Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2024 21(1): 162-171. **OPEN ACCESS**

In vitro and *in vivo* efficacy and safety of *Ferula assa foetida* L.

Abeer Hamad [1,3], Nurah M. Alzamel[1] and Naglaa Elarabany [*1,2]

[1]Biology Department, Faculty of Science and Humanities, Shaqra University, Shaqra 11961, **Saudi Arabia**
[2]Zoology Department, Faculty of Science, Damietta University, New Damietta 34517, **Egypt**
[3]Biology Department, College of Applied and Industrial Science, Bahri University, Khartoum 1660, **Sudan**

***Correspondence: nelarabany@su.edu.sa** **Received: January 03, 2024, Revised: February 16, 2024, Accepted: February 21, 2024 e-Published: February 22, 2024**

Ferula Assa Foetida oleo gum resin (OGR) is a cooking spice and traditional medicine for treating different ailments worldwide. The present study evaluated asafoetida OGR antioxidant properties and its toxic effect on haematological and biochemical parameters to prove its importance as a traditional spice and medicine. The methanolic extract's total phenolic and flavonoid content and qualitative phytochemical screening were measured. OGR was tested for its scavenging properties In vitro by 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and ferric-reducing antioxidant power (FRAP). In vivo, the toxic effect of OGR was evaluated by acute and chronic oral administration of OGR extract. The quantitative phytochemical screening revealed that OGR showed the total phenolic and flavonoid compounds were 429.32± 11.81mg/gm of gallic acid equivalent and 191.93 ± 6.21 mg/gm of catechin equivalent. Moreover, OGR extract was also rich in secondary metabolites with valuable medicinal activities such as coumarins, flavonoids, alkaloids, tannins, triterpenes and steroids. Asafoetida OGR showed promising radical scavenging activity in all assays (IC50 value >42.9 µg/mL). In vivo, OGR extract administration decreased lipid peroxidation in plasma and liver and increased CAT and SOD activities. It also significantly increased ALT, AST, and ALP while no significant changes were found in urea or creatinine. Regarding haematological parameters, OGR decreased RBCs, haematocrit, and platelet levels; no changes happened on WBCs. These findings suggest potential antioxidant properties of F. assafoetida both in vitro and in vivo and had broad safety and low toxicity for short-term use at a dose of 150 mg/kg.

Keywords: Antioxidant, catalase, *Ferula assa-foetida*, lipid peroxidation (MDA), oxidative stress, SOD, haemato-toxicity, biochemical toxicity

INTRODUCTION

Since old times, medicinal herbs and their extracts have been used for different diseases' treatments and have many therapeutic potential due to their phytoconstituents and antioxidant components (Alves and Rosa, 2005; Shahat et al. 2018). Medicinal plants produce bioactive substances, such as antioxidants that counteract free radicals and inhibit microbial growth in other species (Aziman et al. 2014). Moreover, they play a significant role in preventing or controlling diseases because of their antioxidant properties which are usually associated with different polyphenol compounds (Tungmunnithum et al. 2018). Naturally occurring antioxidants like phenolics and flavonoids, which have anti-inflammatory, anti-cancer, and antimicrobial properties, are mostly found in food and inedible plants (Sengul et al. 2009; Ramachandran et al. 2012; Mahato et al. 2018).

Increased reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced during oxidative stress are the main reasons for carcinogenesis (Valko et al. 2006). Elevated free radicals that cause toxicity can essentially be eliminated by substances of plant origin, and numerous compounds with these activities have previously been identified in different herbs (Manjamalai and Grace, 2012; Al-Rimawi et al. 2020). Natural antioxidants, especially those found in plants, are of particular interest to researchers because they have fewer side effects.

Ferula assa foetida L is one of the Umbelliferae family which is widely used as a traditional medicine for many problems in the reproductive system, nervous system, gastrointestinal tract and some tumours (Sirizi et al. 2023). The main bioactive compound in the plant is the resin (OGR), which is considered a folklore medicine in many countries (Abd El-Razek et al. 2001). OGR generally is collected by making an incision between the roots and stems (Ali et al. 2023). This genus extract contains interesting phytochemical features, such as the occurrence of phenolic substances like diterpenes, phenolic acids (cinnamic and benzoic acid derivatives), tocopherols, carotenoids and flavonoids which are very

powerful antioxidants (Yatham et al. 2022). OGR is traditionally used as an analgesic, aphrodisiac, sexual aphrodisiac (Eigner and Scholz, 1990), anticonvulsant, antispasmodic, antiseptic and anthelmintic agent (Sirizi et al. 2023). It is also believed to have properties and increase sexual drive (Eigner and Scholz, 1990).

Asafetida OGR's toxic effects have primarily been studied in parasites and a few protozoan species, which suggests that it has antifungal (Sitara et al. 2008), antiparasitic (Ramadan and Al Khadrawy, 2003; Ramadan et al. 2004; Kumar and Singh, 2006) and antibacterial properties. Moreover, asafetida has been proven to have cytotoxic effects on brine shrimp (Kumar and Singh, 2006).

To evaluate the usefulness of this resin as a medicinal herb, the phytochemical screening and antioxidant activity of the asafoetida OGR was examined by applying different *In vitro* assay systems, such as DPPH, ABTS and FRAP; and *In vivo* to assess the toxic effects of asafetida on haematological and biochemical parameters in male Sprague Dawley rats.

