ARTICLE
Traditional Medicine Research 2023;8(2):9. https://doi.org/10.53388/TMR20220514002

Traditional Indian Medicine

Targeting biosignatures of hyperglycemia and oxidative stress in diabetes comorbid depressive rats: effectiveness of hydroethanolic extract of the whole plant of *Ludwigia octovalvis*

Stuti Pandey[1], Pawan Kumar Pandey[1], Alakh Niranjan Sahu[2], Himanshu Verma[2], Manmath Kumar Nandi[1*]

[1]Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India. [2]Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India.

[*]**Corresponding to:** Manmath Kumar Nandi, Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Ajagara, Varanasi 221005, Uttar Pradesh, India. E-mail: manmathk.nandi1@bhu.ac.in.

Author contributions

Stuti Pandey participated in conceptualization, methodology, and data curation (plant extraction, in vitro and ex vivo studies). Pawan Kumar Pandey participated in writing-original drafts, writing-reviewing and editing. Himanshu Verma participated in data curation (phytochemical screening). Alakh Niranjan Sahu participated in data curation (animal behavioral study) and writing-reviewing. Manmath Kumar Nandi participated in conceptualization, methodology, interpretation, supervision, writing-reviewing and editing.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors are grateful to the Banaras Hindu University, Varanasi, India for providing facilities for the performed experimental work.

Abbreviations

L. octovalvis, *Ludwigia octovalvis* (Jacq.) P.H. Raven; NF-κB, nuclear factor kappa B; HLO, hydroethanolic extract of *Ludwigia octovalvis*; DCD, diabetes comorbid depression; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; QE, quercetin equivalent; SOD, superoxide dismutase.

Peer review information

Traditional Medicine Research thanks all anonymous reviewers for their contribution to the peer review of this paper.

Citation

Pandey S, Pandey PK, Sahu AN, Verma H, Nandi MK. Targeting biosignatures of hyperglycemia and oxidative stress in diabetes comorbid depressive rats: effectiveness of hydroethanolic extract of the whole plant of *Ludwigia octovalvis*. *Tradit Med Res*. 2023;8(2):9. doi: 10.53388/TMR20220514002.

Executive editor: Shan-Shan Lin.

Received: 14 May 2022; **Accepted:** 27 July 2022; **Available online:** 26 August 2022.

Abstract

Background: The prime objective of the current research was to evaluate the whole plant hydroalcoholic extract of *Ludwigia octovalvis* (HLO) against hyperglycemia, and oxidative stress biomarkers in rats induced with diabetes comorbid depression, diabetes comorbid depression (streptozotocin-nicotinamide + electric footshocks). **Methods:** 2,2-Diphenyl-1-picrylhydrazyl assay of HLO versus ascorbic acid was done. Effects of 200 and 400 mg/kg body weight/day HLO doses versus 25 mg/kg body weight/day metformin was studied through insulin, glucose, superoxide dismutase, lipid peroxidation, catalase, and behavioral assessment (forced swim and open field tests). **Results:** IC_{50} values of HLO and ascorbic acid were 33.52 and 27.86 µg/mL respectively. Both the HLO doses showed intended results with respect to oxidative stress biomarkers in diabetes comorbid depression rats in comparison to metformin. Open field test showed better results for HLO in diabetes comorbid depression rats. However, hypoglycemic effects, and forced swim test performance of metformin was slightly higher than the 400 mg dose, followed by the 200 mg dose of HLO. Ethyl gallate, gallic acid, β-sitosterol, and quercetin in HLO might resulted in attenuating diabetic as well as depression biomarkers. **Conclusion:** Inhibition of glucosidase and lipase activity, and AMP-activated protein kinase phosphorylation might be the possible biochemical changes occurred in HLO treated rats.

Keywords: 2,2-diphenyl-1-picrylhydrazyl assay; brain homogenate; insulin; superoxide dismutase content; catalase activity; lipid peroxidation

Highlights

- The whole plant hydroethanolic extract of *Ludwigia octovalvis* (HLO) possess promising effects on metabolic as well as central nervous system activities.
- HLO raises insulin level to exert hypoglycemic effects.
- Antioxidants present in HLO lowered depression related parameters.
- Diabetic subjects are more prone to depression; HLO is capable to supress the pathological biomarker concentrations involved in diabetes comorbid depression.

Medical history of objective

- In India *Ludwigia octovalvis* has medicinal importance from the ancient period. The leaf juice is used to treat cough, cold and intermittent fever. The whole plant and leaf pastes is used in toe fungal infection and geriatric eczema respectively. The whole plant aqueous extract is used to treat diabetes.
- Chinese materia medica describes the medicinal importance of its aqueous decoction in treating crabuncle swelling, stomatitis, fever, mouth ulcer, sore throat and cold.

