50% H_2SO_4 was added into a test tube containing 5ml of crude stem bark extract of *Vitellaria paradoxa*. The mixture was heated in boiling water for 15 minutes and then allowed to cool. It was then neutralized with 10% NaOH and 5ml of Fehling's solution was added and the mixture was boiled again. A brick-red precipitate was formed which indicated the presence of glycosides (23).

Test for alkaloids

The crude stem bark extract of *Vitellaria paradoxa* was dissolved in dilute hydrochloric acid and filtered. The filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). A yellow-coloured precipitate was observed which indicated the presence of alkaloids (24).

Test for cardiac glycosides

One millilitre (1ml) of crude stem bark extract of *Vitellaria paradoxa* was added into a test tube containing 2ml of 3.5% ferric chloride solution and was allowed to stand for one minute. Then 1ml of concentrated H_2SO_4 was poured carefully down the wall of the tube so as to form a lower layer. No reddish-brown ring was

formed at the interface, which indicated the absence of cardiac glycosides (23).

Test for steroids (Salkowski)

Half millilitre (0.5ml) of crude stem bark extract of *Vitellaria paradoxa* was dissolved in 2ml of chloroform. 2ml of sulphuric acid was carefully added, a lower layer was formed. A reddish-brown colour at the interface was observed which indicated the presence of a steroidal ring (23).

Test for saponins glycosides

Two and half millilitre (2.5ml) of crude stem bark extract of *Vitellaria paradoxa* was added into a test tube containing 2.5ml of Fehling's solution A and B. A bluish green precipitate was formed, which indicated the presence of saponins glycosides (23).

Test for balsams

The crude stem bark extract of *Vitellaria paradoxa* was mixed with equal volume of 90% ethanol. About 2 drops of alcoholic ferric chloride solution were added to the mixture. A dark green colour was formed which indicated the presence of balsams (23).

Test for Anthraquines

Half gram (0.5g) of crude stem bark extract of *Vitellaria paradoxa* was mixed and shaken with 10ml benzene and 5ml of 10% ammonia solution. The mixture was shaken again and the formation of a pink colour in the lower phase was observed which indicated the presence of anthraquinones (24).

Test for Volatile oils

One millilitre (1ml) of crude stem bark extract of *Vitellaria paradoxa* was mixed with diluted HCl. A white precipitate was formed which indicated the presence of volatile oils (23).

Induction of Diabetes

A single dose of diabetogenic agent –Alloxan, 150 mg/kg was used to induce diabetes in the rats. The alloxan was dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5), thereafter injected intraperitoneally (25). Only rats with an elevated glucose level of ≥11.1 mmol/L three days post-Alloxan injection were included in the diabetic group before the commencement of treatment. The treatment commenced on the seventh day after the rats were acclimatized and this was considered as the first day of treatment.

Experimental Design

Thirty (30) Wistar rats (both sexes) were allotted to five experimental groups (n = 6 per group) as follows: normal healthy control rats (group I) which were fed orally with physiological saline; diabetic rats (group II) which were fed orally with physiological saline; diabetic rats which were fed orally with 100mg/kg body weight/day of crude stem bark extract of *Vitellaria paradoxa* (group III); diabetic rats which were fed orally with 200 mg/kg body weight/day of crude stem bark extract of *Vitellaria paradoxa* (group IV); diabetic rats which were fed orally with 200mg/kg body weight/day of Metformin (group V). These treatments were continued to the end of the study (for 28days).

Anti-coagulated Blood and Serum Preparation

After the last treatment, the rats were fasted overnight and anaesthetized in a glass jar containing chloroform for painless and rapid death. Blood samples were obtained through cardiac puncture and divided into ethylenediamine tetra acetic acid (EDTA) containers, and plain tubes. Blood collected into plain tubes was allowed to clot and retract and was then centrifuged at 4000 x g for 10 min at 4 °C. The sera were then transferred into labelled sterile serum bottles and tightly capped and stored at −20 °C until used for the measurement of TNF-α, IL-6 and IL-10. Blood from EDTA containers was immediately mixed thoroughly and used for the analysis of leucocytes indices.

Determination of Blood Glucose Level

Glucose level was measured with the Ames One Touch glucometer (One-Touch Basic; Lifescan, Johnson and Johnson, New Brunswick, NJ). In this technique, blood sample was collected from the tip of the tail of each rat using a sterile lancet needle, the blood was passed onto the strip and the estimated blood glucose (mmol/l) was displayed on the screen of the glucometer.