MATERIALS AND METHODS

Ferula assa-foetida oleo gum resin and preparation of extracts:

Ferula assa foetida OGR is a solid brownish mass with the highest purity grade that was purchased from a local market in Mansoura, Egypt. To prepare the methanolic extract, 100g and 200g of dried OGR powder were soaked in 500 ml methanol (95%) for 48h at room temperature. To avoid volatile contents preservation, the temperature was raised to 50°C using a hot plate and kept warm for 5 hours. After cooling, crude methanol extracts were filtered in a vacuum using Whatman filter paper (grade 40) with their half volume (100 g/ 250 ml and 200 g/ 250 ml) solutions were then evaporated and the solid residues were resuspended in 20% methanol, this step was repeated three times and the extract was stored in desiccators for further work.

Phytochemical Screening:

Quantitative Phytochemical Screening:

Determination of Total phenolic content: Folin–Ciocalteu reagent was used to measure the total phenolic content of OGR extract following the method described previously (Kiran et al. 2015). Different dilutions of OGR extract (0.5 mL of 1 mg/mL) and gallic acid as standard were made using dH2O (1:10 dilution). 0.5 ml from each aliquot was added to 2.5 ml and then Folin–Ciocalteu reagent solution (0.2 M), followed by 4 mL of 1 M aqueous sodium carbonate. Each reaction mixture was left for 30, and then the absorbance was read at 765 nm using a UV- -spectrophotometer. A calibration curve was made using gallic acid and the total phenolic content was expressed in mg of gallic acid equivalent (GAE; mg/g of dry mass). All measurements were done in triplicate and the data were analyzed as the mean of three values.

Determination of Total Flavonoids Contents:

The total flavonoid content was determined by a calorimetric method described by (Zhishen et al. 1999). A 100 µl of OGR extract was added to 4 ml of distilled water, followed by 0.3 ml of 5% sodium nitrite. The reaction mixture was allowed to set for 5 min and 0.3 ml of 10% aluminium chloride was added. 2 ml of 1 M sodium hydroxide was added within 6 minutes. 3.3 ml of distilled water was mixed thoroughly with the reaction mixture. The absorbance was measured at 510 nm against a blank using catechin as standard for the calibration curve. The total flavonoid content of OGR extract was expressed in mg catechin equivalents per gram of sample (mg/g). All measurements were performed in triplicate and the values were expressed as means of three values.

Qualitative Phytochemical Screening:

Phytochemical screening for the active constituents was carried out on the methanolic extract of asafoetida OGR according to previous methods (Wall et al. 1952; Sofowora, 1982; Harborne, 1984; Martinez and Valencia, 1999; Gennaro, 2008).

Identification of tannins:

A weight of 0.5 g of OGR extract was dissolved in 10 ml hot saline solution and divided into two test tubes. 2-3 drops of ferric chloride were added to one tube and 2 – 3 drops of gelatin salts reagent to the other one. The presence of tannins is identified by the appearance of a blackish-blue colour in the 1st test tube and turbidity in the 2nd one.

Test of sterols and triterpenes:

A weight of 0.5 g OGR extract was dissolved in 10 of chloroform. 0.5 ml acetic anhydride was added to 5 ml of the solution followed by 3 drops of concentrated sulfuric acid. The gradual appearance of green, blue, and pink to purple colour at the contact zone of the two liquids was taken as evidence of the presence of triterpenes (pink to purple) or sterols (green to blue) in the sample.

Test for Alkaloids:

A weight of 0.5 g of OGR extract was incubated with 5 ml of 2N HCL in a water bath and stirred for 10 minutes, the mixture was then cooled, filtered, and divided into two test tubes. A few drops of Mayer's reagent were added to one tube while a few drops of Valser's reagent were added to the 2nd one. The presence of alkaloids will cause slight turbidity or heavy precipitate in either of the two test tubes.

Tests for Flavonoids:

A weight of 0.5 g of OGR extract was dissolved in 30 ml of 80% ethanol, the mixture was then filtered. One ml of aluminium chloride solution in methanol (1%) was added to 3 ml of the filtrate. The appearance of yellow colour was considered an indicator of flavonoids, flavones, or/and chalcone presence. Another 2 ml of the filtrate was mixed with 0.5 ml of magnesium turning. Changing the colour to pink or red was taken as evidence of flavone existence.

Test for Saponins:

A weight of 0.3 g of OGR extract was mixed with 10 ml of distilled water, and the mixture was vigorously shaken for about 30 seconds. When the foam started to form after the mixture had been left to stand for at least an hour, it was taken as an indicator that saponins were present.

Test for Cumarins:

A weight of 0.2 g of OGR extract was dissolved in 10 ml distilled water. A filter paper with a spot of 0.5N KOH was used to cover the mixture and to saturate it with the vapour. The filter paper was then removed and examined by UV light, the presence of coumarin was determined if the spot adsorbed the UV light.

Test for Anthraquinone glycoside:

A weight of 0.2 g of OGR extract was boiled with a mixture of 9 ml of 0.5N KOH and 1 ml of 3% H_2O_2 solution. The mixture was extracted by shaking with 10 ml of benzene. 5 ml of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was considered an indicator of the alkaline layer that might have a pink or red colour.

Antioxidant Assays:

To determine the antioxidant effects of plant extracts, different antioxidant assays were performed.

2,2′-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay

OGR radical scavenging activity against DPPH radicals was determined according to a previous method (Zhu et al. 2015). In a 96-well plate, 10 µL of positive control solutions (31.25–1000 µM Trolox and Ascorbic acid) were added to 190 µL of methanol solution of DPPH (0.1 mM). After thirty minutes of reaction at room temperature, the absorbance was measured at 517 nm with a microplate reader (Tecan Infinite M200 PRO, TECAN, Männedorf, Switzerland) using methanol as a blank control. All controls and extracts were measured in triplicate. DPPH inhibition percentage was computed and measured according to the equation: Radical scavenging rate (%) = [(AbC − AbS)/ AbC] × 100, where AbC is the blank control absorbance value, Abs is the tested sample absorbance value, and the IC50 value was obtained when DPPH free radicals were inhibited by 50%.

