A pilot study of the effects of Ajwa date seed extract in a diabetic animal with parallel observations on human subjects.

ABSTRACT

Background: most diseases are inequality of the antioxidants, pro-oxidants, and failure in resolved inflammation. Diabetes is a metabolic malfunction associated with inflammation and ends with organs failure. Alternative treatments for diabetes include a lifestyle and an anti-inflammatory diet.
Method: we investigated the effects of dietary Ajwa dates seeds extract (AJSO) against alloxan monohydrate-induced biochemical changes. We analyzed the phytochemical components of AJSO with gas chromatography-mass spectrometry and inductively coupled plasma-mass spectrometry. We determined the effects of oral AJSO on biochemical changes caused by alloxan monohydrate in rats. We also observed biochemical changes in human volunteers with or without Ajwa seed as caffeine-free coffee replacements.
Results: the results and outcomes confirmed the improvement in the rats' biochemical analysis due to AJSO constituents. The volunteers treated with Ajwa seed as caffeine-free coffee replacements showed significant amelioration of inflammatory and diabetes markers.
Conclusion: AJSO had a healthy impact by decreasing the levels of amyloid a, c-reactive protein, lipid peroxidation, nitric oxide, and improving diabetes, lipid and liver profiles in both rats and human volunteers.

Keywords: Diabetes, inflammation, Ajwa dates seeds, amyloid A, C-reactive protein, lipid peroxidation.

1. INTRODUCTION

Diabetes is a metabolic disturbance involving the glucose metabolism and its level in the body [1]. The glycohemoglobin (HbA1c) levels and hyperglycemia are the main clinical and diagnostic tests for diabetes type 2 [1], [2]. Chronic hyperglycemia associated with diabetes can result in sustained inflammation and organ dysfunction including the retina, kidneys, nerves, heart, and blood vessels [1], [2]. Sustained inflammation elevates the prooxidants and inflammatory markers such as c-reactive protein and Amyloid A and affects people's life quality [3].
The prevalence etiologies of diabetes type 2 are a western lifestyle, absence of physical activity, and an energy-dense diet with an individual genetic predisposition. Moreover, the proof has revealed that inflammatory pathways are the principal, standard pathogenetic mediators in the natural path of diabetes beneath the stimulation of the risk aspects represented above. Anti-inflammatory treatments, natural diets, and herbs, with plenty of antioxidant constituents, deliver the most suitable alkaline medium to control chronic hyperglycemia, diabetes complication and sustain inflammation [2], [4].
Date palm seed (Phoenixdactylifera) belongs to the family (Arecaceae) and has had nutritional value as food for years. It is located in hot land such as Saudi Arabia, Eastern lands, and Egypt [5], [6]. The nutrition compositions of date palm seed are phytochemicals, dietary fibers, carbohydrates, sterols, carotenes, and flavonoids [6]. It is used in therapeutic anticancer, antihypertensive, antimutagenic, and antifungal. Moreover, in various countries, date seeds are used as animal feed or caffeine-free coffee substitutes. Recent studies have shown that Date palm seed has various beneficial outcomes, including anti-inflammatory and antidiabetic effects [5], [6].

In this investigation, we examined the outcomes of treatment with a dietary AJSO extract on alloxan monohydrate -induced biochemical modifications in a rat model and maintained parallel observations of human volunteers used Ajwa seed as caffeine-free coffee replacements. Animal models are

broadly utilized in biochemistry investigations, and the outcomes of animal examinations traditionally start the basis of pilot investigations with humans. However, there are situations about the proper scope of animals that likewise respond to physiological triggers to humans. From this viewpoint, our investigation coordinates to an animal pilot study side-by-side with observations on human responses to AJSO against inflammation and diabetic markers. The trial's purpose to analysis the effect of AJSO on indicators of inflammation, lipid peroxidation and diabetes, such as amyloid A, C-reactive protein, blood glucose level, lipid peroxidation, and nitric oxide (NO), induced by monohydrate in rats .
We also observed the differences in the levels of these biochemical indicators and the glycohemoglobin (HbA1c), and the liver, kidney, lipid profile between human volunteers with caffeine-free coffee replacements treatment against inflammation and those without, and corresponded the outcomes to those from the rat experimentation.

2. MATERIALS AND METHODS

The Institution involved in this work approved the study protocol, animal resources, and experimental materials and procedures.

2.1 The Plant Study

2.1.1 The plant materials

We obtained Ajwa dates (Phoenix dactylifera L) from Al Madina, Saudi Arabia for this study, here, Figure 1 shows the experimental design. Ajwa dates seeds were crushed and extracted with a solvent (1: 4 w/v), where the solvent components were hexane and ethanol (2:1). After being put in the solvent for 20 minutes at room temperature (26± 4°C), the extracts were concentrated with a 45-55°C rotary evaporator [6]; we obtained a total AJSO (Ajwa dates (Phoenix dactylifera L) extracts) yield of 21%.

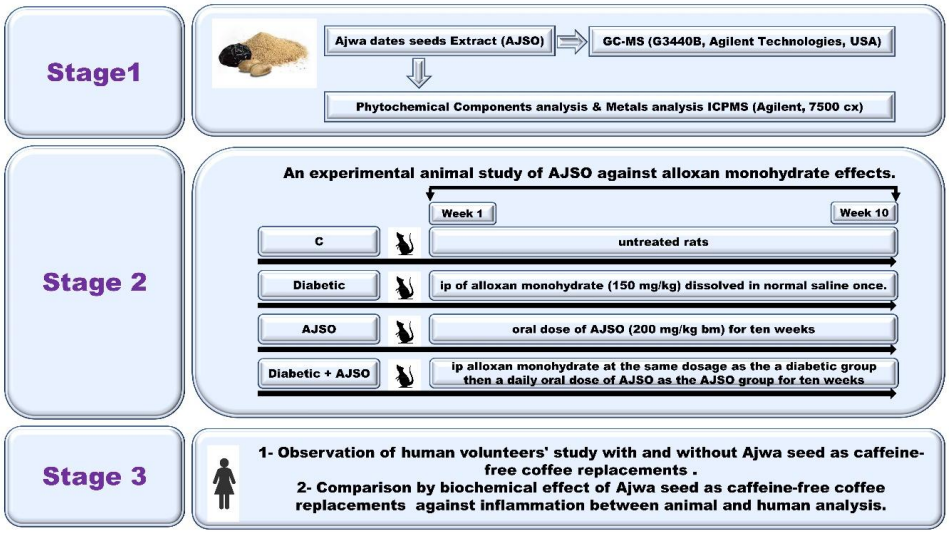


Fig. 1. The Study Design.