Background

The perennial aquatic plant *Ludwigia octovalvis* (Jacq.) P.H. Raven (*L. octovalvis*) fits in Onagraceae family. Traditionally it has been extensively used as herbal tea and uterine tonic. *L. octovalvis* is abundantly found as weeds with rice crops, in wet tropical regions of India, and it comes under threat category medicinal plant as per International Union for Conservation of Nature 2020 [1–3]. Yang et al. reported 50% ethanolic extract of *L. octovalvis* as an antihepatotoxic agent on carbon tetrachloride and D-galactosamine induced cultured rat hepatocytes [4]. Ramírez et al. and Morales et al. has described the inhibitory effects of 60% hydroethanolic extract of *L. octovalvis* on glucosidase and lipase activity, chiefly containing ethyl gallate and gallic acid for exerting antidiabetic effects [5, 6]. Lin et al. interpreted the hypoglycemic, and memory enhancing effects of 70% ethanolic extract of *L. octovalvis*, and β-sitosterol in diabetic mice, by inducing AMP-activated protein kinase phosphorylation [7]. Similarly, 95% ethanolic extract of *L. octovalvis* containing chlorophyll a, activates AMP-activated protein kinase signaling pathway and APO-1/CD95 (a transmembrane receptor belonging to the tumor necrosis factor receptor superfamily) receptor system, exerting apoptotic effects, and anti-proliferative activity on 3T3-L1 (a continuous substrain of Swiss albino mouse developed through clonal isolation) adipocytes [8]. Hydroalcoholic extract of *L. octovalvis* has also been reported to exert anti-inflammatory effects by inhibiting nuclear factor kappa B (NF-κB), along with antibacterial effects [9]. 80% methanol extract of *L. octovalvis* did not exhibited any toxicological effects in BALB/c (an albino strain of tshe house mouse) mice, which denotes its safety prospect [10]. Aqueous extract of *L. octovalvis* showed antibacterial effects against *Streptococcus mutans* [11]. *Staphylococcus aureus* strains were also found susceptible to *L. octovalvis* crude extract [12]. Recently Kannan et al., and Ramasamy et al. investigated *L. octovalvis* silver nanoparticles, and TiO_2 nanocatalyst respectively, collectively for its antimicrobial, photocatalytic, and antibiofilm activity [13, 14]. β-sitosterol and squalene present in *L. octovalvis* 95% ethanolic extract has been reported to produce anti-aging effects in *Drosophila melanogaster* and senescence-accelerated mouse-prone 8 mice [15]. 80% methanolic extract of *L. octovalvis* was proved as immunostimulant against Shiga toxin in BALB/c mice [16]. From the methanolic extract of *L. octovalvis*, Chang et al. reported 3 new oleanane-type triterpenes, (23Z)-coumaroylhederagenin, (23E)-coumaroylhederagenin, and (3Z)-coumaroylhederagenin, which were found cytotoxic against human cancer cell lines [17]. In their next investigation, Chang et al. reported 2 more compounds as (23Z)-feruloylhederagenin and (23E)-feruloylhederagenin [18]. While repeated silica gel chromatography of 95% ethanol extract of *L. octovalvis* favors the presence of ellagic acid, gallic acid, apigenin, luteolin, carrotoside, 2α-hydroxyursolic acid, β-sitosterol, 3,4,8,9,10-pentahydroxydibenzo[b,d]pyran-6-one, short-leaf hematoxylate, quercetin, maltol, potentic acid, and oleanolic acid [19].

Now, it is widely accepted the coexistence of depression with diabetes. One should not omit depression while treating diabetes, and vice versa [20]. Although both these diseases are different, but few biomarkers like insulin resistance, hyperglycemia, and inflammation involved in their pathophysiology has been reported common. Many such factors collectively creates an imbalance between antioxidant system and reactive oxygen species production [21].

Therefore, after comprehensive literature survey done about the effects of *L. octovalvis* on various biomarkers, the present investigation was designed to evaluate the 70% hydroethanol extract of *L. octovalvis* (HLO) in attenuating the biosignatures involved in diabetes comorbid depression (DCD). It has been reported that in streptozotocin-induced diabetic rats, prefrontal cortex oxidative stress plays a great role in producing depressive-like behavior [22]. Therefore, we considered assaying catalase, superoxide dismutase (SOD), lipid peroxidation, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity in this experimental work, beside of behavioral study and plasma glucose, and insulin estimation.

Methods

Plant procurement and validation

L. octovalvis was procured from Rajiv Gandhi South Campus Banaras Hindu University Barkachha Mirzapur, Uttar Pradesh, India. Prof. Nawal Kishore Dubey (Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi) validated *L. octovalvis* with voucher specimen no. Onarga. 2019/1.

Drug and chemical reagents

DPPH and ascorbic acid were bought from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Metformin was procured from Sigma Aldrich (Louis, MO, USA). Nicotinamide was obtained from SD Fine Chemical Ltd. (Mumbai, India), and streptozotocin was obtained from HiMedia (Thane West, India). Rest of chemicals and reagents procured from regional suppliers were of analytical grade.

Laboratory animals

The Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India had supplied 42 healthy adult male Charles Foster albino rats (140–160 g). Throughout the whole experiment all animals were freely fed with Amrut® Rodent Diet (M/s Krishna Valley Agrotech LLP, Pune, Maharashtra, India) and water. The atmosphere provided to all the animals housed in polypropylene rat cages had 24–26 °C temperature, 50 ± 5% humidity, and 12-12 h dark-light cycles for the total time period (7 days prior to- and throughout the experiment). Behavioral experiment was conducted between 09:00 and 14:00. Animal investigational treatments and supervision were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying, Government of India, and were approved by the Institutional Ethics Committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India (Registration No.: Dean/2019/IAEC/1232, dated 05/05/2019).