2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay

Asafoetida OGR ABTS radical scavenging activity was assayed according to a previous method by Zhu et al. (2015). Working ABTS+ (radical cation) (Sigma-Aldrich, USA) solution was prepared by mixing equal quantities of potassium persulfate (4.9 mM in H2O) and ABTS solution (7 mM in H2O), and allowed them to react, for 12-16 hours, in dark at room temperature. Appropriately, ABTS+ solution was diluted with methanol to achieve an absorbance value at 734 nm of 0.70±0.10. In a 96-well plate, the mixture solution consisting of appropriately diluted samples (10µL) or Vitamin C solution (31.25-1000 µM) and ABTS+ solution (190µL) was incubated in the dark for 30 minutes before reading the absorbance at 734 nm. Vitamin C was used as a positive control and methanol as a blank. All controls and extracts were tested in triplicate. ABTS assay IC_{50} value was calculated at different concentrations using the same formula described in the DPPH assay.

Total antioxidant contents (FRAP assay):

The total antioxidant contents of asafoetida OGR were evaluated by ferric-reducing antioxidant power (FRAP) assay according to the method of Niazmand and Razavizadeh (2021). The ferric tripyridyltriazine complex is being reduced to its ferrous by antioxidants. The FRAP solution was freshly prepared by mixing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 10 mM TPTZ solution (2,4,6-tripyridyl-s-triazine in 40 mM HCL), and 2.5 ml of 20 mM FeCl3·6H2O solution. This mixture was incubated for 30 min at 37 °C. About 150 µl of OGR extract was added to 3 ml of FRAP solution and left for 30 min in the dark. The absorbance was read at 593 nm using ferrous sulfate as a reference. FRAP was determined as µmol of Fe2+ equivalents (FE) per gram of dry extract (µmol FE/g dry extract)

Animals and Study Design

Forty Sprague Dawley male rats, with body weight ranging between 110 and 140g (6–8 weeks old) were obtained from URAF and allowed to adapt for two weeks under temperature at 21 ± 1°C in a daylight cycle. Animals were housed in plastic cages and food and water were made available ad libitum. All procedures were performed following the Institutional Animal Care and Use Committee (IACUC). Ethics approval for this study was obtained from the Cairo University Institutional Animal Care and Use Committee (approval number: URAF E-3-23)

Determination of LD_{50}:

To determine the acute toxicity of asafoetida OGR, 20 rats were divided into four groups (n = 5/ group) and were administered aqueous suspension of OGR extract at doses 0 (control) 250, 500, and 1000 mg/kg /day. The control group received 1 ml of distilled water. All rats were observed immediately for 48 hours and mortality was recorded (Hayes, 2001; Devaki et al. 2012).

Chronic toxicity administration:

Twenty rats were divided into five groups (n = 4/each) and four groups were orally administered aqueous suspension of OGR extract at doses of 25, 50, 100, and 150 mg/kg body weight/ day (Bagheri et al. 2015) while the 5th group was assigned as a control group and received 1 ml of distilled water. All groups were treated for 4 weeks.

Serum, plasma and tissue sampling:

At the end of the experiment, all rats were euthanized under diethyl ether anaesthesia. About 4 ml blood samples were drawn using cardiac puncture methodology and centrifuged for 10 min at 2000x g. Finally, plasma samples and sera were collected and stored at -20 for subsequent biochemical analysis. Liver samples were removed and weighed individually and then washed with cold phosphate-buffered saline (PBS), pH 7.4. Liver homogenates (10%) were prepared by homogenizing a specific weight of each liver in 0.1 M phosphate buffer (pH 7.4) using a homogenizer at 4 °C. The homogenates were centrifuged at 1,500 ×g, the obtained supernatants were kept at −80 °C for evaluation of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA). All assays were done using available commercial kits (Biodiagnostics Co.), and a UV spectrophotometer was used to measure the absorbance values of all samples and standards.

Statistical analysis

Statistical analysis was performed using version 20 SPSS (SPSS Inc., Chicago). All data were represented as mean ± SD. ANOVA test followed by Duncan's test was used for different assessments. $P<0.05$ was statistically significant.

RESULTS

Quantitative Phytochemical Screening:

The total phenolic content (TPC) and total flavonoid content (TFC) values of OGR extract are shown in Table 1. According to the results, the methanolic extract of asafoetida OGR has a considerable level of total phenolic content (429.32± 11.81 mg GAE/g extract) and total flavonoids content (191.93 ± 6.21 mg Catechin/g extract).

Table 1: Total phenolic and flavonoid contents of methanolic extract of *Ferula assa-foetida* oleo gum resin

Phytochemicals	Concentration
Total phenolic content (mg GAE/g extract)	429.32± 11.81
Total flavonoids content (mg Catechin/g extract)	191.93 ± 6.21

Qualitative Phytochemical Screening:

In the present study, qualitative phytochemical screening was carried out on methanolic extract of asafoetida OGR. The results in **Table 2** revealed that the extract contained secondary metabolites with higher medicinal properties such as tannins and sterols in high quantities, flavonoids and triterpenes were present in moderate amounts and traces of coumarin, and alkaloids. Anthraquinones, tannins, and saponins were absent.