2.1.2 Phytochemical Components analysis

We followed the method of Bolajoko and Onyeaghala, 2020 for phytochemical Components analysis including total phenolic, tannin, flavonoid and alkaloids with an UV-Vis spectrophotometer 1000 series (CECIL Instruments Limited, Milton Technical centre Cambridge CB24 6AZ, England) [7].

2.1.3 Metals analyses

We followed our previous method for inductively coupled plasma-mass spectrometry ICPMS (Agilent, 7500 cx) after digested process with nitric acid using a microwave digestion system (Milestone, Ethos 1) to analysis K, Na., pb, cd, Al [8]

2.1.4 Gas chromatography–mass spectrometry analysis

We followed our previous method for GC–MS analysis to separate the chemical composition of the AJSO extract using a gas chromatography system (G3440B, Agilent Technologies, USA) [8]. We then searched the WILEY and NIST (National Institute of Standards and Technology) mass spectral libraries to recognize the chemical contents of the extract [8].

2.2 The Rat Study

2.2.1 Animals

We used 40 adult male Sprague-Dawley rats weighing 150–190 g in this study. The rats were tested for their health status at 28 °C and given a standard diet and water daily for two weeks before the study began. After acclimatization, the rats were divided into four groups of 10 animals each. All animal experiments were approved by the Experimental Animal Care Society's Ethics Committee and conformed to the Three Rs (Replacement, Reduction, Refinement) [4]. We complied with all institutional and national regulations for the care and handling of laboratory animals [4]. The four groups of animals contended of the following: a **control group** of untreated rats; a **diabetic group** of rats injected with an intraperitoneal dose of alloxan monohydrate were purchased from Sigma-Aldrich (USA) (150 mg/kg) dissolved in normal saline once. We measured diabetes in rats by analysis glucose level with a glucometer after 80 hours of alloxan monohydrate dose. Experimental should having blood glucose concentration above 290±10 mg/dl were confirmed to be diabetic for further studies [5]. An **AJSO group** of rats treated with a daily oral dose of AJSO at 200 mg/kg bm for ten weeks [5]. And **a diabetic + AJSO group** of rats injected with alloxan monohydrate at the same dosage as the a diabetic group then a daily oral dose of AJSO as the AJSO group for ten weeks [5].
After the treatment period ended, we collected a blood specimen and then serum was sequestered at −30 °C until assay.

2.2.2 Biochemical assays

we estimated the glucose level by colorimetric Assay GOD-POD analysis kit according to the method described by Sarfraz et al, 2017 [5].
We analyzed NO concentration, malondialdehyde (MDA) level, total cholesterol, low-density lipoproteins (LDL cholesterol [LDL-C]), high-density lipoprotein (HDL cholesterol [HDL-C]), triglycerides, the aspartate transaminase (AST) and alanine transaminase (ALT) level according to our previous study [4].
We analyzed Serum amyloid A concentration calorimetrically using a commercial ELISA kit (Tridelta Development Ltd, Maynooth, Ireland) according to Aida et al, (2019) [9].
we measured C-reactive protein (CRP) level with commercially available enzyme-linked immunosorbent assay (ELISA) kits for CRP (Helica Biosystems, Fullerton, Calif., USA), according to Cai et al (2006) [10].

2.3 The human study

2.3.1 Patient population and data collection

From 2016 to 2021, this observational study enrolled 55 females' volunteers. Pregnant women, smokers, and children were also excluded. The selected volunteers were 21–60 years of age female, and had a BMI of 29.90 ± 2.24. The appropriate institutional approved the protocol, which complied with the Helsinki Declaration as revised in 2013. Written informed consent was received from all the volunteers. We divided the volunteers into two groups—Group 1 was not drinking Ajwa seed as caffeine-free coffee replacements (for at least 3 months) and Group 2 was drinking Ajwa seed as caffeine-free coffee replacements (for at least 3 months). We collected the data for the investigation of parameters in this observational study. PCR for Familial Mediterranean Fever Gene Mutation (FMF) analysis was negative for all the volunteers.

2.3.2 Biochemical assays of volunteers' serum

We withdraw blood after an overnight fast (12 hours). We estimated biochemical analysis the total cholesterol, the low-density lipoproteins (LDL cholesterol [LDL-C]), the high-density lipoprotein (HDL cholesterol [HDL-C]), the triglycerides(T.G), the aspartate transaminase (AST), the alanine transaminase (ALT), the creatinine, the urea, the uric acid , the troponin T and the glycohemoglobin (HbA1c) levels according to our previous study using commercially available kits by Roche/Hitachi Cobas e 601 analyzer (Roche Diagnostics, Mannheim, Germany) utilizing electrochemiluminescence immunoassay and Roche/Hitachi Cobas c 501 analyzer (Roche Diagnostics, Mannheim, Germany). We measured Familial Mediterranean Fever Gene Mutation (FMF) by PCR.17 Autoimmunity Tests. We analyzed Amyloid A protein and C-reactive protein by Roche/Hitachi Cobas c 501 analyzer (Roche Diagnostics, Mannheim, Germany) [3].

We analysis the NO level using process that depended on the estimation of the nitrite level in serum according to Griess's method. We measured the MDA in serum following the method of Ohkawa et al. as mentioned before in our study [4].

2.4 Statistical analyses

Data were analyzed according to our previous study using IBM SPSS software version 20.0 **(**IBM Corp, Armonk, NY**).** The significance level of the tests was set at 5% [4].

3. RESULTS

Table 1 shows the quantified phytochemicals, and metal contents of AJSO (Ajwa dates (Phoenix dactylifera L) extracts.