Plant extract

Ethanol and water (70:30 v/v) was used for the Soxhlet extraction of *L. octovalvis* [7]. Briefly, the coarse powder of whole plant of *L. octovalvis* was placed in the Soxhlet apparatus, and the extract was concentrated. Preliminary phytochemical studies of HLO was carried out, followed by their quantitative estimation through spectroscopy. High-performance thin-layer chromatography profiling of HLO was

performed to determine the major secondary metabolites. Ultraviolet-visible spectrophotometry was performed for the quantitative estimation of total phenolic (at 650 nm), and total flavonoid (at 510 nm). Total phenolic and flavonoid content present in HLO was estimated as 5.15 mg/g gallic acid equivalent (GAE) and 43.9 mg/g quercetin equivalent (QE) respectively. High-performance thin-layer chromatography profiling of HLO confirmed the presence of tannic acid, lupeol, quercetin, gallic acid and stigmasterol.

In vitro DPPH free radical scavenging assay

DPPH assay was performed to calculate the antioxidant activity of HLO by using ascorbic acid as the positive control [23]. Methanolic solution of DPPH looks purple or violet colored, but on adding antioxidant to it the color changes to yellow. HLO and ascorbic acid stock solutions in methanol, each with 10 mg/mL concentration was prepared. 10 µL to 80 µL of HLO and ascorbic acid were transferred in separate test tubes, and the volume was made up to 1 mL with methanol. Further, 2 mL freshly prepared methanolic DPPH solution (0.01 mM) was added to each dilution of HLO and ascorbic acid. All the prepared mixtures were vortexed, incubated, and kept in dark for half an hour. After then, the absorbance of the mixture was measured at 517 nm by using a double-beam ultraviolet-visible spectrophotometer (UV-1800 Shimadzu). 80% (v/v) methanol was used as blank. The assay was performed in triplicate. Following formula was used for DPPH scavenging activity calculation. The result was reported in IC_{50} values of HLO as well as of ascorbic acid.

$$\text{Scavenging activity (\%)} = \frac{Absorbance\ (control) - Absorbance\ (sample)}{Absorbance\ (control)} * 100$$

Inducing non-insulin dependent diabetes

Streptozotocin powder was dissolved in citrate buffer (pH 4.5, 0.1 M), beside of preparing normal saline nicotinamide solution separately [24]. Further, the rats were intraperitoneally injected with 120 mg/kg body weight (b.w.) single dose of nicotinamide, followed by injecting 65 mg/kg b.w. single dose of streptozotocin via the same route. The nondiabetic control group rats received only normal saline + 0.1 M citrate buffer vehicle. Purchased rat fodders and sucrose solution was freely suppled to every animal. Blood samples from rat tail-nick was collected after 72 h of streptozotocin-nicotinamide administration, to evaluate the insulin and glucose levels. Rats which attained minimum of 250 mg/dL blood glucose level were judged as diabetic, and were selected for further comorbid depression induction.

Experimental design and drug treatment

Forty-two rats were divided in seven groups having 6 animals in each group. The control group rats were treated with 1 mL/kg b.w., per os (p.o.) vehicle. The seven groups were named as control, diabetic control, depression control, DCD control, DCD + HLO 200 (DCD rats treated with 200 mg/kg b.w., p.o. HLO), DCD + HLO 400 (DCD rats treated with 400 mg/kg b.w., p.o. HLO), and DCD + metformin (DCD rats treated with 25 mg/kg b.w., p.o. metformin). For dosing freshly prepared 25 mg/mL metformin in distilled water was used. HLO and metformin dosing was initiated after development of comorbid depression in the diabetic rats. In-house validated method was employed for dosing metformin and HLO at different interval of time.

Inducing depression in diabetic rats

To induce depressive-like behavior in diabetic rats, both diabetic and nondiabetic rats were placed in a grid floored black box. Five 2 mA electric footshocks were given to diabetic rats for 2 ms duration, at the intervals of 10 s. However, nondiabetic rats were left untreated [25].

Assessing depression by forced swim test

For 10 days all the rats were treated with drug or vehicle. On the 11th day they were individually assessed for depression by forced swim test, 1 h after drug or vehicle treatment. For this, a 45 cm long cylindrical chamber of diameter 40 cm was filled with water up to 38 cm at ambient temperature, and single rat was let to swim in the chamber for 15 min. The maximum time limit set for reading the immobility period was 5 minutes [26].

Open field test

Individual rat was exposed to open field test to evaluate spontaneous motor activity in HLO and metformin treated DCD rats [27]. The dimensions of the open field test equipment (an opaque Plexiglas cage) was 44 × 33 × 20 cm^3. The bottom of the cage was comprised of 12 squares (11 × 11 cm^2). A digital camera was used to record the activities of all the animals. The animals were randomly positioned in the corners of the cage. The 3 variables, crossing, rearing and grooming of the rats were observed. Where, crossing is the rate of the number of squares passed-through by a rat through its rear legs, while rearing that time period (in seconds) for which a rat reside in the cage in vertical posture, and grooming of a rat is the time period of sprucing act (in seconds). A five minute halt was practiced after each rat's assessment to eliminate the previous rat's odor, by wiping the open field cage with ethanol (10% v/v).