Neutrophils to Lymphocyte Ratio (NLR) Calculation

Anti-coagulated whole blood from rats was processed and the white blood cell count measured using an automated complete blood cell counter (model XT 2000i; sysmex, Kobe, Japan), which simultaneously provided values for total white blood cell count, absolute neutrophils count and absolute lymphocyte count. Neutrophils to Lymphocytes Ratio were calculated as a simple ratio of absolute neutrophils count to absolute lymphocyte count (26).

Serum Cytokines Determination

The serum concentration of TNF-α, IL-6 and IL-10 were measured using a sandwich- enzyme-linked immunosorbent assay (ELISA) technique with a kit (E-Lab Science Technology, USA). The procedure was performed according to the manufacturer's instructions.

Statistical Analysis

Data generated was analysed using Microsoft Excel broadsheet and INSTAT 3 software program (Graph Pad Software Inc., La Jolla, CA, USA). Results were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was performed and Tukey *post hoc* test was used for comparisons of means of the various groups. Differences of *p* value less than or equal to 0.05 were considered significant.

Results

The results of the phytochemical screening of the crude stem bark extract *Vitellaria paradoxa* (Table 1) indicated that with the exception of cardiac glycosides which were absent, the crude stem bark extract *Vitellaria paradoxa* was found to contain saponins, glycosides, alkaloids, steroids, volatile oil, flavonoids, tannins, saponins-glycoside and balsams. Figure 1 shows the distribution of baseline fasting blood glucose (BFBG) at day 1 and final fasting blood glucose (FFBG) at day 28 in Alloxan induced Wistar rats. The BFBG levels in group I was significantly ($p<0.05$) lower compared to groups II, III, IV, and V. The FFBG level in group I significantly lower ($p<0.05$) compared to group II and III. No significant difference ($p>0.05$) was observed in FFBG level in group I compared group IV and V. The FFBG level in group II was significantly ($p<0.05$) higher when compared to the diabetic treated groups. The FFBG level in group III was significantly ($p<0.05$) higher compared to groups IV and V. Finally, FFBG level in group IV was significantly ($P<0.05$) lower compared to group V.

Table 2 shows the effect of treating diabetic rats with crude stem bark extract of *Vitellaria paradoxa* on NLR and serum concentration of IL-6, IL-10 AND TNF-α. The NLR in group I was significantly ($p < 0.05$) higher compared to group II and the diabetic treated groups. The NLR in group II decreased significantly ($P<0.05$) compared to groups III and group IV. However, no significant difference was observed between the DM group treated with *Vitellaria paradoxa* for the value of NLR compared to the group treated with metformin (DM + 200 Metformin) group. Furthermore, no significant difference ($P>0.05$) was observed for the value of NLR in group III compared to group IV. The mean value of NLR in group III increased significantly ($P<0.05$) com pared to group V. Finally, the NLR in group IV increased significantly ($P<0.05$) compared to group V.

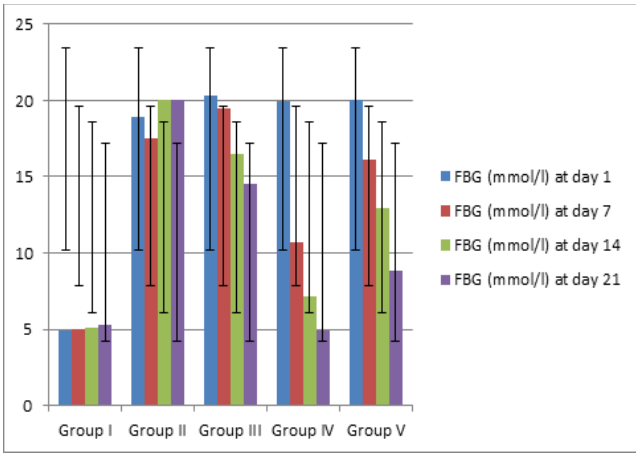

Figure 1: Effect of crude stem bark extract of *Vitellaria paradoxa* and Metformin on blood glucose level in Alloxan induced diabetic Wistar Rats.