Table 1: analysis of phytochemical components and metals of AJSO

Components	Concentrations
Phytochemical	
Tannins (g/100g TAE)	Not detected
Polyphenols (g/100g GAE)	2.76
Terpenoids (mg/kg)	0.30
Flavonoids (mg/100g CE)	2.80
Alkaloid (mg/g AE)	0.02
Metals	

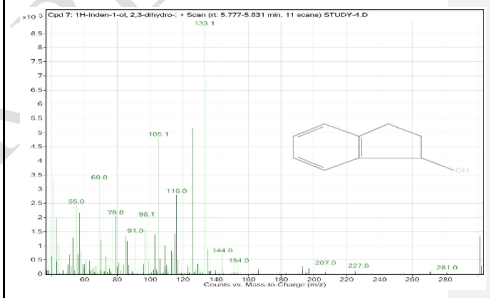
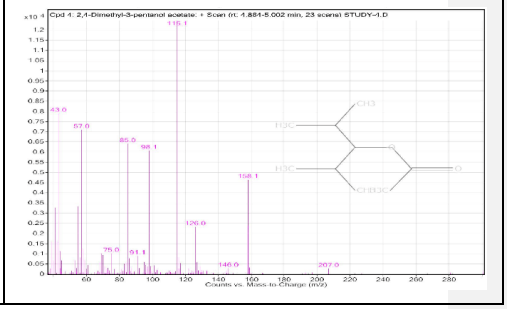
Na(mg/kg)	86.2
K(mg/kg)	5729.1
Cd (mg/kg)	Not detected
Pb (mg/kg)	Not detected
Al(mg/kg)	Not detected

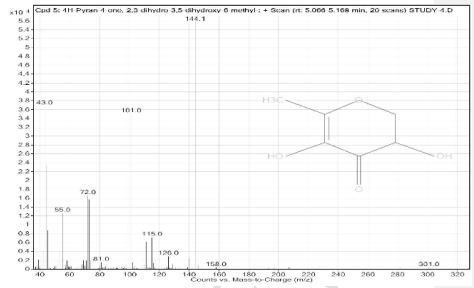
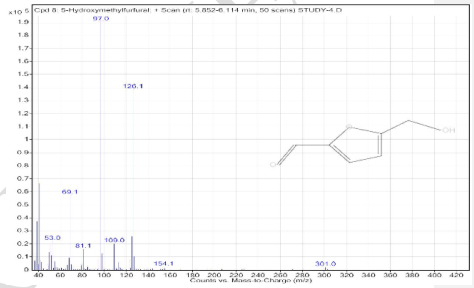
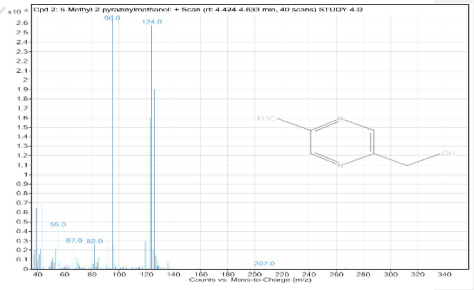
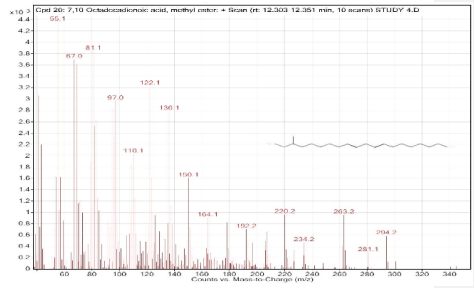
Abbreviations* tannic acid equivalent (TAE)- gallic acid equivalents (GAE)- catechin equivalents (CE)- atropine equivalent (AE)

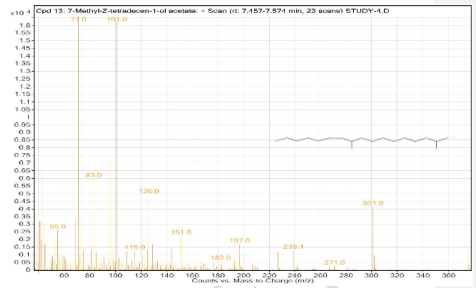
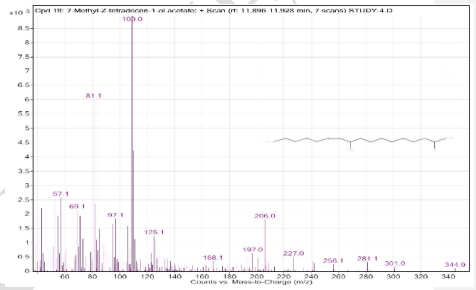
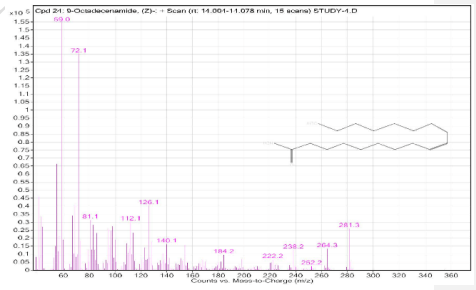
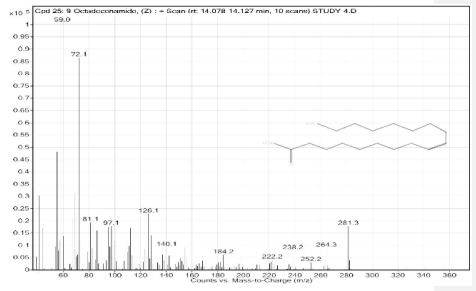
Table 2 shows the chemical composition of AJSO (Ajwa dates (Phoenix dactylifera L) extracts identified by GC-MS [6].