Collection of blood

Prior to blood sampling the rats were sedated via treating them with carbon dioxide-ambient air mixture (80:20) for 2 min in an anaesthetizing gas chamber [28]. Retro-orbital puncture method was employed to collect the blood samples. For the separation of blood plasma, the blood samples were centrifuged at 1,500 g for 10 min at 4 ˚C. Then after, glucose and insulin was quantified in the blood plasma.

Plasma glucose and insulin quantification

Autospan Glucose (ARKRAY Healthcare Pvt. Ltd., Mumbai, India) was used as an enzymatic assay kit comprised of oxidase/peroxidase reagent for the colorimetric analysis of glucose at 505 nm. DRG Insulin ELISA Kit (DRG International, Inc., Springfield, NJ, USA) was used to estimate insulin at 450 nm. Both glucose and insulin were analyzed by iMark™ microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA).

Brain homogenate preparation

After decapitation of rats, the brain was anatomized to collect the prefrontal cortex, weighed, and was swiftly freeze preserved at −80 ˚C in liquid nitrogen. Next, 0.02 M cold phosphate buffer (pH 7.4) was prepared. The volume of ice-cold phosphate buffer taken was 10 times that of prefrontal cortex weight for homogenization. A glass tissue homogenizer was used to thaw the brain tissues, followed by its cold centrifugation (4 ˚C) at 12,000 g × 45 min. Then after the clear supernatant was separated out, and was kept preserved at −80˚C, in anticipation of oxidative stress assay [29].

Ex vivo oxidative stress assessment in the prefrontal cortex

The prefrontal cortex homogenate was assayed for catalase activity, SOD content, and lipid peroxidation to evaluate the extent of oxidative stress [30]. The catalase activity was assayed by using catalase assay kit (Sigma-Aldrich, Burlington, MA, USA), and the absorbance was measured at 520 nm. SOD content in the brain supernatant was assayed by using SOD assay kit (Sigma-Aldrich, Burlington, MA, USA), and the absorbance was measured at 450 nm. While the lipid peroxidation assay was performed by using lipid peroxidation malondialdehyde assay kit (Sigma-Aldrich, Burlington, MA, USA), and the absorbance was measured at 532 nm. Beside these 3 assays, protein content in the brain homogenate was also evaluated. All the colorimetric absorbance were measured by using iMark™ microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA). All the protocols followed throughout the assays were in accordance with the manufacturer's catalogue.

Statistical analysis

Calculations were presented for n = 6, and the results were furnished as mean ± standard error of the mean. Whole data analysis had gone through Tukey's multiple comparison test and one-way analysis of variance equipped with GraphPad Prism 9.0.0.121 (GraphPad

Software, San Diego, CA, USA). The statistical significance in the results was expressed as $p < 0.05$.

Results

Behavioral studies

Forced swim test. After 10 days treatment with drug or vehicles, the diabetic rats were assessed for the presence of comorbid depression, through forced swim test and the immobility period was recorded (Figure 1A). Non-diabetic control rats were immobile for only 39.3 ± 8.6 s, while DCD control rats showed maximum immobility period of 159 ± 30.6, followed by depression control rats with immobility period of 114 ± 8.2 s ($p < 0.05$). The immobility period of diabetic control rats was found 82.5 ± 11 s. Drug treatment was found to reduce the immobility period by DCD + HLO 200, DCD + HLO 400, and DCD + metformin group rats as 124.6 ± 11.74 s, 95.5 ± 7.28 s, and 70 ± 8.6 s respectively ($p < 0.05$), in comparison to DCD control rats.

Open field test of HLO treated rats in relation to others. After 10 days dosing of drug/vehicle, open field test was performed to assess the activeness of the treated rats (Figure 1B). DCD rats showed lower open field activity (crossing: 15.1 ± 1.1 sec; rearing: 3.0 ± 0.1; grooming: 2.8 ± 0.3) as compared with the nondiabetic controls (crossing: 30.1 ± 0.2 sec; rearing: 9.6 ± 1.2; grooming: 10.1 ± 0.3) ($p < 0.05$). Open field activity in depression control rats was lower (crossing: 18.1 ± 1.3 sec; rearing: 6.0 ± 1.0; grooming: 5.8 ± 0.2) as compared with nondiabetic controls rats ($p < 0.05$). While diabetic control rats exhibited lower activity (crossing: 17.6 ± 1.3 sec; rearing: 5.6 ± 0.2; grooming: 6.3 ± 0.3) as compared with nondiabetic controls rats ($p < 0.05$). DCD + HLO 400 mg rats displayed higher open field activity (crossing: 23.1 ± 2.4 sec; rearing: 5.8 ± 0.7; grooming: 6.5 ± 1.0) as compared with DCD + HLO 200 mg rats (crossing: 21 ± 2.4 sec; rearing: 4.3 ± 1.0; grooming: 5 ± 0.8) ($p < 0.05$). DCD control rats showed higher immobility periods (crossing: 15.1 ± 1.1 sec; rearing: 3.0 ± 0.1; grooming: 2.8 ± 0.3) as compared with DCD metformin 25 mg/kg rats (crossing: 25.1 ± 2.4 sec; rearing: 7.8 ± 0.7; grooming: 8.5 ± 1.0) ($p < 0.05$).

