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Hepatoprotective, Antihyperglycemic and Cytotoxic Activities of *Jacaranda acutifolia* Leaf Extract

Nada M Mostafa[1], **Omayma A Eldahshan**[1], **Hesham A El-Beshbishy**[2] **and Abdel Nasser B Singab**[1*]

[1]*Department of Pharmacognosy, Ain Shams University, Abbassia, Cairo, Egypt*

[2]*Department of Medical Laboratories Technology, Faculty of Applied Medical Sciences, Taibah University, Al-Madinah Al-Munwarah, Saudi Arabia*

[*]**Corresponding author:** Abdel Nasser B Singab, Department of Pharmacognosy, Ain Shams University, Cairo, Egypt, Tel: 01005036231; E-mail: nasersingab@hotmail.com

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Abstract

Objective: Leaves methanol extract of *Jacaranda acutifolia* Humb. and Bonpl. (JA) family Bignoniaceae was subjected to phytochemical investigation as well as antioxidant, hepatoprotective, cytotoxic and antihyperglycemic activities evaluation.

Key findings: Eight compounds were identified: luteolin-7-O-β-D-glucuronide, luteolin-7-O-β-D-glucoside, aesculetin, luteolin, verbascoside, luteolin-7-O-β-D-glucuronide methyl ester, apigenin-7-O-β-D-glucuronide methyl ester and apigenin. JA revealed a potent antioxidant activity *in vitro* superior to vitamin E (DPPH assay; EC_{50} of 0.43 mg/mL). A potential cytotoxic activity was produced against hepatocellular (HepG2) and cervical (HeLa) carcinoma cells with IC_{50} of 6.05 and 16.7 µg, respectively. Treatment with JA extract inhibited the rise in alanine aminotransferase and aspartate aminotransferase by 33.6% and 36.8% respectively, reduced thiobarbituric acid by 35.7% and decreased the tamoxifen-induced elevation in tumor necrosis factor alpha (TNF-α) level by 42.86%. JA extract elicited a significant decrease in fasting blood glucose by -59.26%.

Conclusions: *Jacaranda acutifolia* could be a natural source for antioxidant, hepatoprotective supplements and could provide a basis for a potential cytotoxic agent. The compounds isolated are responsible at least in part for the observed effects.

Keywords: *Jacaranda acutifolia*; Hepatoprotective; Antihyperglycemic; Cytotoxic

Introduction

Genus Jacaranda distributes around the world and includes 49 species. The main identified constituents are flavonoids, triterpenes, quinones, and acetosides [1]. Members of this genus are well known in traditional ethnobotany for their promising pharmacological activities [2].

Jacaranda species were used, traditionally, to treat rheumatism, leishmaniasis as well as venereal infections and gastrointestinal disorders. *Jacaranda acutifolia* have shown diuretic and astringent activities [3]. Leaves and barks could be applied directly to the wounds or in the form of decoction or infusion, as they are considered as disinfectant [4]. The bark could be used as astringent and diuretic and in the treatment dermatitis, syphilis and diseases related to urinary tract diseases [5].

Jacaranda acutifolia leaves contain verbascoside, jacaranone, phenyl acetic-β glucoside, scutellarein-7-glucoronide and hydroquinone [6]. Flowers contain anthocyanins which are responsible for their violet color [5]. A novel biflavonoid [kaempferol (6→8″) apigenin] was isolated from the leaves [7]. It exerted a promising cytotoxic activity against breast carcinoma cell line (MCF-7). The main components of leaf volatile oil of Jacaranda were methyl linolenate (26.7%), 1-octen-3-ol (10.8%), methyl phenyl acetate (9.9%), beta-linalool (5.5%) and palmitic acid (4.7%). n-Hexane extract revealed similar composition, but with other compounds as homogentisic acid p-benzoquinone, phenyl acetic acid and resorcinol. The oil showed activity against *Staphylococcus aureus, Escherichia coli, Candida albicans, Salmonella typhimurium* and *Shigella flexneri* where n-hexane extract showed moderate activities against many microorganisms [8]. The total phenolic contents of *Jacaranda acutifolia* were calculated as 17.20 mg/g gallic acid equivalents. The ethyl acetate fraction possessed the highest antioxidant activities, estimated by DPPH radical scavenging (EC_{50}=0.049 mg/mL) and ferric ion reducing activities (EC_{50}=0.125 mg/mL) [9].

The present study was undertaken to evaluate antioxidant, cytotoxic, hepatoprotective and antihyperglycemic activities *Jacaranda acutifolia* leaves and to identify compounds responsible for the observed effects.

Materials and Methods

Plant materials

Jacaranda acutifolia (Humb. and Bonpl.) leaves (JA), Bignoniaceae were collected from Merryland Botanical Garden, Cairo, Egypt, and air-dried. They were authenticated by Prof. Abd El Salam Mohamed Al-Nowiahi, Professor of Taxonomy, Faculty of Science, Ain Shams University. Voucher specimens of JA (PHG-P-JA-201) was deposited at Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Processing of *Jacaranda acutifolia* leaves

The intact air-dried plant materials (2 Kgs) were boiled in distilled water for 2 hours then filtered while hot. The water extract was lyophilized, and then extracted with methanol. The methanol portions were distilled off in rotary evaporator at 55˚C till dryness. The extract was concentrated until constant weight (87 g) then kept in vacuum desiccators over anhydrous $CaCl_2$.

