ID 3033

Antioxidant and Protective Effects of Bioactive Compounds Contained In the Hydro-ethanolic extract of Olive Pomace against Cellular Alterations Induced by Streptozotocin in Diabetic Rats

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ABSTRACT

The phenol-rich olive pomace (OP) extract showed significant benefits in treated diabetic rats, improving body weight (BW)and blood sugar levels. It notably reduced transaminases and lipid levels while enhancing hepatic antioxidant status and lowering malondialdehyde (MDA) content. Histopathological analysis demonstrated a decrease in hepatic morphological changes control.

Keywords: phenol, histopathological, diabetic, antioxidants, olive pomace.

1. Introduction

Phenolic compounds (PC) noted for their antioxidant properties, have shown potential in reactivating insulin secretion, challenging assumptions about irreversibility in diabetes. These compounds play a crucial role in preventing oxidative damage, with OP highlighted for its rich content. Our study aims to investigate oxidative damage in the liver during streptozotocin-induced diabetes and assess changes in redox status. We evaluate the antioxidant, cytoprotective, and antidiabetic effects of PC extracted from OP in diabetic rats treated with HEE at 350 mg/kg. Assessment includes antioxidant enzyme activity, lipid peroxidation, insulinemia, and blood sugar levels.

2. Experimental

2.1 Plant Material and Preparation of Hydro-Ethanolic Extract

The OP, identified by the PPABIONUT laboratory at the University of Tlemcen, was collected, air-dried, and stored. Subsequently, the dried OP was ground, mixed with an ethanol-water solution, stirred, filtered, concentrated, and dried, resulting in a concentrated extract suitable for both in vitro and in vivo experiments.

2.2 Quantification of total phenolic content and evaluation of the antioxidant activity

Total phenolic content was assessed using the Folin-Ciocalteu method, outlined by Singleton and Rossi. Antioxidant activity was determined spectrophotometrically via DPPH assay, described by [1].

2.3 Animal Material and Induction of Diabetes

Diabetes was induced in 24 Wistar rats via stz, divided into control, untreated diabetic, and two treatment groups orally for 28 days. Weight gain and blood glucose were monitored, and after 28 days, rats were sacrificed. Blood organs were collected for biological and histopathology analysis.

DOI: 10.21467/abstracts.169 ISBN: 978-81-970666-4-1 (eBook)

2.4 Glycemic, weight trends and biochemical analyses in experimental rats

BW and glycemia were measured to assess the progression of diabetes in diabetic rats compared to controls. Total cholesterol, TG, and HDLc levels, as well as plasma activities of AST and ALT, were determined using enzymatic methods with colorimetric enzyme kits from Sigma-Aldrich.

2.5 Determination of oxidative stress parameters

Oxidative stress parameters were assessed through various methods: MDA, Catalase and, Superoxide dismutase SOD

2.6 Histopathological study

Livers were preserved for histopathological analysis to reveal structural changes, under a light microscope.

2.7 Statistical analysis

The results were presented as mean ± standard deviation (SD) and analyzed using SPSS software. Statistical significance was determined using ANOVA followed by Tukey post hoc tests. Significance levels were denoted as $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$, with NS indicating non significance for $P>0.05$.

3. Results and Discussion

3.1 Yield, quantification of total phenols and evaluation of antioxidant activity

The polyphenol extraction rate from OP is 8.66%, with a total phenolic content of 90.139±15.54 mg GAE/g dry matter and an EC_{50} value of 1.7±0.02 mg GAE/m in the DPPH assay, highlighting its potential as a source of health-beneficial antioxidants [2].

3.2 Effect of OP hydro-ethanolic extract on blood glucose evolution and weight growth in diabetic rats

Increase in body weight and reduction in blood glucose levels suggest a therapeutic potential of OP extract in diabetic rats. Consistent with prior research, OP increased body weight and lowered blood sugar levels, possibly due to its antioxidant activities and enhanced insulin secretion, as supported by earlier studies [3].