Data expressed as mean ± SD; n = 6, I: normal healthy control rats, II: diabetic rats, III: diabetic rats treated with 100mg/kg body weight of crude stem bark extract of *Vitellaria paradoxa*, IV: diabetic rats treated with 200mg/kg body weight of crude stem bark extract of *Vitellaria paradoxa*, V: diabetic rats treated with 200mg/kg body weight of Metformin. $P < 0.05$ by Tukey multiple comparison analysis

The concentration of IL-6 in group I was significantly ($p < 0.05$) lower compared to group II. No significant difference ($P>0.05$) was observed in the concentration of IL-6 in group I compared to diabetic treated groups. The concentration of IL-6 in group II was significantly ($P<0.05$) higher compared to the diabetic treated groups. No significant difference ($P>0.05$) was observed in the concentration of IL-6 among the diabetic treated groups. The concentration of TNF-α in group I was significantly ($p < 0.05$) lower compared to group II and the diabetic treated groups. The concentration of TNF-α in group II was significantly ($P<0.05$) higher compared to groups III and group IV. However, there was no significant difference ($P>0.05$) observed in the concentration

Table 1: Phytochemical screening of crude stem bark extract of *Vitellaria paradoxa*

Phytochemical	TEST	Result
Saponins	Foam test (23).	+
Glycosides	Modified Borntrager's Test (23)	+
Alkaloids	General test: Dragendorff reagent (24)	+
Steroids	Liebermann-Burchardt Test (23).	+
Volatile oil	Based on Formation of white precipitates (23)	+
Flavonoids	Alkaline Reagent Test (22)	+
Tannins	Gelatin test (23)	+
Saponins glycosides	Froth Test (23)	+
Balsams	Based on colour intensity (23)	+
Anthraquines	Borntrager's Test (22)	+
Cardiac glycoside	Legal's Test (23).	-

"+" represent presence of phytochemicals, "-"represent absence of phytochemicals

Table 2: Effect of crude stem bark extract of *Vitellaria paradoxa* on NLR, IL-6, TNF-α and IL-10 among the groups

Groups	NLR	IL-6 (pg/ml)	TNF -α (pg/ml)	IL-10 (pg/ml)
I	1.85 ± 0.09	23.20 ± 3.46	490.10 ± 7.17	146.61 ± 12.02
II	0.04 ± 0.01 [a]	154.13 ± 22.69 [a]	1204.05 ± 150.91 [a]	72.16 ± 4.02 [a]
III	0.33 ± 0.02 [ab]	43.26 ± 13.82 [b]	943.95 ± 4.02 [ab]	113.42 ± 2.9 7[ab]
IV	0.37 ± 0.01 [ab]	29.45 ± 2.60 [b]	680.64 ± 5.09 [abc]	134.87 ± 2.70 [abc]
V	0.04 ± 0.02 [acd]	37.47 ± 5.67 [b]	1117.60 ± 8.14 [acd]	122.98 ± 1.1 [abd]
p-value	0.0001	0.009	0.0001	0.0019

Data expressed as mean ± SD; n = 6, I: normal healthy control rats, II: diabetic rats, III: diabetic rats treated with 100mg/kg body weight of crude stem bark extract of *Vitellaria paradoxa*, IV: diabetic rats treated with 200mg/kg body weight of crude stem bark extract of *Vitellaria paradoxa*, V: diabetic rats treated with 200mg/kg body weight of Metformin. [a]*p< 0.05* when compared with group II, [b]*p< 0.05* when compared with group III, [c]*p< 0.05* when compared with group IV, [d]*p< 0.05* when compared with group V by Tukey multiple comparison analysis.

TNF-α in group II compared to group V. The concentration of TNF-α in group III increased significantly (P<0.05) compared to group IV. The concentration of TNF-α in group IV decreased significantly (P<0.05) compared to group V.

The concentration of IL-10 in group I was significantly ($p < 0.05$) higher compared to group II and the diabetic treated groups. The concentration of IL-10 in group II was significantly (P<0.05) lower compared to the diabetic treated groups. The concentration of IL-10 in group III was significantly (P<0.05) lower compared to group IV and group V. while the concentration of IL-10 in group IV increased significantly (P<0.05) compared to group V.

Discussion

The need to evaluate the effects of crude stem bark extract of *Vitellaria paradoxa* on interleukin-6, interleukin-10 and tumour necrosis factor-alpha in Alloxan-induced diabetic rats was prompted by its wide range use as a local medicine (12, 16, 13).