Asafoetida OGR extract antioxidant activities

In this study, we used different assays (DPPH, ABTS, and FRAP) to assess and compare the antioxidant potential of Asafoetida. The IC_{50} values for 100g/250 ml OGR extract were 42.91±1.4 and 83.6± 4.2 µg/mL for DPPH and ABTS, respectively. On the other hand, the dose of 200 g/250 ml was more active and had IC_{50} values of 34.46±0.9 and 78.6±3.2 µg/mL, respectively. Similarly, the reducing capacity of asafoetida OGR increased with increasing concentration, FRAP level was 1.87 ± 0.05 and 2.21 ± 0.01 µmol FE/g dry extract at 100g/ 250 ml and 200/ 250 ml, respectively (Table 3).

Table 2: Phytochemical Screening of Methanolic Extract of asafoetida oleo gum resin

Saponins	Cumarins	Alkaloids	Flavonoids	Tannins	Sterols	Triterpenes	Anthraquinone
-	+	+	++	+++	+++	++	-

Key: +++ High ++ Moderate + Trace - Negative

Table 3: Antioxidant activity of Asafoetida OGR. Values are the mean of three replicates ± SE; DPPH= 2,2 diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid); radicals of DPPH and ABTS were inhibited by 50%, the *maximal-half* inhibitory level (IC_{50}) was obtained. FRAP [µM Fe (II) equivalent] method.

Parameter	OGR (100 g/250 ml)	OGR (200 g/250 ml)
DPPH IC_{50} (µg/mL)	42.91±1.4	34.46±0.9
DPPH activity (%)	87.7± 3.2	93.52± 4.1
ABTS IC_{50} (µg/mL)	83.6± 4.2	78.6±3.2
ABTS activity (%)	62.32± 2.5	88.59±2.1
FRAP (µmol FE/g dry extract)	1.87 ± 0.05	2.21 ± 0.01

Acute toxicity results:

All rats showed normal activity, no signs of toxicity, and no mortality was recorded following acute oral administration of various dosage doses of asafetida, which demonstrated that the methanol extract of asafoetida OGR is not lethal up to a dose of 1000 mg/kg.

Effect of asafoetida OGR extract on biochemical parameters:

The results of serum, liver, and kidney functions are shown in Table 4. No significant changes were found in urea and creatinine concentrations between the control and all treated groups with asafetida OGR. Moreover, AST, ALT, and ALP were significantly elevated in the asafetida 150 mg/kg group compared to other treated and control groups ($P < 0.05$).

Effect of asafoetida OGR extract on haematological parameters

Table (5) shows that RBC, platelet concentrations, and HCT % significantly decreased after 4 weeks of OGR administration, while no significant change was found in WBC concentration.

Effect of asafoetida OGR extract on plasma and liver lipid peroxidation

The concentrations of MDA in both liver and plasma are shown in **Table 6**. The MDA levels in both the liver and plasma of the treated groups decreased compared to the control group.

Effect of asafoetida OGR extract on antioxidant enzyme activity in rat liver

As shown in Figure 1, administration of asafoetida OGR extract at doses of 25, 50, 100, and 150 mg/ kg/day elevated SOD in plasma (1.51± 0.10, 0.77± 0.12, 1.89± 0.27 and 1.90± 0.27 U/min, respectively) as well as in the liver (1.21±0.09 1.21± 0.09, 2.18± 0.31 and 2.23± 0.12 U/ g tissue, respectively), the changes in SOD activity was not dose-dependent. On the other hand, the changes in plasma CAT activity for administered doses of OGR extract (25, 50, 100, and 150 mg/ kg/day) were 4.42%, 46.50 %, 64.83 %, and 118.,80 % of the control respectively while liver CAT activity changed by 9.75 %, 51.20 %, 56.00 %, and 94 % of control, respectively (Figure 2).

Table 4: Effect of asafetida OGR methanolic extract on liver and kidney biochemical measurements in male Sprague Dawley rats (n=4).

Groups	Urea (g/dL)	Cr. (mg/dL)	AST (U/ml)	ALP (U/ml)	ALT (U/mL)
Control	9.21±0.48	0.68±0.08	30.0 ±2.47	70.57± 2.92	28.92±1.12
Asafetida 25 mg/kg	11.68± 0.84	0.49± 0.06	23.40±3.33	72.22± 2.29	28.02± 1.64
Asafetida 50 mg/kg	11.34± 0.39	0.78± 0.08	30.60±1.21	75.54± 2.71	24.60± 2.64
Asafetida 100 mg/kg	10.06± 0.58	0.66± 0.11	34.00±2.77	74.16± 5.43	24.02±1.99
Asafetida 150 mg/kg	11.68±0.76	1.51±0.18	46.57±2.90*	112.10±11.45*	50.69±3.26*

*****Represents significant changes at P<0.05 when compared to control values. Values are means±SE. SE= Standard error of the mean, Cr= Creatinine, AST =Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP= Alkaline phosphatase.