HLO treatment reduced plasma glucose and enhanced insulin concentrations

After 28 days of dosing, all rats were assessed for plasma glucose and plasma insulin concentrations.

Glucose levels. Plasma glucose levels in the nondiabetic control, and diabetic control rats were calculated as 84.6 ± 11.5 mg/dL, and 433.5 ± 57.7 mg/dL respectively ($p < 0.05$) (Figure 2A). DCD control rats showed 418.8 ± 65.3 mg/dL glucose concentration. In depression control rats it was 91.6 ± 19.2 mg/dL concentration of glucose ($p < 0.05$). After drug treatment, DCD + HLO 200, DCD + HLO 400, and DCD + metformin rats showed significantly reduced plasma glucose level of 189.5 ± 33.7 mg/dL, 120 ± 13.69 mg/dL, and 96.5 ± 11.8 mg/dL respectively, as compared to DCD control rats ($p < 0.05$). Metformin and HLO 400 dose exhibited better hypoglycemic results in relation to HLO 200 dose.

Insulin levels. The plasma insulin levels of nondiabetic control, and diabetic control rats were 17.3 ± 2.6 μIU/mL, and 5.6 ± 1.7 μIU/mL respectively ($p < 0.05$) (Figure 2B). However in depression control, and DCD control rats the plasma insulin levels were calculated as 14.4 ± 2.0 μIU/mL, and 4.4 ± 1.1 μIU/mL respectively ($p < 0.05$). Following drug treatment, DCD + HLO 200, DCD + HLO 400, and DCD + metformin rats showed uplifted plasma insulin levels of 6.0 ± 1.8 μIU/mL, 12.1 ± 1.3 μIU/mL, and 13.4 ± 1.7 μIU/mL respectively as compared to DCD control rats ($p < 0.05$). Metformin and HLO 400 dose showed better plasma insulin uplifting as compared to the HLO 200 dose.

Antioxidant validation of HLO

DPPH scavenging activity of HLO versus ascorbic acid. In vitro non-enzymatic DPPH assay was performed to evaluate scavenging activity of HLO. HLO inhibited 85.3%–48.67% free radicals at 266.66–33.33 μg/mL concentration range (Figure 3). However ascorbic acid as the positive control displayed 83.95%–14.25% DPPH scavenging activity at 50–10 μg/mL concentration range. IC_{50} values of HLO and ascorbic acid were calculated as 33.52 and 27.86 μg/mL respectively. HLO showed comparable DPPH scavenging activity in relation to the standard ascorbic acid.

Ex vivo oxidative stress assessment in the prefrontal cortex. The extent of oxidative stress in the prefrontal cortex region of the brain was studied in rats by estimating catalase activity, SOD content, and lipid peroxidation. Because in both the cases of diabetes and DCD, oxidative stress is the key concurred hallmark.

Catalase activity: the nondiabetic control, and diabetic control rats showed catalase activity of 54.2 ± 8.0, and 19.2 ± 2.9 μmol H_2O_2/min/mg protein ($p < 0.05$) (Figure 4A). However depression control, and DCD control group showed catalase activity of 38.3 ± 3.5, and 12.2 ± 2.1 μmol H_2O_2/min/mg protein respectively ($p < 0.05$). Increase in catalase activity upon drug treatment DCD + HLO 200, DCD + HLO 400, and DCD + metformin groups were calculated as 26.3 ± 2.6, 40.2 ± 5.5, and 28.5 ± 5.2 μmol H_2O_2/min/mg protein respectively ($p < 0.05$). HLO 400 dose was found to increase the catalase activity at highest extent in relation to others.

SOD content: the nondiabetic control, and diabetic control rats exhibited SOD content of 33.1 ± 5.0, and 11.3 ± 1.3 IU/mg protein respectively ($p < 0.05$) (Figure 4B). While, SOD content of depression control, and DCD control groups were calculated as 26.6 ± 4.5, and 6.8 ± 1.9 IU/mg protein respectively ($p < 0.05$). Drug treated DCD + HLO 200, DCD + HLO 400, and DCD + metformin groups showed increase in SOD contents of 19.1 ± 2.4, 27.3 ± 4.9, and 12.2 ± 1.6 IU/mg protein mg/kg respectively, in relation to DCD control group ($p < 0.05$). The SOD assay was better evaluated for HLO 400 dose in relation to others.

Lipid peroxidation: malondialdehyde content observed was taken as the measure of lipid peroxidation in the rat prefrontal cortex (Figure 4C). The nondiabetic control, and diabetic control rats exhibited lipid peroxidation as 8.3 ± 2.1, and 25.0 ± 4.4 nmol malondialdehyde/mg protein respectively ($p < 0.05$). While depression control, and DCD control rats showed lipid peroxidation as 9.3 ± 2.5, and 28.8 ± 3.5 nmol malondialdehyde/mg protein respectively ($p < 0.05$). Drug treated DCD + HLO 200, DCD + HLO 400, and DCD + metformin rats showed declined lipid peroxidation as 19.33 ± 2.7, 9.63 ± 2.1, and 16.6 ± 2.7 nmol malondialdehyde/mg protein respectively ($p < 0.05$). HLO 200 dose showed better dropped lipid peroxidation as compared to others.