Experimental animals

Female Sprague-Dawley rats (120-140 g) were used. Animals were maintained under standard conditions of temperature (24 ± 5˚C) and relative humidity (55 ± 5%), with a regular 12 h light: 12 h dark cycle, and allowed free access to standard laboratory food and water 7 days before starting the experiment and during the whole period of the experiment.

All animals were fed with common pellet diets and water ad libitum. All stressful conditions were avoided. All animals were treated humanely in accordance with the guidelines for animal's care set by the World Health Organization. Experimental protocol was approved by the local committee for animal care, Taibah University, Al-Madinah Al-Munwarah, Saudi Arabia.

Fraction II (24.8 g) was re fractionated over a cellulose column and eluted with distilled water followed by 50% methanol in water, then neat methanol. Sub-fraction A yielded compounds 1, 2 and 3, while sub-fraction B yielded compound 4. Compounds were further purified by preparative PC.

Fraction III (26.9 g) was re-fractionated over a polyamide column. Elution started with distilled water then gradually decreasing polarity by addition of methanol in water with different proportions, followed by neat methanol. Sub-fractions were pooled together to give five main subfractions: A-E. Sub-fraction B (30% MeOH in water) yielded compound 5 by preparative PC on Whatmann No. 3 MM and 6% acetic acid as solvent system, then purified on a column packed with sephadex LH-20. Sub-fraction C (50% MeOH in water) yielded two compounds 6 and 7 which were isolated by preparative PC using BAW and 6% acetic acid as solvent systems individually.

Fraction IV eluted with 75% methanol in water (8.3 g) was re-fractionated over a sephadex LH-20 column using water and methanol with decreasing polarity. One major compound appeared in neat methanol subfraction and yielded compound 8.

In vitro antioxidant assay (DPPH assay)

The hydrogen atom or electron donation ability of JA methanol extract was measured as a result of the bleaching of purple-colored methanol solution of DPPH· [10].

Cytotoxicity and cell viability assay (MTT assay)

Sensitivity of HepG2 and HeLa cells to JA leaves extract were determined using MTT cell viability assay [11].

Hepatoprotective activity

Experimental animals' treatment

Animals were randomly assigned to three groups (6 rats in each): Group I: Normal control rats were injected daily (i.p.) with normal saline for seven successive days. Group II: Rats were injected daily (i.p.) with TAM for seven successive days at a dose of 45 mg/kg/day in normal saline. Group III: Rats were treated daily i.p. with JA extract at a dose of 20 mg/kg/day in normal saline for 7 successive days then i.p. injection of TAM at a dose of 45 mg/kg/day in normal saline for 4 successive days starting at day 4-7.

Dosing volume was kept at 10 mL/kg. After the last treatment by 24 h, animals were subjected to light ether anesthesia and blood samples were collected by orbital puncture in serum-separating tubes. The blood was centrifuged at 3000 rpm for 10 min to separate the serum. Sera were used for measurement of ALT and AST activities.

Anaesthetized animals were scarified by cervical dislocation. The liver was rapidly dissected out, washed in ice-cold isotonic saline and blotted between two filter papers. Part of each liver was used to prepare 10% (w/v) liver homogenates in ice-cold 0.1 M potassium phosphate buffer, pH 7.5, and stored at -70 ˚C for subsequent analysis.

Antihyperglycemic activity

Induction of diabetes mellitus: Diabetes was induced in the rats by a single (i.p.) injection of freshly prepared STZ (60 mg/kg b.w.) in normal saline. Two days after STZ administration, blood samples were obtained from the tips of the rat's tail and the fasting blood glucose (FBG) levels determined using OneTouch® Ultra® glucometer (Life Scan, USA) to confirm diabetes. The diabetic rats exhibiting blood glucose levels above 190 mg% were included in this study [12]. The biochemical effects of the extracts were compared to GLB.

Experimental animals' treatment: In the present study animals were divided into four groups (eight rats in each): Group I: Normal control rats received 0.1 mL DMSO and 0.5 mL 5% Tween 80 for 7 days by oral lavage. Group II: STZ-diabetic rats received 0.1 mL DMSO and 0.5 mL 5% Tween 80 for 7 days by oral lavage. Group III: Diabetic rats orally received GLB (20 mg/kg/day) in 0.1 mL DMSO and 0.5 mL 5% Tween 80 for 7 days by oral lavage [13]. Group IV: STZ-diabetic rats orally received JA extract dissolved in 0.1 mL DMSO and 0.5 mL 5% Tween 80 at a dose of 20 mg/kg/day for 7 days by oral lavage [14]. On the 8th day, the fasting rats were subjected to light ether anesthesia and killed by cervical dislocation. Trunk blood was collected into heparinized chilled tubes containing NaF. Serum was separated by centrifugation at 4˚C and stored at -20˚C until further use.

Statistical analysis

Results are reported as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall P-value was found statistically significant ($P<0.05$), further comparisons among groups were made according to post hoc Tukey's test.