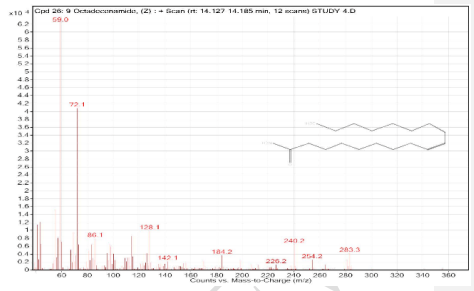
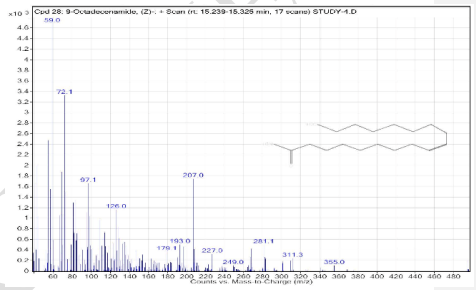
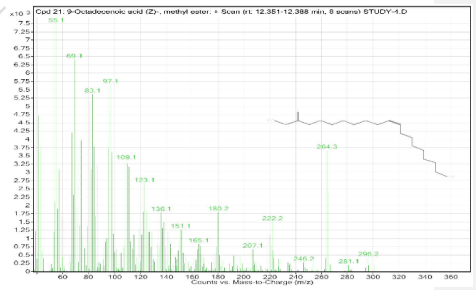
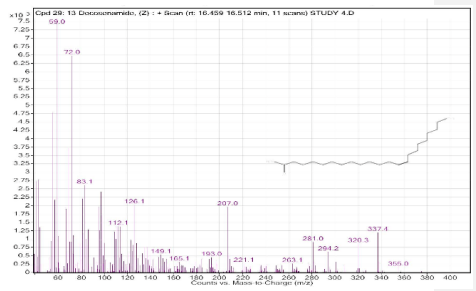
Table 2: Gas chromatography–mass spectrometry analysis

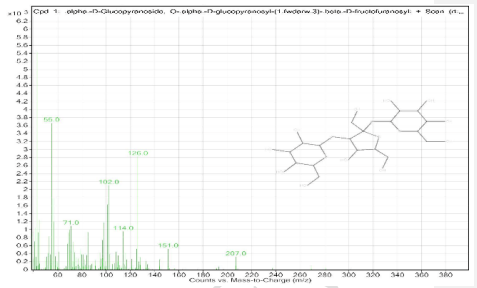
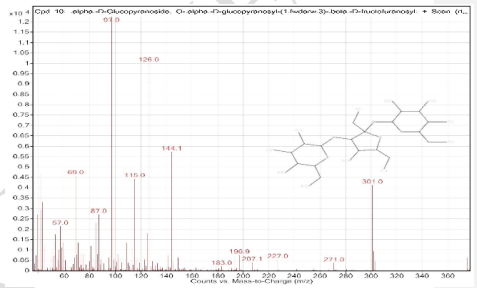
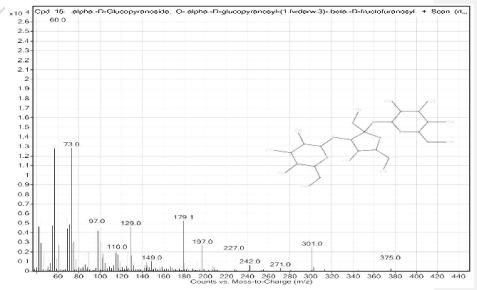
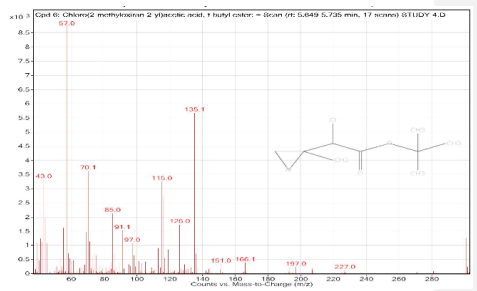
Comment [HL1]: Its better to move this table to supplementary materials

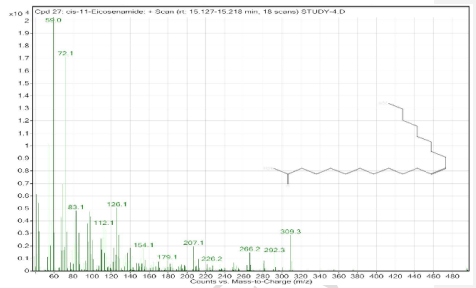
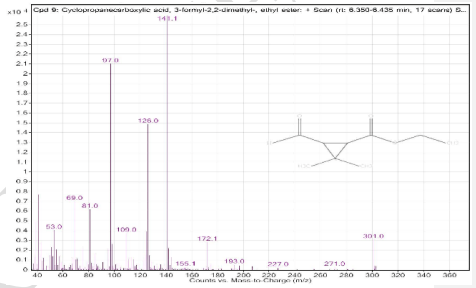
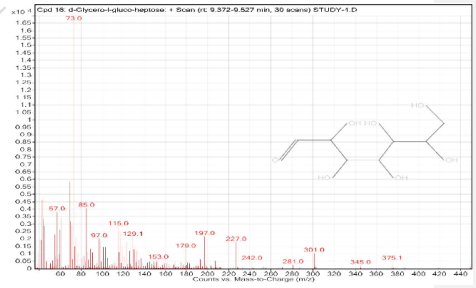
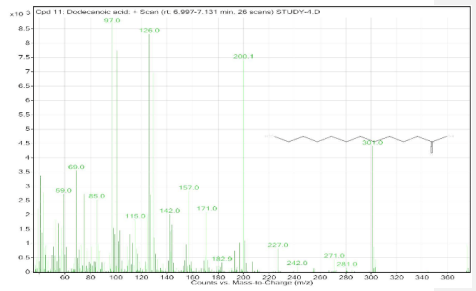
No.	Name	Base Peak	RT	Chromatogram
1.	1H-Inden-1-ol, 2,3-dihydro-	133.1	5.815	
2.	2,4-Dimethyl-3-pentanol acetate	115.1	4.938	