Table 5: Effect of asafetida OGR methanolic extract on haematological measurements in male Sprague Dawley rats (n=4)

Groups	WBCs ($\times 10^3$/µl)	RBCs ($\times 10^6$/µl)	HCT (%)	PLT ($\times 10^3$/µl)
Control	16.46± 2.35	72± 3.83	37.4± 1.85	734± 64.14
Asafetida 25 mg/kg	13.5± 1.52	67.9± 1.82*	34.7± 1.05*	685±26.74*
Asafetida 50 mg/kg	12.7± 2.10	61± 2.60*	34.9± 1.22*	680±25.41*
Asafetida 100 mg/kg	10.5± 1.92	62.07± 1.72*	32.8±1 .33*	682±63.98*
Asafetida 150 mg/kg	14.7± 1.16	66.7± 1.87*	33.7± 0.82*	648±45.32*

*Represents significant changes (P<0.05) when compared to control values. Values are means±SE. SE= Standard deviation of the mean, WBCs = White blood cells, RBCs = Red blood cells, HCT = Hematocrit, PLTs = Platelets

Table 6: Effect of asafetida OGR methanolic extract on haematological measurements in male Sprague Dawley rats (n=4)

Groups	Plasma MDA(nmol/ml)	Liver MDA(nmol/ml)
Control	35.04± 5.28^{a}	48.92± 5.32^{a}
Asafetida 25 mg/kg	29.82± 5.28^{a}	38.10±6.06^{ab}
Asafetida 50 mg/kg	32.66± 5.78^{a}	24.27± 4.35^{bc}
Asafetida 100 mg/kg	12.19± 2.46^{b}	8.52± 3.32^{c}
Asafetida 150 mg/kg	26.15± 5.28^{ab}	15.78±2.53^{bc}

Superscript letters represent significant changes (P<0.05) when compared to control values. Values are means±SE. SE= Standard deviation of the mean, MDA = Malondialdehyde.

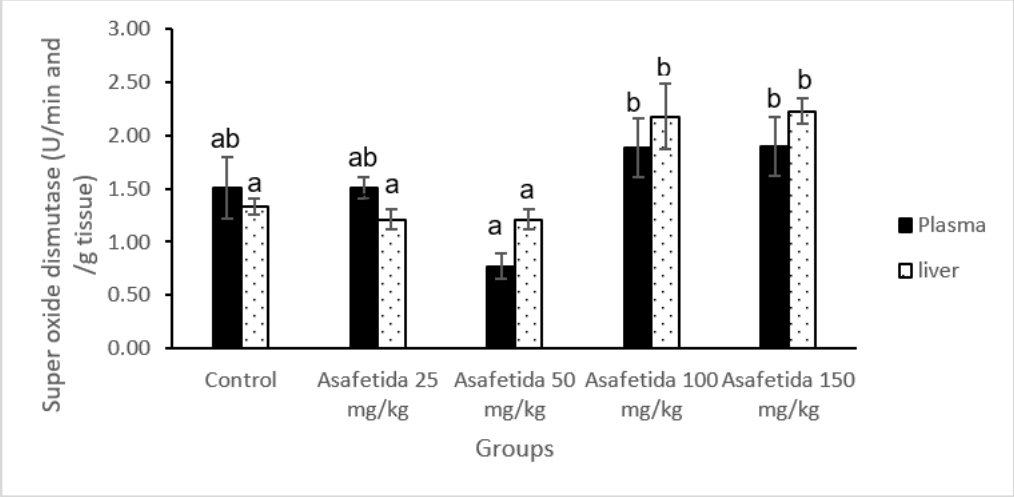

Figure 1: Superoxide dismutase activity (SOD) in plasma (U/min) and liver (U/min/gm tissue) of male Sprague Dawley rats in control, four groups treated with different dosages of asafoetida OGR methanolic extract (25, 50, 100, and 150 mg/kg bw). Bars represent means± SE, and letters on each bar indicate significantly different groups.

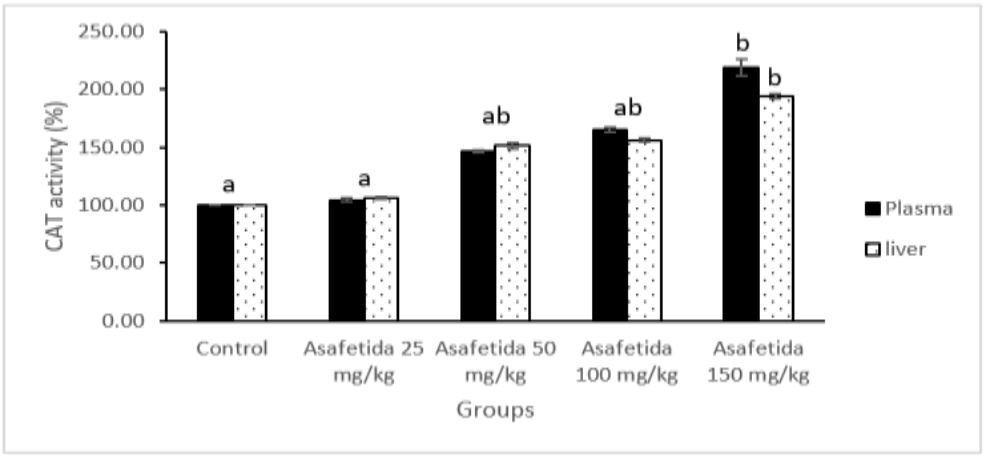

Figure 2: Changes in Catalase activity (CAT) in plasma and liver of male Sprague Dawley rats in four groups treated with different dosages of asafoetida OGR methanolic extract (25, 50, 100, and 150 mg/kg bw) in comparison to the control group (100%). Bars represent means± SE, and letters on each bar indicate significantly different groups.

DISCUSSION

The redox properties of specific compounds are necessary for neutralizing free radicals, the removal of free oxygen species, and the breakdown of peroxides. Natural antioxidant alternatives are safer and have fewer adverse effects than synthetic antioxidants. Medicinal plants, which are the primary source of natural antioxidants have been thoroughly studied. Herbal substances with strong antioxidant activity can aid in the prevention and treatment of diseases brought on by oxidative stress, such as diabetes and cardiovascular disease (Noroozi et al. 2008). Novel agents can be found for usage in both pharmaceutical and food industries by conducting studies on these naturally occurring antioxidants and evaluating their effectiveness compared to synthetic molecules.