Discussion

Free radical scavenging activity of HLO was found comparable to ascorbic acid. So, it may be concluded that HLO possess good antioxidant activity, which might had attenuated the common oxidative stress biomarkers involved in diabetes and depression [22].

Although the immobility period of diabetic control group was slight less than the HLO treated rats, improvisation in immobility period was encountered in the highest dose receiving DCD + HLO 400 group, in comparison to depression control, and DCD control groups. Similar results were obtained in open field activity test, and DCD + HLO 400 group showed betterment in DCD. Therefore, HLO may be considered as promising agent in attenuating depressive-like behavior.

Perhaps DCD + HLO 200 group did not met the hypoglycemic effects as observed in DCD + metformin group, but DCD + HLO 400 group exhibited significant results in the proximity of DCD + metformin group, with reduced glucose, and enhanced insulin levels. Thus, the results reestablishes the influential antidiabetic effects of *L. octovalvis* [4–6].

The results obtained from catalase activity, SOD, and lipid peroxidation studies of prefrontal cortex region revealed that the hydro-ethanolic extract of *L. octovalvis* has good antioxidant activity in the management of diabetes comorbid depression rats, and it could be exploited in other neurological disorders where oxidative stress is involved in the pathophysiology pathways.

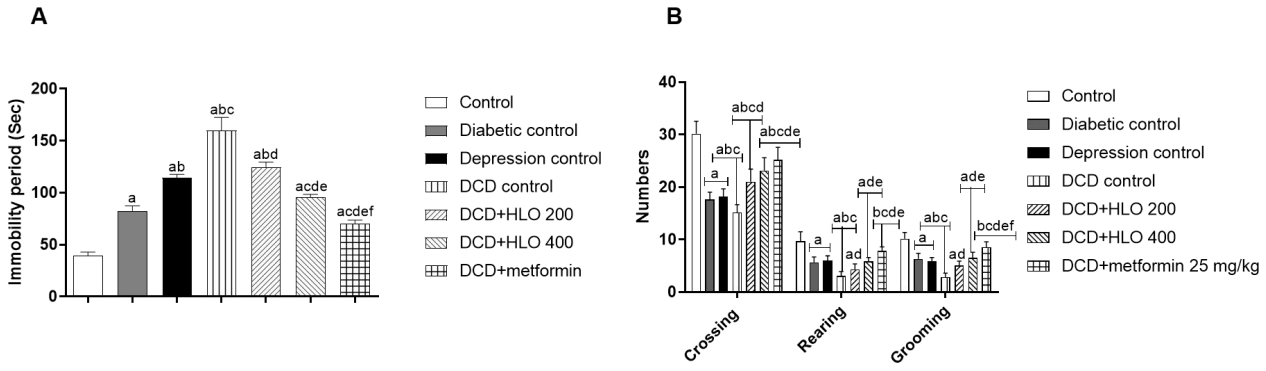

Figure 1 Comparative immobility period study by forced swim test to assess the depression levels. In comparison to DCD control rats, the decline in immobility period shown by DCD + HLO 200, DCD + HLO 400, and DCD + metformin rats were 22.02%, 40.23%, and 56.19% respectively (A), open field test variables study (B). Data are stated from independent triplicate tests as the mean ± standard error of the mean ($p < 0.05$). [a]$p < 0.05$, compared with control group; [b]$p < 0.05$, compared with diabetic control group; [c]$p < 0.05$, compared with depression control group; [d]$p < 0.05$, compared with DCD control group; [e]$p < 0.05$, compared with DCD + HLO 200 group; [f]$p < 0.05$, compared with DCD + HLO 400 group. DCD, diabetes comorbid depression; HLO, hydroethanolic extract of *Ludwigia octovalvis*.