The qualitative phytochemical analysis of crude stem bark extract of *Vitellaria paradoxa* revealed the presence of certain phytochemicals including saponins, glycosides, alkaloids, steroid, volatile oil, flavonoids, tannins, saponins-glycoside, balsams and anthraquines which are known to possess biological activities including anti-diabetes (19). The antidiabetic activities of the crude stem bark extract of *Vitellaria paradoxa* may be attributed to the presence of some of the above phytochemicals either acting in synergy or individually.

The doses of the extract (100 mg/kg b.w. and 200 mg/kg b.w) significantly ($p = 0.0019$) reduced the fasting blood glucose of the Wistar rats after 28 days of oral administration when compared with normal control group. These results may be attributable to the chemical compounds present in the extract that have the capacity to mimic the action of insulin or to stimulate its secretion by the β-cells of the islets of Langerhans (27). The other possible mechanisms of the natural products action may be the regeneration of β cells of the islets of Langerhans, the transport of blood glucose in peripheral tissue, the stimulation of glucose uptake by peripheral tissues, the inhibition of endogenous glucose production or the activation of gluconeogenesis in the liver and muscles (27) which may be as a result of the polyphenols' synergistic effects as seen in this study. The higher dose of 200mg/kg b.w. of the crude stem bark extract of *Vitellaria paradoxa* significantly *(p<0.05)* reduced fasting blood glucose in the rats more than the dose of 100 mg/kg b.w. of the crude stem bark extract of *Vitellaria paradoxa.* This suggests the crude stem bark extract of *Vitellaria paradoxa* has hypoglycaemic property in a dose dependent manner. This result is consistent with a study that, reported that the antidiabetic effect was found to be increased with increasing concentrations of the *Vitellaria paradoxa* extract. Comparatively, treatment with 200 mg/kg b.w. of the crude stem bark extract of *Vitellaria paradoxa* reduced fasting blood glucose more than treatment with 200 mg/kg b.w. of the Metformin (28). It has been previously reported that, the antidiabetic activity of most natural products is due to the contents of phenolics and flavonoids (9) as some of the contents identified in the crude stem bark extract of *Vitellaria paradoxa* in this study.

In this study, hyperglycaemic condition in the rats significantly increased the serum IL-6 level. This finding concurs with other studies (10, 29, 30). These similarities may be due to similarity in the animal model and type of disease induced. A clinical study on human subjects also indicated that serum IL-6 levels in patients with type 2 diabetes mellitus was up regulated (8, 31). However, another study demonstrated that IL-6 levels of type 1 DM patients did not differ significantly, as compared to controls (32). It has been established that the elevated levels IL-6 in diabetes and its associated complications are as a result of hyperglycaemia and IL-6 has been considered to be the link between inflammation and insulin resistance (6). IL-6 induces a number of glucocorticoid receptors, increase the concentration of circulating glucagon, and adipose paracrine effect to decrease insulin action. It has been reported that IL-6 causes insulin resistance through affecting both the proximal and distal events in hepatic insulin receptors (IR) signal transduction (33). IL-6 is also capable of causing inhibition of insulin action in primary hepatocytes. Pre-treatment of primary hepatocytes with IL-6 markedly inhibited both insulin-induced protein kinase B (Akt) activation and glycogen synthesis. Therefore, this suggests that hepatocytes in general are physiologic targets for the inhibitory effect of IL-6 on insulin signalling (33). The results from the current study showed that crude stem bark extract of *Vitellaria paradoxa* of both doses down-regulated the elevated concentration of IL-6 in diabetes, possibly, through down regulating some pro-inflammatory genes. The observed effect suggests that crude stem bark extract of *Vitellaria paradoxa* possess some immunomodulatory properties. The effects displayed by crude stem bark extract of *Vitellaria paradoxa* may be due to the high content of flavonoids. Zhao et al. and Kim et al.

reported that flavonoids significantly reduced IL-6 by suppressing NF-κB activity. Research by (32) showed that phytochemicals decrease the concentration of IL-6 in patients with diabetes.