Several present studies showed that the existence of phenolic and flavonoid components in medicinal plants is typically positively correlated with their antioxidant activity (Rai and Kon, 2013; Wong-Paz et al. 2015). The cytotoxic effect is shown by phytochemicals such as tannins, flavonoids, and alkaloids (Blois, 1958). Tannin has growth inhibitory activity, anticancer (Chowdhury et al. 2017) and antihelmintic properties (Bailly and Vergoten, 2020) while flavonoids could prevent cardiovascular diseases and cancer (Ballard and Marostica, 2019). The tested OGR extract showed high levels of phenolic and flavonoid compounds which have significant antioxidant activities and could be used as anticancer, antibacterial, and antiseptic agents, and antioxidants.

Thus, in this study, the *In vitro* antioxidant potentials of the OGR extract were evaluated by DPPH, ABTS, and FRAP assays. In a dose-dependent manner, OGR extract showed high DPPH and ABTS radical scavenging activity. These results are in line with Niazmand and Razavizadeh (2021) who reported that both leaf and gum extracts had DPPH scavenging activity (IC_{50} = 84.00 ± 1.07 and 22.72 ± 0.35; respectively). Moreover, Martinez and Castillo (Martínez et al. 2019) reported that the DPPH scavenging of pomegranate and rosemary extracts was 92.55%, 81.29%, and 77.96%, respectively. FRAP level of asafoetida OGR extract in the current study was close to those results by Niazmand and Razavizadeh (2021) who found that FRAP levels in asafoetida leaf and gum extracts were 2.57 ± 0.07 and 1.12 ± 0.02; respectively. On the other hand, FRAP level was lower than those reported by Martinez and Castillo (Martínez et al. 2019) in ethanolic extracts of *Thymbra spicata* and *Thymus vulgaris* (14.19 and 26.93 µM Fe+2/g, respectively).

Asafetida is used widely as a cooking spice and treats many diseases. Although there is no experimental data about the toxicity effects of asafetida, it was confirmed that intake of higher doses of asafetida can cause lip swelling, diarrhoea, discomfort, and headache (Amiri et al. 2023).

The non-toxic properties and oral safety of asafetida at the chosen dose were validated because there were no fatalities in treated rats. Any compound or medication with an estimated oral LD_{50} higher than 1,000 mg/kg could be regarded as having minimal toxicity (Clarke and ML, 1997). This suggests that asafetida OGR might be safe at doses up to 1,000 mg/kg body weight in terms of fatality.

In chronic oral toxicity studies, Sprague Dawley rats were used to evaluate the safety and nutritional value of *F. assa-foetida* oleo gum resin. Oral administration of asafetida OGR significantly increased AST, ALT, and AST activities in treated rats when compared to the control group (Table 4). These enzymes are markers of liver function and not found in the serum but probably leak during tissue damage and are therefore a good tool in clinical diagnosis (Lal et al. 2022). The elevation in serum ALT, AST, and ALP activity is considered a sensitive indicator of hepatic damage (Akanji and Yakubu, 2000; Appidi et al. 2009).

In the current study, no significant changes were found in urea and creatinine levels after administration of asafetida. Blood urea and creatinine levels usually increase in case of destruction of glomeruli which decreases the glomerular filtration rate (GFR) resulting in chronic renal failure (Al-Habori et al. 2002). Since the urea and creatinine levels did not change, asafetida OGR could be considered not nephrotoxic.

Haematological data in this study showed no significant modifications of white blood cells, on the other hand, RBCs, HTC, and platelets significantly decreased in all treated groups compared to the control group. Changes in RBCs, HTC, and platelets indicate that asafetida OGR might have some moderate effect on the haematological parameters. Similar results were found where asafetida declined all haematological parameters in male Wistar rats (Bagheri et al. 2015). Since the hematopoietic system is very sensitive towards toxic substances (Harper, 1973), the interference of OGR extract on this organ is obvious and its use in the pharmacological field needs to be thoroughly evaluated.

Lipid peroxidation is one of the principal factors involved in cellular damage caused by free radicals and is also a marker of oxidative stress (Dehghan et al. 2007). It could cause several adverse effects by increasing membrane rigidity, decreasing cellular elasticity, reducing RBC survival, and neurodegenerative disorders (Abdollahi et al. 2004). The prevention of lipid peroxidation by antioxidants may be due to their scavenging abilities (Dehpour et al. 2009).

The current *In vivo* data show that lipid peroxidation decreased in OGR extract-treated groups. Antioxidants enzymes such as SOD and CAT, are depleted in the removal of superoxide ions and hydroxyl radicals. SOD scavenging activity is functioning only if it is completed by CAT action. Interestingly, the OGR extract

decreased MDA concentration and increased SOD and CAT activities in both plasma and liver of the treated groups. These results are in line with those of Ghasemi (Ghasemi et al. 2021) who found that the methanolic extract of *F. szowitsiana* root reduced malondialdehyde (MDA), but boosted SOD and CAT levels confirming the plant's antioxidant properties.