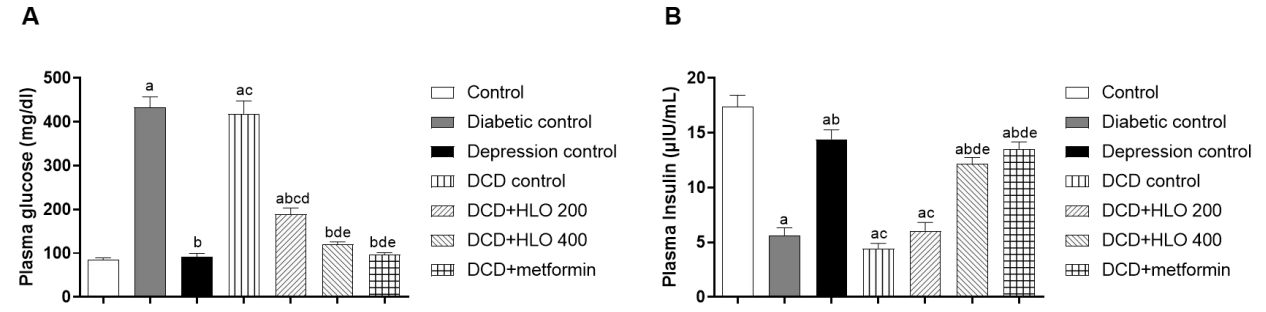

Figure 2 Effect of HLO and metformin on plasma glucose concentration in DCD control rats and **plasma insulin assessment in response to HLO and metformin.** DCD + HLO 200, DCD + HLO 400, and DCD + metformin rats exhibited 54.75%, 71.34%, and 76.95% decline in plasma glucose concentration respectively, as compared to DCD control rats (A). DCD + HLO 200, DCD + HLO 400, and DCD + metformin rats exhibited raised insulin level by 36.36%, 175.00%, and 204.54% respectively, in relation with DCD control rats (B). Data are stated from independent triplicate tests as the mean ± standard error of the mean ($p < 0.05$). [a]$p < 0.05$, compared with control group; [b]$p < 0.05$, compared with diabetic control group; [c]$p < 0.05$, compared with depression control group; [d]$p < 0.05$, compared with DCD control group; [e]$p < 0.05$, compared with DCD + HLO 200 group; [f]$p < 0.05$, compared with DCD + HLO 400 group. DCD, diabetes comorbid depression; HLO, hydroethanolic extract of *Ludwigia octovalvis*.

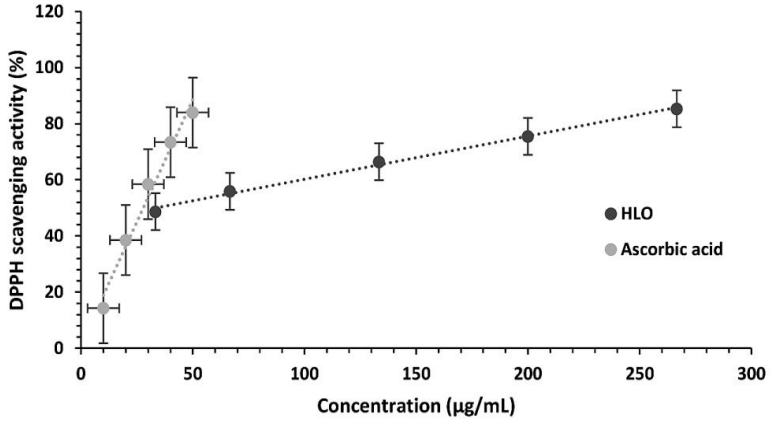

Figure 3 DPPH free radical scavenging activity of HLO and ascorbic acid. IC_{50} values: HLO = 33.52 µg/mL; ascorbic acid = 27.86 µg/mL. Data are stated from independent triplicate tests as the mean ± standard deviation ($p < 0.05$). DPPH, 2,2-diphenyl-1-picrylhydrazyl; HLO, hydroethanolic extract of *Ludwigia octovalvis*.

Both the HLO doses significantly showed outstanding effects in empowering catalase activity as well as the SOD content, with vanished lipid peroxidation, as compared to metformin. Thus, HLO also qualified as a good antioxidant phytomedicine, assayed through ex vivo study of prefrontal cortex of DCD rats.

The possible mechanism of action of HLO is illustrated in Figure 5, which supports the inhibition of pancreatic lipase and α-glucosidase theory for its antidiabetic effects, and AMP-activated protein kinase phosphorylation in the brain neuronal cells for its antidepressant effect.

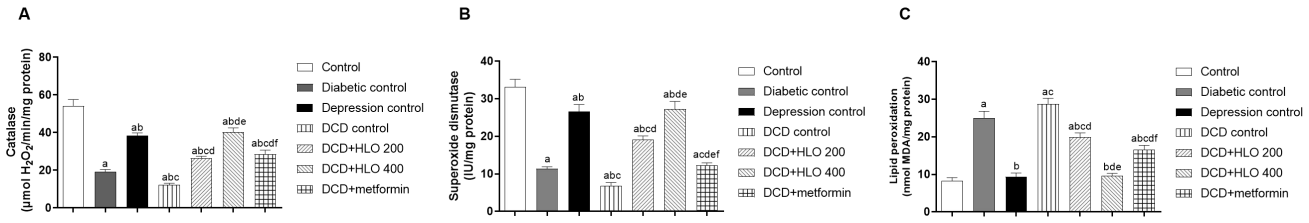

Figure 4 Elevated catalase activity (A), effects of HLO and metformin on superoxide dismutase content (B), and HLO and metformin effects on lipid peroxidation (C) in the brain homogenate of rats. 115.57% in DCD + HLO 200 group, 229.50% in DCD + HLO 400 group, and 133.60% in DCD + metformin group, as compared to DCD control group (A). SOD content was raised by 180.88%, 301.47%, and 79.41% in case of DCD + HLO 200, DCD + HLO 400, and DCD + metformin groups respectively, in relation with DCD control group (B). Metformin lowered lipid peroxidation by 42.36%, but it was declined by 66.56% and 32.88% in case of DCD + HLO 400 and DCD + HLO 200 groups respectively, when compared with DCD control group (C). Data are stated from independent triplicate tests as the mean ± standard error of the mean ($p < 0.05$). [a]$p < 0.05$, compared with control group; [b]$p < 0.05$, compared with diabetic control group; [c]$p < 0.05$, compared with depression control group; [d]$p < 0.05$, compared with DCD control group; [e]$p < 0.05$, compared with DCD + HLO 200 group; [f]$p < 0.05$, compared with DCD + HLO 400 group. HLO, hydroethanolic extract of *Ludwigia octovalvis*; DCD, diabetes comorbid depression; DPPH, 2,2-diphenyl-1-picrylhydrazyl; SOD, superoxide dismutase; MDA, malondialdehyde.