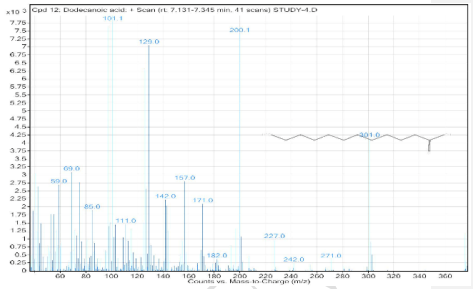
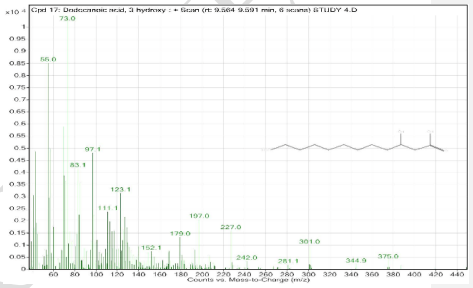
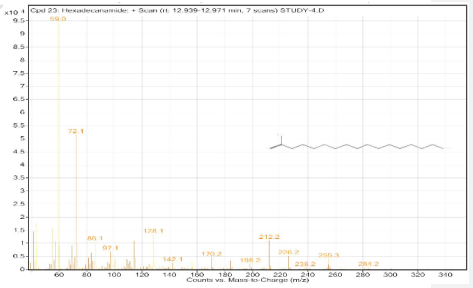
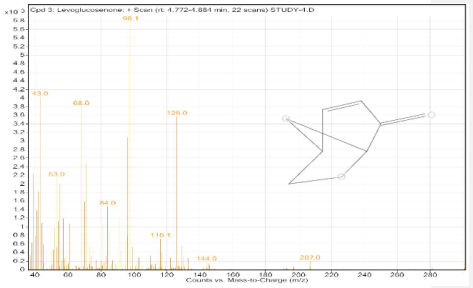
No.	Name	Base Peak	RT	Chromatogram
3.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.1	5.13	
4.	5-Hydroxymethylfurfural	97	5.981	
5.	5-Methyl-2-pyrazinylmethanol	95	4.526	
6.	7,10-Octadecadienoic acid, methyl ester	55.1	12.34	

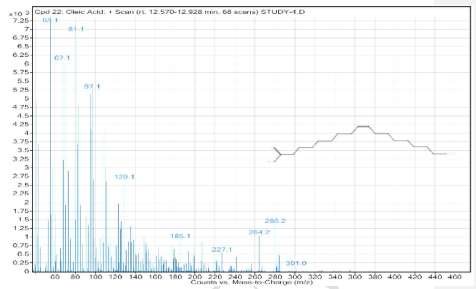
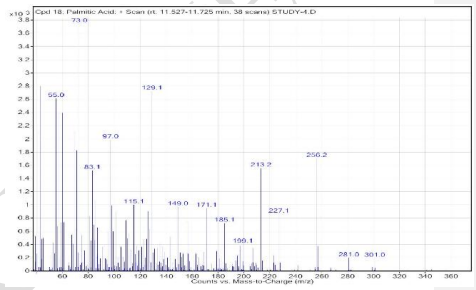
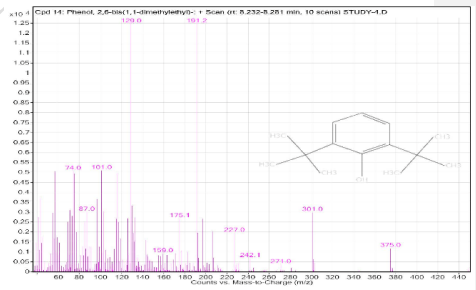
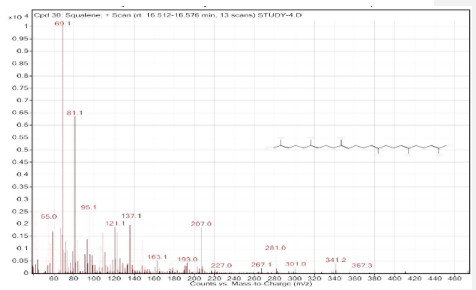
No.	Name	Base Peak	RT	Chromatogram
7.	7-Methyl-Z-tetradecen-1-ol acetate	71	7.516	
8.	7-Methyl-Z-tetradecen-1-ol acetate	109	11.907	
9.	9-Octadecenamide, (Z)-	59	14.036	
10.	9-Octadecenamide, (Z)-	59	14.089	

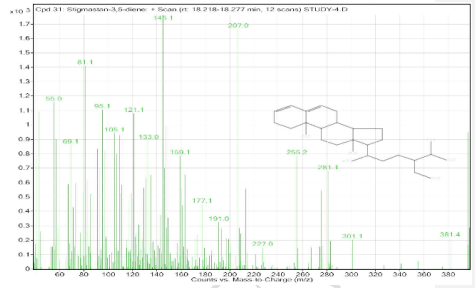
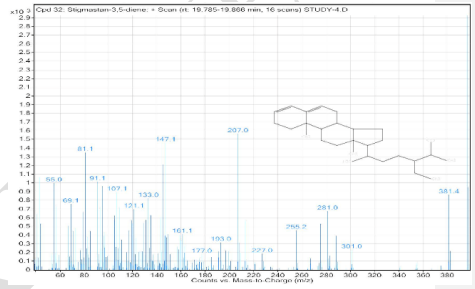
No.	Name	Base Peak	RT	Chromatogram
11.	9-Octadecenamide, (Z)-	59	14.143	
12.	9-Octadecenamide, (Z)-	59	15.26	
13.	9-Octadecenoic acid (Z)-, methyl ester	55.1	12.367	
14.	13-Docosenamide, (Z)-	59	16.48	

No.	Name	Base Peak	RT	Chromatogram
15.	.alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	43	4.226	
16.	.alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	97	6.751	
17.	.alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	60	8.575	
18.	Chloro(2-methyloxiran-2-yl)acetic acid, t-butyl ester	57	5.676	

No.	Name	Base Peak	RT	Chromatogram
19.	cis-11-Eicosenamide	59	15.143	
20.	Cyclopropanecarboxylic acid, 3-formyl-2,2-dimethyl-, ethyl ester	141.1	6.371	
21.	d-Glycero-l-gluco-heptose	73	9.457	
22.	Dodecanoic acid	97	7.088	

No.	Name	Base Peak	RT	Chromatogram
23.	Dodecanoic acid	101	7.232	
24.	Dodecanoic acid, 3-hydroxy-	73	9.58	
25.	Hexadecanamide	59	12.955	
26.	Levoglucosenone	98	4.836	

No.	Name	Base Peak	RT	Chromatogram
27.	Oleic Acid	55.1	12.688	
28.	Palmitic Acid	73	11.554	
29.	Phenol, 2,6-bis(1,1-dimethylethyl)-	191.2	8.27	
30.	Squalene	69.1	16.539	

No.	Name	Base Peak	RT	Chromatogram
31.	Stigmastan-3,5-diene	145.1	18.245	
32.	Stigmastan-3,5-diene	396.4	19.828	

*Notes for Tables 2: RT: Retention times (minutes); PA: peak area (%). GC–MS investigation was done for the Hexane: ethanolic (2:1) extracts and run at Agilent Technologies (G3440B, USA). The composition of Ajwa seed extract **AJSO** were recognized by computer simulations in commercial libraries of Wiley and NIST (National Institute of Standards and Technology).