Free-radical scavenging activity of the purified compounds

Free-radical scavenging activity of the purified compounds (1-8) against stable DPPH was determined by DPPH-TLC assay method [15]. One µL (1 mg/mL in methanol) of each compound was spotted on precoated silica plate (silica 60 F254) and dried. Solvent system was dichloromethane and methanol (7: 3) with 0.1 mL formic acid. TLC plate was dried and sprayed with 2% DPPH solution dissolved in methanol and then air dried. After 15 min, yellow-colored spots were observed against a purple background (positive antioxidant activity).

Results and Discussion

Phytochemical investigation

Phytochemical investigation of the methanol extract of JA leaves resulted in isolation and identification of 8 compounds (Figure 1): luteolin-7-O-β-D-4C1-glucuronopyranoside which was first to be isolated from family Bignoniaceae: aesculetin (first to be isolated from the genus Jacaranda): while Luteolin-7-O-β-D-4C1-glucopyranoside, Luteolin-7-O-β-D-4C1-glucuronide methyl ester, Apigenin-7-O-β-D-4C1-glucuronide methyl ester, verbascoside, luteolin, and apigenin were first to be isolated from the species.

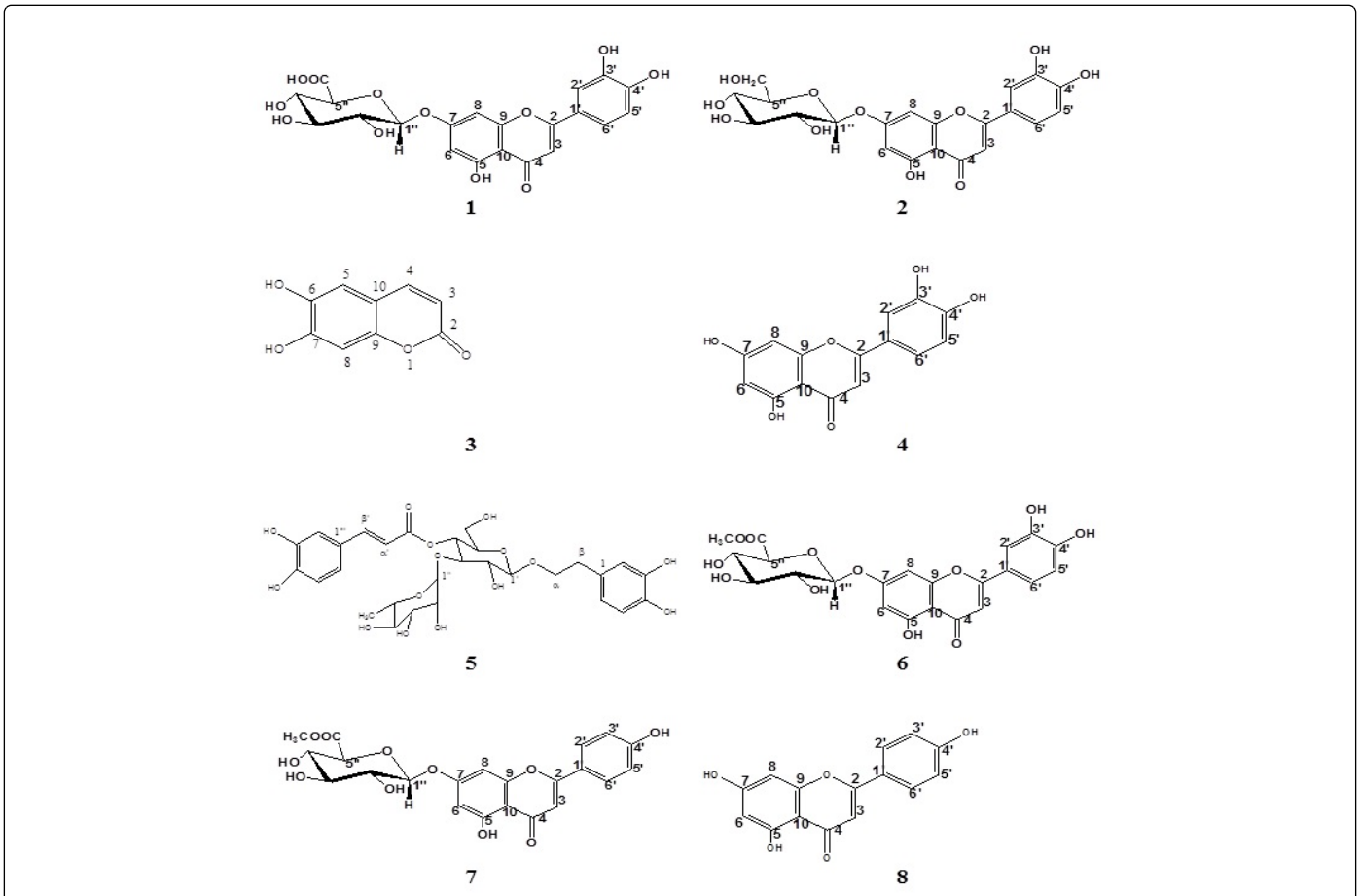

Figure 1: Compounds Isolated from *J. acutifolia* leaves extracts.