CONCLUSIONS

These findings suggest potential antioxidant properties of F. asafoetida both *In vitro* and *In vivo* by scavenging free radicals, reducing cellular lipid peroxidation, increasing the activity of antioxidant enzymes, and total antioxidant power. It has broad safety and low toxicity for short-term use at a dose of 150 mg/kg.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/

Author contributions

Conceptualization, N.E., A.H. and N.M.A.; Data curation, N.E., A.H. and N.M.A.; Formal analysis, N.E., A.H. and N.M.A.; Funding acquisition, N.E. and A.H.; Investigation, N.E., A.H. and N.M.A.; Methodology, N.E., A.H. and N.M.A.; Project administration, N.E.; Resources, N.E., A.H. and N.M.A.; Software, N.E., A.H. and N.M.A.; Supervision, N.E.; Validation, N.E., A.H. and N.M.A.; Visualization, N.E., A.H. and N.M.A.; Writing—original draft, N.E.; Writing—review and editing, A.H. and N.M.A. All authors have read and agreed to the published version of the manuscript.

Funding statement

This research received no external funding.

Institutional Review Board Statement

All procedures were performed following the Institutional Animal Care and Use Committee (IACUC). Ethics approval for this study was obtained from the Cairo University Institutional Animal Care and Use Committee (approval number: URAF E-3-23).

Informed Consent Statement

Not applicable.

Data Availability Statement

All of the relevant data are presented in the paper

Acknowledgements

The author would like to thank the Deanship of Scientific Research at Shaqra University for supporting this work

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abd El-Razek M H, Ohta S, Ahmed A A, Hirata T, (2001). Sesquiterpene coumarins from the roots of Ferula assa-foetida. Phytochemistry 58, 1289-1295.

Abdollahi M, Mostafalou S, Pournourmohammadi S, Shadnia S, (2004). Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to malathion. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 137, 29-34.

Akanji M, Yakubu M, (2000). α-Tocopherol protects against metabisulphite-induced tissue damage in rats. Nig J Biochem Mol Biol 15, 179-183.

Al-Habori M, Al-Aghbari A, Al-Mamary M, Baker M, (2002). Toxicological evaluation of Catha edulis leaves a long-term feeding experiment in animals. J. Ethnopharmacol. 83, 209-217.

Al-Rimawi F, Jaradat N, Qneibi M, Hawash M, Emwas N, (2020). Free radicals and enzymes inhibitory potentials of the traditional medicinal plant Echium angustifolium. European Journal of Integrative Medicine 38, 101196.

Ali A N M, Raghad J F, Nuaman R S, Mahmod Z S, Alamery N D, (2023). Anticancer Potentiality of Ferula assa-foetida Gum Extract against CT-26, HT-29, SW742 and WiDr Colorectal Cancer Cell Lines. South Asian Res J Bio Appl Biosci 5, 1-6.

Alves R R, Rosa I L, (2005). Why study the use of

animal products in traditional medicines? J Ethnobiol Ethnomed 1, 1-5.

Amiri M M, Garnida Y, Almulla A F, Abduljabbar A S, Jalil A T, et al. (2023). Herbal therapy for haemorrhoids: An Overview of Medicinal Plants Affecting Hemorrhoids. Advancements in Life Sciences 10, 22-28.

Appidi J, Yakubu M, Grierson D, Afolayan A, (2009). Toxicological evaluation of aqueous extracts of Hermannia incana Cav. leaves in male Wistar rats. African Journal of Biotechnology 8.

Aziman N, Abdullah N, Noor Z M, Kamarudin W S S W, Zulkifli K S, (2014). Phytochemical profiles and antimicrobial activity of aromatic Malaysian herb extracts against food-borne pathogenic and food spoilage microorganisms. J. Food Sci. 79, M583-M592.

Bagheri S M, Yadegari M, Mirjalily A, Rezvani M E, (2015). Evaluation of toxicity effects of asafetida on biochemical, haematological, and histological parameters in male Wistar rats. Toxicol. Int. 22, 61.

Bailly C, Vergoten G, (2020). Esculentosides: insights into the potential health benefits, mechanisms of action and molecular targets. Phytomedicine 79, 153343.

Ballard C, Marostica M, (2019). Health benefits of flavonoid. Bioactive Compounds. Sawston. Cambridge: Woodhead Publishing.

Blois M S, (1958). Antioxidant determinations by the use of a stable free radical. Nature 181, 1199-1200.

Chowdhury S, Poddar S K, Zaheen S, Noor F A, Ahmed N, et al. (2017). Phytochemical screening and evaluation of cytotoxic and hypoglycemic properties of Mangifera indica peels. Asian Pacific Journal of Tropical Biomedicine 7, 49-52.

Clarke E, ML C, (1997). Landers Veterinary Toxicology in London. London: Bailliere Tindall 2, 456-461.

Dehghan G, Shafiee A, Ghahremani M H, Ardestani S K, Abdollahi M, (2007). Antioxidant potential of various extracts from Ferula szovitsiana. in relation to their phenolic content. Pharm. Biol. 45, 691-699.

Dehpour A A, Ebrahimzadeh M A, Fazel N S, Mohammad N S, (2009). Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition. Grasas Aceites 60, 405-412.

Devaki K, Beulah U, Akila G, Gopalakrishnan V, (2012). Effect of aqueous extract of Passiflora edulis on biochemical and haematological parameters of Wistar albino rats. Toxicol. Int. 19, 63.

Eigner D, Scholz D, (1990). Das Zauberbüchlein der Gyani Dolma. Pharmazie in unserer Zeit 19, 141-152.

Gennaro L, (2008). Extraction technology for medicinal and aromatic Plants. United Nations Industrial Development Organization and the International Centre for Science and High Technology, 6-8.