Figure 2 shows the results for the different parameters studied, given as mean ± SD for 10 rats. Figure 2 shows that alloxan monohydrate initiated prooxidants, lipoperoxidation, hyperlipidemia, hyperglycemia and inflammation in animals, which was indicated by a significant ($p \leq 0.05$) elevation in the levels of NO (174.1%), MDA (660.9%), Amyloid A (800.0%), and CRP (239.2%), compared to the control group. This was linked to a significant ($p \leq 0.05$) increased in total cholesterol (192.9%), triglycerides (101.2%), LDL-C (133.8%), AST (93.4%), ALT (462.5%), and glucose (455.5%) related to the control group, while the diabetic group showed a significant 16.4% decline in HDL-C approximately to the control group (Figure 2a, b, c, d).

The treatment of AJSO after alloxan monohydrate injection (Diabetic +AJSO group) significantly ($p \leq 0.05$) decreased the studied parameters NO, MDA, Amyloid A, CRP, and glucose concentrations by 47.2%, 34.8%, 55.6%, 47.4%and 60.0%, respectively, compared to treatment only with alloxan monohydrate (Figure 2). This was linked with a significant ($p \leq 0.05$) decreased in total cholesterol, triglycerides, LDL-C, AST, and ALT by 46.6%, 42.6%, 30.5%, 37.3%, and 73.4%, respectively, compared to the levels in the diabetic group (Figure 2a, b). As well as, the Diabetic +AJSO group presented a significant 10.6% increase in the HDL-C serum concentration compared to that of the diabetic group, $p \leq 0.05$; Figure 2a). On the other hand, the rats treated only AJSO for ten weeks (the AJSO group) showed minor variation in the levels of glucose (↑1.1%) and HDL (↑.2%) (Figure 2). This was associated with no changes in the remaining studied parameters compared to the levels in the control group.

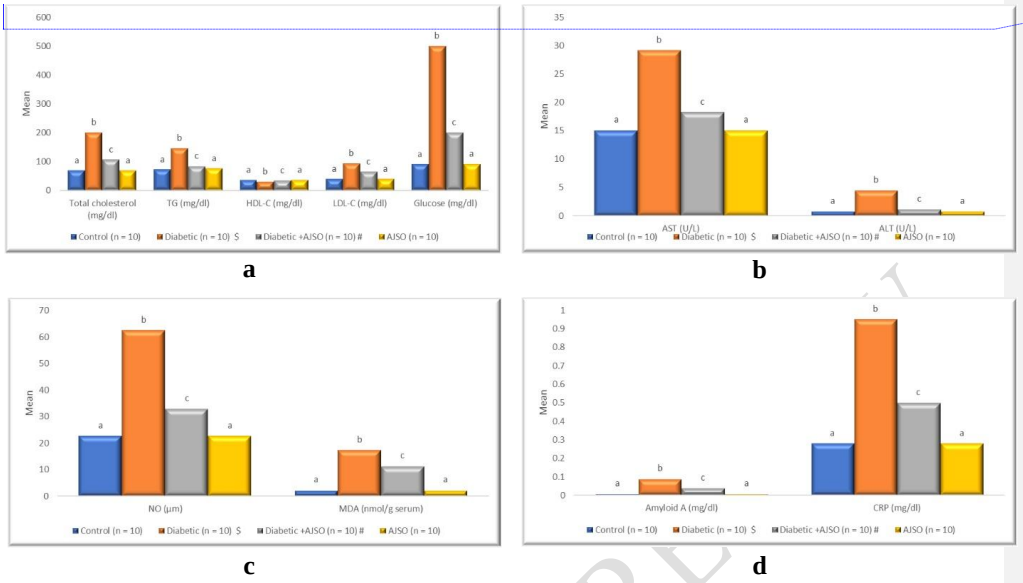


Fig. 2. Biochemical comparison of serum of the different groups in the animal study

(a) Total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and glucose; **(b)** aspartate transaminase (AST) and alanine transaminase (ALT); and **(c)** MDA = malondialdehyde; NO = nitric oxide; **(d)** Amyloid A, CRP = C-reactive protein levels in four rat groups; Control = untreated rats, a diabetic group = rats injected with an intraperitoneal dose of alloxan monohydrate (150 mg/kg) dissolved in normal saline once; the AJSO group =rats treated with a daily oral dose of the AJSO at 200 mg/kg bm for ten weeks; the diabetic + AJSO group = rats injected with alloxan monohydrate at the same dosage as the diabetic group plus a daily oral dose of AJSO as the AJSO group for ten weeks. Data are given as the mean ± SD for 10 rats. Statistical significance was set at $p \leq 0.05$; means denoted by the same letters are not significantly different. $ = % change from the control group, # = % change from the Diabetic group.

Figure 3. illustrates biochemical comparison of the two groups in the human study. Group 2 showed a significant decrease in the concentration of serum NO (34.7%), MDA (43.9%), amyloid A (79.6%), CRP (60.9%), HbA1c (33.8%), and Troponin T (16.4%) compared to Group 1 (Figure 3 a, d, e). This was connected with a significant decline ($p \leq 0.05$) in serum total cholesterol (14.2 %), TG (28.1%), LDL-C (6.1%), AST 8.8%, and ALT (11.6%) compared to Group 1 (Figure 3b and c). Group 2 showed 8.5% increase in HDL-C than Group 1 (Figure 3 b). Furthermore Group 2 showed 32% decrease in serum creatinine (16.4%), urea (45.4%), uric acid (23%) levels compared to Group 1 ($p \leq 0.05$; Figure 3 e). Figure 4 compares the biochemical effect of AJSO between the animal study and the human study. The percent of change Diabetic +AJSO group to Diabetic in total cholesterol, TG, HDL, LDL, AST, and ALT, showed (↓46.6%, ↓42.6%, ↑10.6 %, ↓30.5%, ↓37.3%, ↓73.4% respectively) as compared to group1 to group 2(↓14.2%, ↓28.1%, ↑8.5%, ↓6.1%, ↓8.9%, ↓11.6% respectively). Also, the percent of change between Diabetic +AJSO group to Diabetic in NO, MDA, amyloid, CRP, and glucose/HbA1c equal to (↓47.2%, ↓34.8%, ↓55.6%,↓ 47.4%, ↓60.0 % respectively) as compared to group1 to group2 showed (↓34.7%, ↓43.9%, ↓79.6%, ↓60.9%, ↓33.9% respectively), ($p \leq 0.05$; Figure 4).