Structure elucidation of the compounds isolated

Luteolin-7-O-β-D-4C1-glucuronopyranoside, compound 1: UV λmax (nm) in MeOH: 258, 267, 356. 1HNMR: 7.44 (1H, d, J=2.1 Hz, H-2′), 7.43 (1H, dd, J=8.7, 2.1 Hz, H-6′), 6.88 (1H, d, J=8.7 Hz, H-5′), 6.78 (1H, d, J=2.1 Hz, H-8), 6.73 (1H, s, H-3), 6.43 (1H, d, J=2.1 Hz, H-6), 5.13 (1H, d, J=7.5 Hz, H-1′′), 3.7 (1H, d, J=9 Hz, H-5′′), 3.2-3.6 (m, sugar protons) [16].

Luteolin-7-O-β-D-4C1-Glucopyranoside, compound 2: UV λmax (nm) in MeOH: 256, 264 sh, 351. 1HNMR: 7.5 (1H, d, J=2.1 Hz, H-2'), 7.55 (1H, dd, J=8.7, 2.1 Hz, H-6'), 6.9 (1H, d, J=8.7 Hz, H-5'), 6.85 (1H, d, J=2.1 Hz, H-8), 6.8 (1H, s, H-3), 6.5 (1H, d, J=2.1 Hz, H-6), 5.13 (1H, d, J=7.5 Hz, H-1''), 3.2-3.8 (m, sugar protons) [17].

6, 7 Dihydroxycoumarin, (Aesculetin), compound 3 : UV λmax (nm) in MeOH: 283, 334. 1HNMR: 7.85 (1H, d, J=9.6 Hz, H-4), 6.98 (1H, s, H-5), 6.74 (1H, s, H-8), 6.15 (1H, d, J=9.6 Hz, H-3). (-)-ESI-MS: 177.01 [M-H]-, 355.02 [2M-H]$^-$ [18].

Luteolin (5, 7, 3', 4'-Tetrahydroxyflavone), compound 4: UV kmax (nm) in MeOH: 253, 267, 348. 1HNMR: 7.4 (1H, d, J=2.2 Hz, H-2'), 7.45 (1H, dd, J=8.3, 2.2 Hz, H-6′), 6.9 (1H, d, J=8.3 Hz, H-5′), 6.65 (1H, s, H-3), 6.45 (1H, d, J=2.5 Hz, H-8), 6.2 (1H, d, J=2.5 H-6) [13].

3,4-Dihydroxy-β-phenylethyl-l-O-α-L-rhamnopyranosyl-1→3)-4-O-caffeoyl-β-D-glucopyranoside, Verbascoside (Acetoside), compound 5: UV kmax (nm) in MeOH: 287, 304, 331 nm. 1HNMR: 7.42 (1H, d, J=16.2 Hz, H-β'), 6.95 (1H, d, J=2 Hz, H-2'''), 6.94 (1H, dd, J=8.1, 2 Hz, H-6'''), 6.86 (1H, d, J=8.1, H-5'''), 6.63 (1H, d, J=2 Hz, H-2), 6.61 (1H, dd, J=8.1, 2 Hz, H-6), 6.47 (1H, d, J=8.1, H-5), 6.06 (1H, d, J=16.2 Hz, H-α'), 5.02 (1H, d, J=1.5 Hz, H-1''), 4.34 (1H, d, J=7.8 Hz, H-1'), 0.96 (3H, d, J=6 Hz, H-6''). 13CNMR: 18.1 (C-6''), 36.0 (C-β), 62.7 (C-6'), 70.3 (C-3''), 70.4 (C-5''), 70.4 (C-4'), 71.0 (C-α), 71.6 (C-2''), 74.4 (C-4''), 74.4 (C-2'), 74.4 (C-5'), 79.0 (C-3'), 101.2 (C-1''), 102.2 (C-1'), 114.5 (C-5'''), 115.4 (C-2'''), 115.8 (C-α'), 116.2 (C-2), 116.2 (C-5), 121.4 (C-6), 122.4 (C-6'''), 128.2 (C-1'''), 130.2 (C-1), 144.9 (C-4), 145.5 (C-3), 146.2 (C-β'), 146.4 (C-3'''), 149.2 (C-4'''), 165.6 (C=O). (-)-ESI-MS: 623.61 [M-H]-, 623.64 [M]$^+$ [19,20].

Luteolin-7-O-β-D-4C1-glucuronide methyl ester, compound 6: UV λmax (nm) in MeOH: : 254, 285 sh, 348. 1HNMR: 7.5 (1H, dd, J=8.3, 2.2 Hz, H-6'), 7.4 (1H, d, J=2.2 Hz, H-2'), 6.9 (1H, d, J=8.3 Hz, H-5'), 6.8 (1H, d, 2.5 Hz, H-8), 6.7 (1H, s, H-3), 6.4 (1H, d, 2.5 Hz, H-6), 5.3 (1H, d, J=7.5 Hz, H-1''), 4.2 (1H, d, J=9 Hz, H-5''), 3.6 (3H, s, OMe), 3.2-3.8 (m, sugar protons). Dept-90: 71.2 (C-4''), 72.6 (C-2''), 75.0 (C-5''), 75.2 (C-3''), 94.4 (C-8), 98.9 (C-1''), 99.2 (C-6), 103.1 (C-3), 113.4 (C-2'), 115.8 (C-5'), 121.2 (C-6'). Dept-135: 51.9 (OMe). (-)-ESI-MS: 475 $[M-H]^-$ [13].