This study provides evidence of an increase concentration of TNF-α in diabetic group on distilled water only (positive control) group and this finding is in line with previous reports by other studies (10, 29). The reasons for the increase in TNF-α level in this research, may be due to increase in oxidative stress in the diabetic rats' model as previously reported (10, 33). Oxidative stress increases the expression of the pro-inflammatory gene by oxidant-mediated activation of transcription factors (34). Reactive oxygen species (ROS) are important in the inflammatory response through the increases of redox-sensitive transcription factors, alteration of histone acetylation or deacetylation and thus pro-inflammatory gene expression (35). The resultant activation of TNF-α is well acknowledged to cause damage to renal cells by enhancing renal hypertrophy, hemodynamic imbalance and albumin permeability (36) The harmful effects of these responses lead to the development of renal disease in patients with T2DM, hence resulting in the progression of renal failure. TNF-α increases retinal endothelial permeability by down regulating the expression of tight junction proteins and the increased permeability can lead to rupturing of the brain retinal barrier (BRB) (37). TNF-α can damage insulin receptor (IR) and insulin receptor substrate (IRS) and then it can inhibit insulin signal thereby causing insulin resistance (35). TNF-α stimulated the expression of SOCS (suppressor of cytokine signal) which bonded either IRS1 or IRS2 and mediated damage and as a result, insulin could not take glucose into the muscle cells and adipose tissue, therefore glucose levels in blood plasma would increase (35). The serum TNF-α concentration was decreased after the crude stem bark extract of *Vitellaria paradoxa* treatment at both doses. The decrease of TNF-α by crude stem bark extract of *Vitellaria paradoxa* was expected due to it high content of phytochemicals. According to Giriwono et al. polyphenolic compounds down-regulate TNF-α in the plasma. Another reason for the decrease in TNF-α could be due to the increase in regulatory cytokine (IL-10) level observed in this present study in response to pro-inflammatory cytokine (TNF-α). This study showed that giving crude stem bark extract of *Vitellaria paradoxa* to rats may tend to have ability for decreasing inflammatory compounds as observed. However, a study reported a decrease in inflammation of pancreatic β cells closely associated with the increased pro-insulin synthesis, improved insulin sensitivity and pancreatic β cell mass (33). We propose that the observed lower TNF-α by crude stem bark extract of *Vitellaria paradoxa* may delay the onset of diabetic nephropathy, retinopathy, neuropathy and hepatopathy.

In this study, hyperglycaemia induced a significant downward regulation of serum IL-10 concentration and, this finding is in line with the other similar studies using diabetic animal model (38, 39). The higher value of Th1 cytokines (TNF-α) observed in this study may lead to the skewing of Th2 cytokines (IL-10) response (40). The mean ratio of TNF-α to IL-10 was 16.67 in the diabetic untreated rats, and the value was generally higher than 3.6 in the non-diabetic rats. In the diabetic untreated rats, we observed relatively high cytokine ratios (TNF-α/IL-10) demonstrating predominance of pro-inflammatory TNF-α over anti-inflammatory IL-10. This indicates that diabetic rats with low concentration of IL-10 had less adequate control of their inflammatory response. Treatment of diabetic rats with crude stem bark extract of *Vitellaria paradoxa* upregulated the concentration of IL-10 with the higher dose being more effective. These effects led us to suggest that the treatment with 200 mg/kg of crude stem bark extract of *Vitellaria paradoxa* hastens the switch from inflammatory to anti-inflammatory responses and dominance of Th2 on Th1. Crude stem bark extract of *Vitellaria paradoxa* produced its anti-inflammatory effects probably through inhibiting the production of TNF-α and blocking TNF-α-mediated inflammation (41). This effect may be directly mediated via the prevention of activation of the extracellular signal-related kinase (ERK), c-Jun NH2-terminal kinase (JNK), c-Jun, and nuclear factor-κB (NF-κB), which are potent inducers of inflammatory gene expression and protein secretion or indirectly via the stimulation of peroxisome proliferator-activated receptor-γ (PPARγ) activity, thereby antagonizing NF-κB or activator protein-1 (AP-1) transcriptional activation of inflammatory genes.

Conclusion

In conclusion, this study has validated the folkloric claim of anti-diabetic and anti-inflammatory properties of crude stem bark extract of *Vitellaria paradoxa* in experimental diabetic Wistar rats after repeated oral administration for 28 days. The study also revealed that administration of crude stem bark extract of *Vitellaria paradoxa* at higher dose may be more beneficial. It is recommended that the work done in experimental rats in the current study should be replicated in higher animals. Further studies to explore the full immunomodulatory effect of crude stem bark extract of *Vitellaria paradoxa* in diabetes are also suggested.

Disclosure statement

There is no conflict of interest to be declared in this study.

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