Comment [HL2]: The marker of graph should be a, b, c, d (in this figure a,b, c,a)

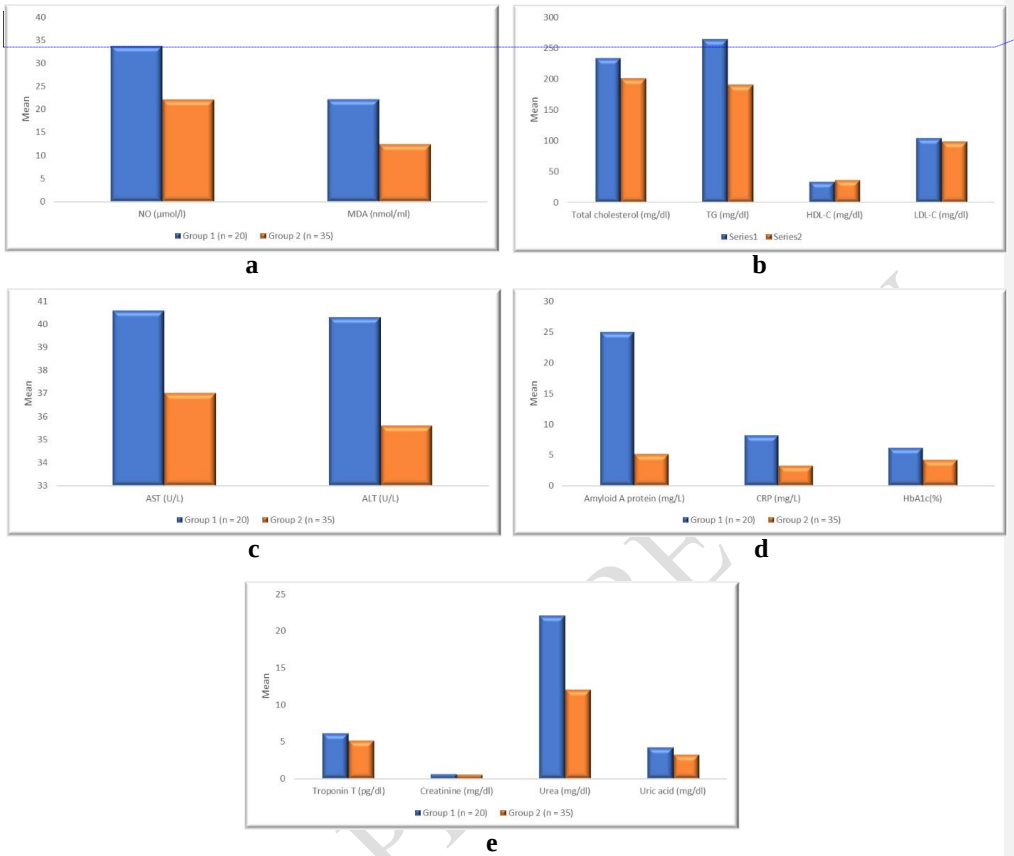


Fig. 3. Biochemical comparison of the two groups in the human study.

(a) nitric oxide (NO), malondialdehyde (MDA) levels**(b)**Total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) and **(c)** aspartate transaminase (AST) and alanine transaminase (ALT) levels**(d)** Amyloid A, C-reactive protein (CRP), HbA1c **(e)** Troponin T , Creatinine , Urea, Uric acid in Group 1 (without drinking Ajwa seed as caffeine-free coffee), Group 2 (with drinking Ajwa seed as caffeine-free coffee replacements). Data are shown as mean ± SD for 55 volunteers (group 1: n =20; group 2: n=35) Statistical significance was set at $p \leq 0.05$.

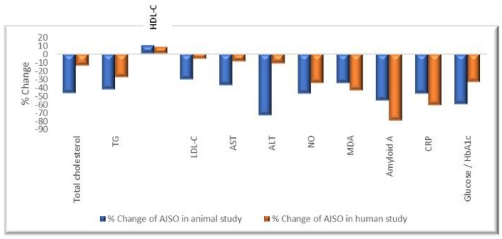


Fig. 4. Comparison of the biochemical effect of AJSO between the animal and human studies.

Total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate transaminase (AST), alanine transaminase (ALT), nitric oxide (NO), malondialdehyde (MDA), amyloid, C-reactive protein (CRP), and glucose/HbA1c levels were compared between the percent of

Comment [HL3]: Please add standard deviation bar in all the chart

change between (Diabetic +AJSO) and Diabetic in rat study compare with the percent of change between (group 1) to (group 2) in human study a diabetic group = rats injected with an intraperitoneal dose of alloxan monohydrate (150 mg/kg) dissolved in normal saline once; AJSO group =rats treated with a daily oral dose of AJSO at 200 mg/kg bm for ten weeks; a diabetic + AJSO group = rats injected with alloxan monohydrate at the same dosage as a diabetic group plus a daily oral dose of AJSO as the AJSO group for ten weeks. Group 1 (without drinking Ajwa seed as caffeine-free coffee Group 2 (with drinking Ajwa seed as caffeine-free coffee replacements). Data are shown as mean ± SD for 55 volunteers (group 1: n =20; group 2: n=35) Statistical significance was set at $p \leq 0.05$.