Apigenin-7-O-β-D-4C1-glucuronide methyl ester, compound 7: UV λmax (nm) in MeOH: 252, 336.. 1HNMR: 7.9 (2H, d, J=8.6 Hz, H-2', 6'), 6.9 (2H, d, J=8.6 Hz, H-3', 5'), 6.8 (1H, d, J=2.5 Hz, H-8), 6.67 (1H, s, H-3), 6.5 (1H, d, J=2.5, H-6), 5.2 (1H, d, J=7.5 Hz, H-1''), 4.2 (1H, d, J=9 Hz, H-5''), 3.6 (3H, s, OMe), 3.2-3.8 (m, sugar protons). Dept-135: 128.5 (C-2',6'), 115.9 (C-3',5'), 103.0 (C-3), 99.0 (C-1''), 99.3 (C-6), 94.5 (C-8), 75.3 (C-3''), 75.1 (C-5''), 72.6 (C-2''), 71.2 (C-4''), 51.9 (OMe) [21].

Apigenin ((5, 7, 4'- Trihydroxyflavone), compound 8: UV Spectral data, λmax (nm) MeOH: : 266, 355. 1H-NMR: 6.19 (1H, d, J=2.5 Hz, H-6), 6.49 (1H, d, J=2.5 Hz, H-8), 6.79 (1H, s, H-3), 6.92 (1H, d, J=8 Hz, H-3', 5'), 7.93 (1H, d, J=8 Hz, H-2', 6') [22].

Antioxidant activity

Results of methanol leaves extract of JA revealed a potent antioxidant activity *in vitro* (DPPH assay), where they showed EC_{50} of 0.43 mg/mL, compared to vitamin E at the same concentrations (Table 1). The powerful antioxidant capacity, which is superior to vitamin E, could be attributed to the presence of phenolics. Apigenin antioxidant capabilities lie in the H+ donating potential of its aromatic OH-group. It has the ability to reverse the oxygen radical-generated DNA damage from hydrogen peroxide, superoxide radicals or singlet oxygen [23]. It reduces cyclosporine-A (CsA) induced changes in total antioxidants in rats [24]. The improvement of glutathione peroxidase enzyme levels in apigenin treated group further proves the apigenin antioxidant effect [25]. Luteolin and its glycosides are reactive against hydrogen peroxide radicals. It possesses DNA protective capacity against free radicals [26]. Luteolin-7-O-β-D-glucuronide methyl ester from the ethyl acetate fraction of Lycopi herba has shown a protective effect against pro-oxidant reactive species and low-density lipoprotein peroxidation [24]. Verbascoside was the major antioxidant constituent of Phlomis lychnitis L. methanol extract (Lamiaceae) in rat pheochromocytoma cells (PC 12) exposed to H_2O_2 [24].

% DPPH inhibition		Concentration (mg/mL)
Vitamin E	*J. acutifolia* extract	
15	25	0.2
22	45	0.4
27	75	0.6

Table 1: Changes of percentage inhibition of DPPH by different concentrations of *J. acutifolia* leaves extract and vitamin E.

Cytotoxic activity

JA extract showed potential cytotoxic activity against hepatocellular (HepG2) and cervical (HeLa) carcinoma cells with IC_{50} of 6.05 and 16.7 µg, respectively (Figure 2 A and B). The cytotoxic activities could be due to the presence of apoptotic and anti-proliferative flavonoids and other phenolics in the extract [26,27]. A cytotoxic biflavonoid compound was previously isolated from the same extract, which exhibited anticancer activity against breast cancer [7].

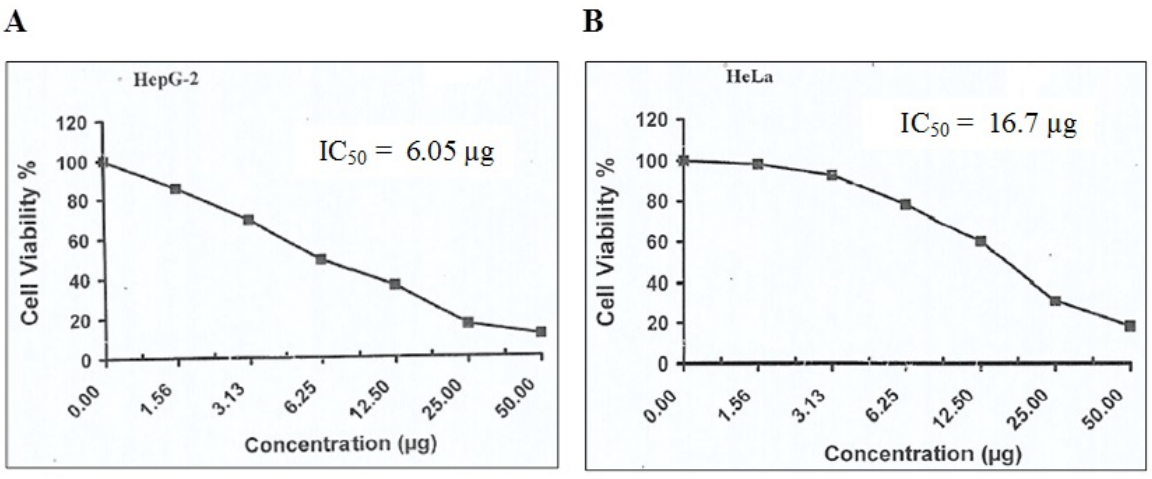

Figure 2: Cytotoxic effect of *J. acutifolia* leaves extract on HepG2 (A) and HeLa (B) cell lines using MTT cell viability assay.