3.3 Effect of OP HEE on biochemical parameters in diabetic rats

Table 1 shows that treated diabetic rats had improved liver function (lower AST & ALT) and blood lipid profile (cholesterol & TG), potentially due to the antioxidant effects of OP extract, as observed in other studies [4].

Table 1: *Effect of OP HEE on biochemical parameters.*

Blood parameters	C	DnTr	DTr350	DTrMref
ASAT (UI/l)	75.42±0.76	108.93±2.89***	77.24±2.73***	80.94±2.30
ALAT (UI/l)	34.37±0.84	76.12±4.43***	50±3.24***	35.68±1.31
Cholesterol (mg/dl)	30.86±3.45	88.47±3.82***	37.87±1.30	64.84±1.6***
HDLc (mg/dl)	41.54±0.92	28.07±0.27*	35.79±5.49	33.67±2.24
TG (mg/dl)	66.41±4.30	93.28±11.28***	75.13±3.64	78.12±5.3

3.4 Effect of HEE of OP on oxidative stress parameters

The effect of HEE on oxidative stress parameters (Table 2) shows that OP extract reduces liver MDA and CARP levels, enhances antioxidant enzyme activities, and decreases tissue-level lipid peroxidation, consistent with previous research indicating olive pomace's potent antioxidant properties [4]

ISBN: 978-81-970666-4-1
Series: AIJR Abstracts
DOI: 10.21467/abstracts.169

Table 2: *Effect of GO hydro-ethanolic extract on oxidative stress parameters in diabetic rats.*

In the liver	Cat (U/min/g)	SOD (µmol/min/g)	MDA (µmol/g)	CP (µmol /g)
C	15.45 ± 0.11	0.69 ± 0.05	0.77 ± 0.02	0.28 ± 0.02
DTr350	57.18 ± 0.65***	0.54 ± 0.04***	1.97 ± 0.55	0.28 ± 0.02
DTrMref	20.99 ± 2.3*	0,59 ± 0.04**	0.44 ± 0.15	0.26 ± 0.05
DnTr	12.09 ± 0.5***	0.129 ± 0.03***	4.97 ± 0.6***	0.33 ± 0.02***

3.5 Effect of Hydro-ethanolic Extract on Histological Changes in the Liver of Diabetic (Figure 1)

Untreated diabetic rats exhibit liver tissue alterations including vascular congestion and inflammatory cell infiltration. However, liver sections from diabetic rats treated with HEE of OP show restored tissue architecture, resembling that of control rats.

- (A) Control rats: normal liver architecture. (B) and (C) Selected micrographs of liver sections from diabetic rats treated with HEE extract, metformin showed normal architecture. The central lobe veins (CLV) observed in (D) a selected micrograph of STZ-treated diabetic rat liver sections showing vascular congestion (VC) with the presence of inflammatory cells (IC) between the recurrent trabeculae.

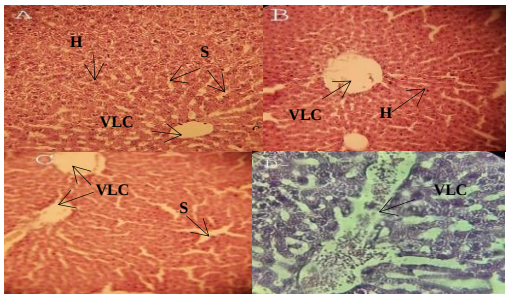

Figure.1: Photomicrographs of liver.

4. Conclusions

The pharmacological investigation of olive pomace aimed at treating diabetes mellitus, revealing antioxidant potential via DPPH assay. In vivo study demonstrated antihyperglycemic effects, confirmed by biochemical tests, and indicated a chemoprotective capacity against STZ. This protective effect maintained liver cell redox balance despite STZ's pro-oxidant impact, guarding against cytolysis and preventing diabetic complications, underscoring the significance of these results.

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ISBN: 978-81-970666-4-1

Series: AIJR Abstracts

DOI: 10.21467/abstracts.169