Ghasemi Z, Rezaee R, Aslani M R, Boskabady M H, (2021). Anti-inflammatory, antioxidant, and immunomodulatory activities of the genus Ferula and their constituents: A review. Iranian Journal of Basic Medical Sciences 24, 1613.

Harborne J, (1984). Phytochemical methods Second edition. By Chapman and Hall, London and New York1984 37, 19-25.

Harper H, (1973). Review of physiological chemistry. Los Altos, California. Lange Medical Publications.

Hayes A W, (2001). Principles and Methods of Toxicology 4Th Ed.

Kiran C R, Rao D B, Sirisha N, Rao T R, (2015). Assessment of phytochemicals and antioxidant activities of raw and germinating Ceiba pentandra (kapok) seeds. Journal of Biomedical Research 29, 414.

Kumar P, Singh D, (2006). Molluscicidal activity of Ferula asafoetida, Syzygium aromaticum and Carum carvi and their active components against the snail Lymnaea acuminata. Chemosphere 63, 1568-1574.

Lal P, Ganjur D, Siddiqui A W, Chhetri P, (2022). Hematological, Biochemical, and Histological Measures in Wistar Male Rats Used to Assess Asafetida's Toxicity. Revista Electronica de Veterinaria 23, 53-57.

Mahato N, Sharma K, Sinha M, Cho M H, (2018). Citrus waste derived nutra-/pharmaceuticals for health benefits: Current trends and future perspectives. Journal of Functional Foods 40, 307-316.

Manjamalai A, Grace V, (2012). Antioxidant activity of essential oils from Wedelia chinensis (Osbeck) in vitro and in vivo lung cancer-bearing C57BL/6 mice. Asian Pacific Journal of Cancer Prevention 13, 3065-3071.

Martinez A, Valencia G, (1999). In Manual de prácticas de Farmacognosia y Fitoquímica. St edition. Medellin: Universidad de Antioquia 2003.

Martínez L, Castillo J, Ros G, Nieto G, (2019). Antioxidant and antimicrobial activity of rosemary, pomegranate and olive extracts in fish patties. Antioxidants 8, 86.

Niazmand R, Razavizadeh B M, (2021). Ferula asafoetida: chemical composition, thermal behaviour, antioxidant and antimicrobial activities of leaf and gum hydroalcoholic extracts. Journal of Food Science and Technology 58, 2148-2159.

Noroozi S, Mosaffa F, Soltani F, Iranshahi M, Karimi G, et al. (2008). Antigenotoxic effects of the disulfide compound persicasulfide A (PSA) on rat lymphocytes exposed to oxidative stress. Planta Med., 32-36.

Rai M, Kon K, (2013). Fighting multidrug resistance with herbal extracts, essential oils and their components. Academic Press.

Ramachandran S, Rajasekaran A, Manisenthilkumar K,

(2012). Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of Terminalia paniculata bark in diabetic rats. Asian Pacific journal of tropical biomedicine 2, 262-268.

Ramadan N I, Abdel-Aaty H E, Abdel-Hameed D M, El Deeb H K, Samir N A, et al. (2004). Effect of Ferula assafoetida on experimental murine Schistosoma mansoni infection. J Egypt Soc Parasitol 34, 1077-1094.

Ramadan N I, Al Khadrawy F M, (2003). The in vitro effect of Assafoetida on Trichomonas vaginalis. J Egypt Soc Parasitol 33, 615-630.

Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, et al. (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pakistan Journal of Pharmaceutical Sciences 22.

Shahat A A, Ullah R, Alqahtani A S, Alsaid M S, Husseiny H A, et al. (2018). Hepatoprotective effect of Eriobotrya japonica leaf extract and its various fractions against carbon tetra chloride induced hepatotoxicity in rats. Evidence-Based Complementary and Alternative Medicine 2018.

Sirizi M A G, Alizadeh Ghalenoei J, Allahtavakoli M, Forouzanfar H, Bagheri S M, (2023). Anticancer potential of Ferula assa-foetida and its constituents, a powerful plant for cancer therapy. World journal of biological chemistry 14, 28-39.

Sitara U, Niaz I, Naseem J, Sultana N, (2008). Antifungal effect of essential oils on in vitro growth of pathogenic fungi. Pak J Bot 40, 409.

Sofowora A, (1982). Medicinal Plants and Traditional Medicine in West Arica, John Wily and Sons. New York 256.

Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A, (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines 5, 93.

Valko M, Rhodes C, Moncol J, Izakovic M, Mazur M, (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160, 1-40.

Wall M E, Eddy C R, McClennan M L, Klumpp M E, (1952). Detection and estimation of steroidal sapogenins in plant tissue. Anal. Chem. 24, 1337-1341.

Wong-Paz J E, Contreras-Esquivel J C, Rodríguez-Herrera R, Carrillo-Inungaray M L, López L I, et al. (2015). Total phenolic content, in vitro antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. Asian pacific journal of tropical medicine 8, 104-111.

Yatham P, Shukla D, Srivastava A K, Pragadheesh V S, Kumar D, (2022). Purification and identification of anticancer organosulfides from Ferula assa-foetida gum: integrative analysis employing GC/GC-MS/RP-HPLC/NMR. Natural product research 36, 2869-2874.

Zhishen J, Mengcheng T, Jianming W, (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555-559.

Zhu M-Z, Wu W, Jiao L-L, Yang P-F, Guo M-Q, (2015). Analysis of flavonoids in lotus (Nelumbo nucifera) leaves and their antioxidant activity using macroporous resin chromatography coupled with LC-MS/MS and antioxidant biochemical assays. Molecules 20, 10553-10565.