Hepatoprotective activity

Serum ALT and AST in rats treated with TAM were elevated by 264.8% and 190% respectively, when compared to the control group. Treatment with JA extract inhibited the rise in ALT and AST by 33.6% and 36.8% respectively as shown in Figure 3A and 3B.

Oxidative status

Figure 3C TAM treatment significantly elevated TBARS (155.6%) as compared to control group. On the other hand, treatment of the combination group with JA extract significantly reduced TBARS by 35.7% as compared with the corresponding TAM-treated group.

Effec on TNF

Treatment of rats with TAM significantly increased the TNF-α hepatic level by 437.5%, when compared with control group. While, JA extract treatment in the combination group significantly decreased the TAM-induced elevation in TNF-α level by 42.86% (Figure 3D).

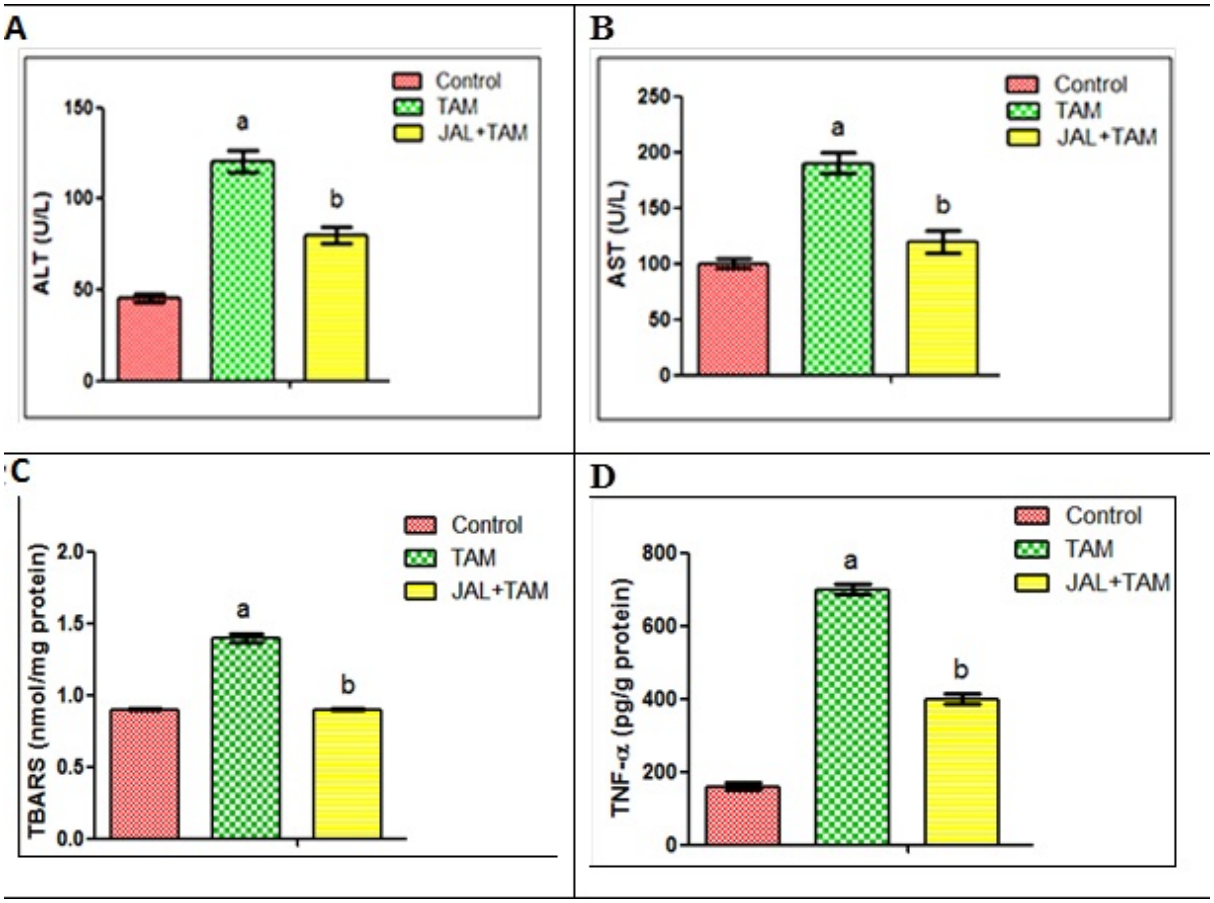

Figure 3: Effect of *J. acutifolia* (JAL) leaves extracts on TAM-induced alteration in rat serum ALT (A), AST (B), hepatic TBARS (C) and TNF-α level (D). Values are expressed as mean ± SEM. a- Significantly different from the corresponding control group at $p<0.05$. b-Significantly different from corresponding TAM-treated group at $p<0.05$.

Our results showed that treatment of rats with TAM significantly elevated hepatic lipid peroxidation expressed as TBARS which was supported by Albukhari et al. findings [28].