4. DISCUSSION

Diabetes type 2 is associated with hyperglycemia and inflammatory reaction activation that increases death rates in the world [11]. The diabetic complication is marked by increased c- reactive protein, amyloid deposition [12], [13], and accumulation of different reactive oxygen species (ROS) [12], [13] is an indicator of oxidative stress such as MDA and NO.; the result in this study for the group with alloxan monohydrate -induced Diabetes, which revealed increased oxidative stress and the inflammatory markers compared to the control group, agrees with this. This concern may lead to the diseases initiation backgrounds [14], [15], which may boost pathological disorders such as immune disorders, liver, heart, and kidney function disruptions, including fatty liver and hyperlipidemia, nephritis especially in individuals with raised genetic susceptibility, and other issues [12], [16]–[18].

Our results shown in Figure 2 concur with previous reports that diabetes activates inflammatory responses. We found higher levels of amyloid A, C-reactive protein, lipid peroxidation, nitric oxide, lipid and liver profiles, and glucose in the Diabetes group than in the control group in the rat study and in Group 1 volunteers than in Group 2 volunteers (Figure 3).

A previous investigation noted that hyperglycemia involves the inflammatory marker and metabolic processes in humans [19], [20]. Our results are in agreement with this and revealed an elevation in HbA1c, and metabolic functions of the liver, kidney, heart, and lipid profile as well as serum amyloid A, C-reactive protein, lipid peroxidation, nitric oxide in the Group 1 volunteers compared to Group 2 (Figures 2 and 3) [21].

To initiate diabetes and insulin resistance [22] that can trigger prooxidant and inflammatory reactions, we injected the animals in this investigation with alloxan monohydrate once; after the confirmed diabetes in rats examination glucose levels with a glucometer above 290 ±10 mg/dl. Insulin resistance was a main hallmark for the etiology and pathogenesis and development of type 2 diabetes mellitus (T2DM) [23], [24]. The outcome of insulin resistance is connected with low-grade tissue-specific inflammatory reactions induced by multiple pro-inflammatory and oxidative stress triggers pro-inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-1 beta, interleukin-6, and others. Chronic vulnerability to pro-inflammatory intermediates promotes the activation of cytokine signaling proteins such as C-reactive protein and amyloid A [3], [4], [24] which agree with the current study. The inflammatory protein reaction is eventually obstructing the hemostasis of insulin signaling receptors in β-cells of pancreatic islets and causes constant pain and many diabetes complications [25], [26]. Amyloid genesis develops in patients with active, chronic inflammatory diseases, such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, fibromyalgia, and other [3], [27], [28]. The deposition of amyloid is different tissue associated with increased release of the proinflammatory cytokines interleukins, tumor necrosis factor and c reactive protein. Abnormal amyloid in organs shows amyloidosis and plays a role in different diseases and immune responses, and active constant pain. Furthermore, amyloidosis is a dysfunction of protein that results in the deposition of insoluble amyloid fibrils in tissues, which causes organ dysfunction and ended with the failure of many organs [3], [29], [30]. The present study reported that the elevation of amyloid A protein and c reactive protein results from hyperglycemia and diabetes complications. A current investigation represented an elevation in all studied parameters in both the diabetic groups in the control group in the animal experiment and Group 1 than in Group 2 human observation study.

Treatment of diabetes involves insulin medicine, a healthy diet, and an active lifestyle. The use of herbal medicine is considered the most popular option recently [6]. Date palm (Phoenixdactylifera) is a constituent of the palm family (Arecaceae) found in Saudi Arabia [31]–[33]. Our recent study reported that Date palm seeds contain components such as complex carbohydrates, beneficial phytochemicals, sterols, healthy fatty acids, carotenes, and flavonoids that have numerous medicinal activities and are utilized as animal feed or coffee substitutes that agree with the present study [6], [31]–[33]. The present study reported that AJSO included polyphenols, terpenoids, flavonoids, and alkaloids (table 1 and 2). Our current study also reported that AJSO contains essential nutrients such

as Na and K, with the absence of heavy toxic metals (Table 1) which consider Ajwa seed extract a healthy edible natural herbal medicine. These results agree with the previous investigation that reported that Ajwa seed is used as an adsorbent treatment for low-cost detoxification herb powder [34].

Our previous study reported that Ajwa seed extract has a synergetic inhibitory as anti-inflammatory and antiapoptotic also through docking experiment binding free energy with specific receptor as a beneficial antioxidant [4]. As we reported before that Ajwa date seed oil includes oleic, palmitic, sterol, phytochemistry, and fatty acids and their by-products; these have antioxidant, anticancer, antibacterial, and antilipidemic effects [6]. These compounds also have antioxidant and anti-inflammatory, neurological, sedation, and cosmetic effects. These polyphenols play an important role as antioxidants to capture free radicals [6], [35]. This effect was shown in our study where a decline in the serum studied parameters after treatment with Ajwa seed extract in both rats and humans. As well as after oral administration of AJSO to rats following diabetic induction, there is a decrease in amyloid A, C-reactive protein, lipid peroxidation, nitric oxide, lipid and liver profiles, and glucose in the Diabetic +AJSO group than in the diabetic group. Furthermore, there are improvements in the levels of HbA1c, and metabolic functions of the liver, kidney, heart, and lipid profile as well as serum amyloid A, C-reactive protein, lipid peroxidation, and nitric oxide in the Group 2 volunteers compared to Group 1. This may be due to the combination of Ajwa seed phytochemical synergetic beneficial effect through the decrease of hyperlipidemia, and hyperglycemia, lipid peroxidation, inflammation process, and improvement in biological profiles.

5. CONCLUSION

Comment [HL4]: Please add some quantitative data in conclusion.

We confirmed control of alloxan monohydrate-induced diabetes, oxidative stress, and inflammation by AJSO extract. Where, there was a decline in amyloid A, C-reactive protein, lipid peroxidation, nitric oxide, lipid and liver profiles, and glucose after treatment with AJSO extract compared to the rats treated with alloxan monohydrate. The outcomes of the animal investigation were promoted by rapprochement of results for human volunteers drinking Ajwa seed as caffeine-free coffee replacements with those for undrinking volunteers. We finalize AJSO extract is the preliminary point therapy for the prevention/control of inflammation and diabetic complication.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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