The pretreatment with JA extract significantly suppressed TAM-induced elevation in serum ALT and AST levels. Thus, the extract protects against TAM-induced hepatocyte injury indicating the ability to stabilize the liver cell membranes [29]. The significant increase in hepatic LPO as assessed by the high level of TBARS explains the observed leakage of cellular ALT and AST into the circulation, which suggests hepatocellular damage. In this study, JA leaves extract pretreatment significantly reduced the TAM-induced hepatic LPO. It was reported that pretreatment with apigenin was able to suppress the elevation of AST and ALT in a dose-dependent manner *in vivo* and reduce the damage of hepatocytes *in vitro* caused by CCl4 in blood serum. The hepatoprotective activity of apigenin is possibly due to its antioxidant properties, acting as scavengers of ROS [30]. A protective effect of acteoside on CCl_4-induced hepatotoxicity has been previously reported [31]. Coumarins have shown to possess potential hepatoprotective activities [32]. Thus, the isolated flavonoids, coumarin and acetoside of *J. acutifolia* leaves extract may be responsible for the observed hepatoprotective effect.

TNF-α is a proinflammatory cytokine that is associated with liver injury in many experimental models and human diseases [33]. In the current study, treatment of rats with TAM resulted in a 4-fold increase in TNF-α level. In accordance with our results, Omar et al. reported that TAM administration to rats resulted in a significant increase in TNF-α level [34]. Additional support comes from the reports indicating that pathology of TAM-induced liver injury includes inflammation resembling that of alcoholic hepatitis [35]. However, pretreatment with JA leaves extract significantly inhibited the rise in the TNF-α level. The anti-inflammatory activity of JA leaves extract is probably due to the presence of apigenin, luteolin and verbascoside. Apigenin inhibits collagenase activity and suppresses lipopolysaccharide-induced NO production, attenuates LPS-induced

cyclooxygenase-2 expression and reduces the TNF-α in rheumatoid arthritis [36].

Luteolin has been reported as a potent selective inhibitor of COX-2. The inhibition is attributed to its down regulation of the mRNA expression of COX-2 in inflammatory responses [37]. It inhibits arachidonic acid [38]. Luteolin and its glycosides have also inhibitory activities against enzymes, involved in inflammation, for the synthesis of thromboxane and leukotriene [39]. Verbascoside has been reported to demonstrate anti-inflammatory effects [40,41]. These findings support our results indicating the hepatoprotective, antioxidant and anti-inflammatory activities exhibited by JA leaves.

Thus, pretreatment with our extract protects rats against TAM-induced hepatotoxicity by preserving cellular integrity, preventing oxidative stress and lipid peroxidation, enhancing antioxidant enzymes activities and inhibition of the hepatic inflammation.

Antihyperglycemic activity

Fasting Blood Glucose (FBG) Level: Injection of STZ into rats exhibited significant elevations in FBG level by +278%, P<0.05, as compared to normal control rats. Administration of GLB to STZ-diabetic rats produced significant declines in the elevated FBG level by -28.57%, P<0.05, as compared to STZ-diabetic rats. Administration of JA leaves extract to STZ-diabetic rats elicited a significant decrease in FBG by -59.26% (P<0.05), as compared to STZ-diabetic rats (Figure 4A). Serum insulin level: Injection of STZ into rats exhibited significant decline in serum insulin level by -52.6 %, P<0.05, as compared to normal rats.

Administration of GLB to STZ-diabetic rats produced significant declines in FBG level by -28.57%, P<0.05, associated with a significant increase in serum insulin level by +22.2%, as compared to STZ-diabetic rats.

Administration of JA leaves extract to STZ-diabetic rats elicited a significant increase in serum insulin by +22.2% (P<0.05), as compared to STZ-diabetic rats (Figure 4B).

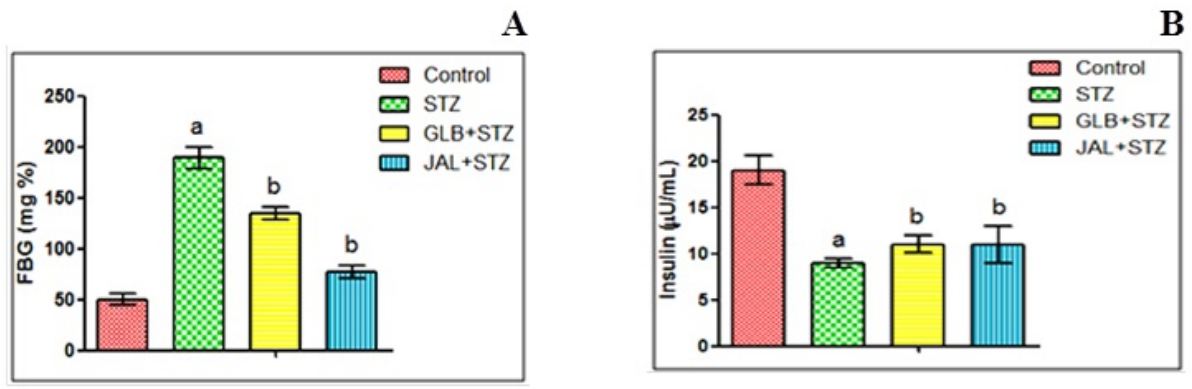

Figure 4: Effect of *J. acutifolia* leaves extracts on STZ-induced alteration in rat FBG level (A) and serum insulin level (B). Values are expressed as mean ± SEM. a-Significantly different from the corresponding control group at p<0.05. b-Significantly different from corresponding STZ-treated group at p<0.05.

Oral administration of JA leaves extract attenuated hyperglycemia and was superior to GLB, in lowering FBG levels but was equivalent in activity to GLB in elevating serum insulin level. Luteolin and luteolin-7-O-β-D-glucoside, which were identified from JA extract, may be responsible for the potent antihyperglycemic effect. Both compounds have inhibitory activities against α-glucosidase and α-amylase. It was reported that luteolin was stronger in inhibitory potency than acarbose, suggesting that it has the possibility to suppress postprandial hyperglycemia in patients with non-insulin dependent diabetes mellitus [40].

Free-radical scavenging activity of the purifie compounds

All the tested compounds showed DPPH-TLC radical scavenging activity as indicated from the deep yellow colored spot developed against a purple background (Figure 5). Thus, we can conclude that JA leaves are a rich source of natural antioxidant.

Conclusion

The results obtained revealed that methanol extracts of JA leaves showed potent *in-vitro* and *in-vivo* antioxidant activity. It confirmed the hepatoprotective and anti-inflammatory and antihyperglycemic activities of the extract.

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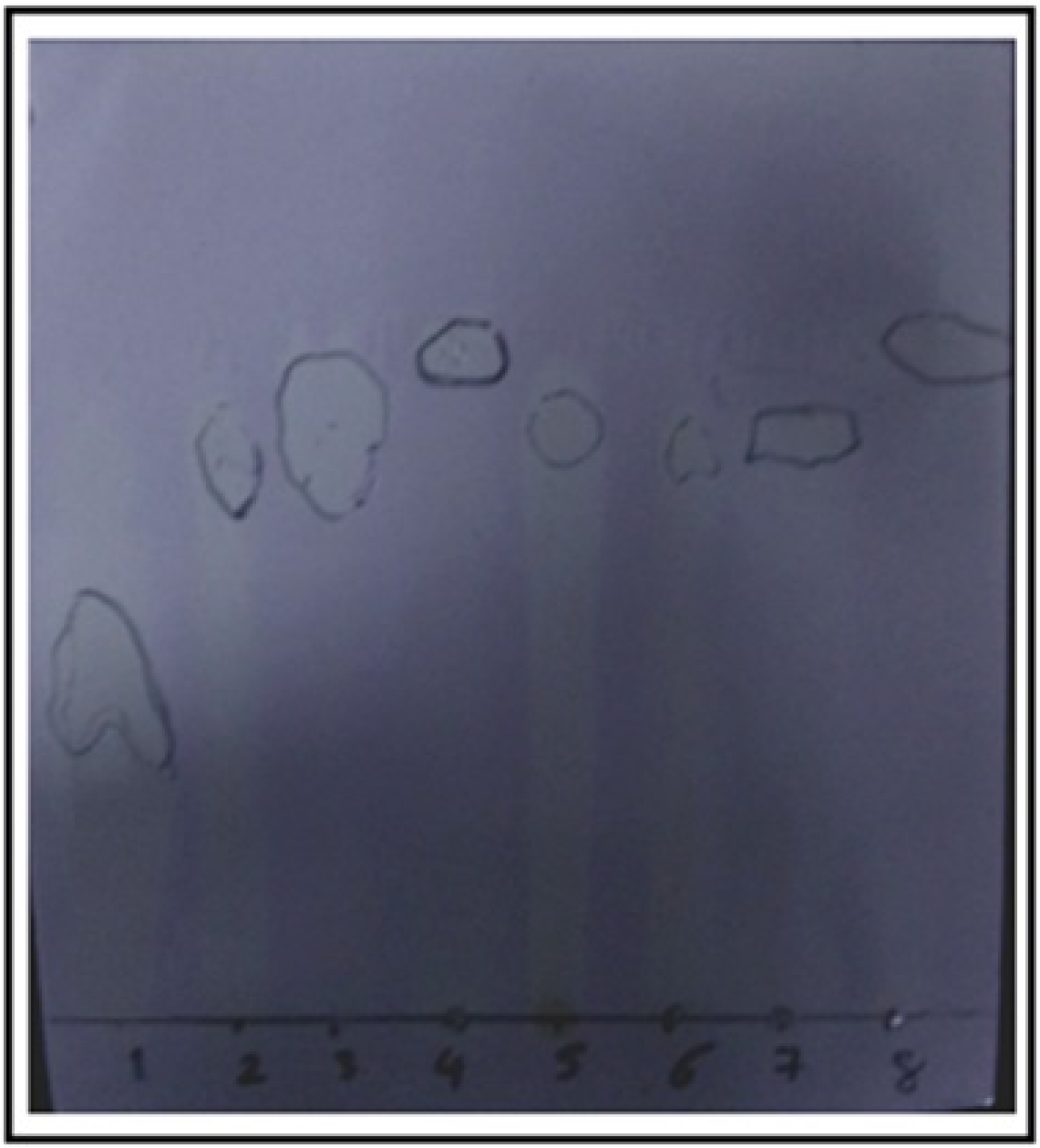

Figure 5: DPPH-TLC showing radical scavenging activity of the isolated compounds.

Conflic of Interest

The authors declare that they have no conflicts of interest to disclose.

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