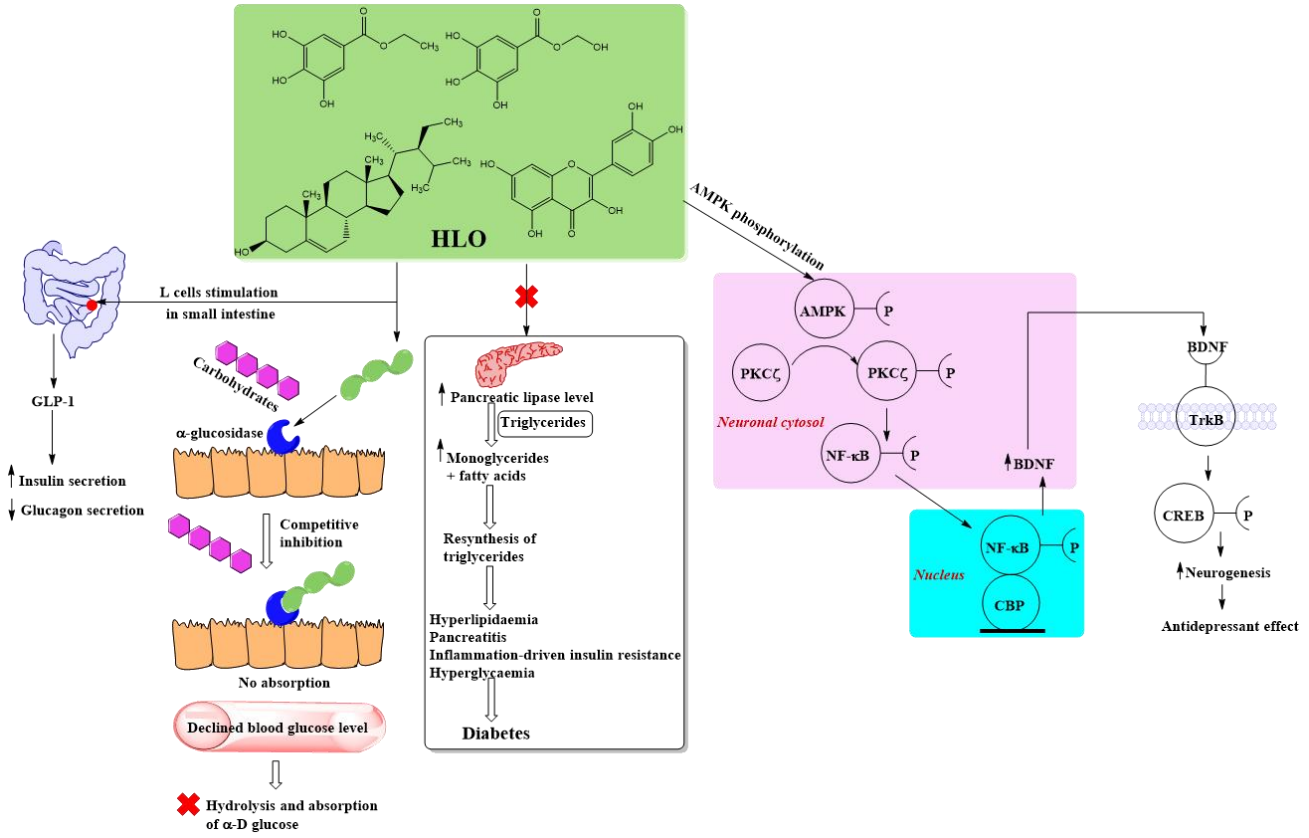

Figure 5 Mechanism of action of HLO to act as antidiabetic and antidepressant agent. HLO phytoconstituents by inhibiting excessive pancreatic lipase and α-glucosidase tone-up the insulin and glucose levels. While the brain neuronal AMPK phosphorylation by HLO suggests the pathway to lower depression like behaviour. HLO, hydroethanolic extract of *Ludwigia octovalvis*; AMPK, AMP-activated protein kinase; NF-κB, nuclear factor kappa B; GLP-1, glucagon-like peptide 1; AMPK, AMP-activated protein kinase; PKCζ, protein kinase C zeta type; NF-κB, nuclear factor kappa B; BDNF, brain-derived neurotrophic factor; CREB, cyclic adenosine monophosphate response element binding protein; CBP, CREB binding protein; TrkB, tyrosine kinase receptor B.

Conclusion

The results obtained from the present study suggests the positive therapeutic effects of *L. octovalvis* extract in diabetic as well as depressive rats. In vitro, and ex vivo studies favors the utilization of HLO in attenuating the focused causes of DCD. It is needful to assess different *L. octovalvis* extracts to treat other metabolic disorders which affects central nervous system health.

References

1. Dennis PA. Herbal medicine among the miskito of Eastern Nicaragua. *Econ Bot*. 1988;42:16–28. https://doi.org/10.1007/bf02859024
2. Mitra S, Karmakar A, Das S, Barik A. Attraction of the potential biocontrol agent Altica cyanea by volatile compounds of three species of Ludwigia weeds from rice fields. *Entomol Exp Appl*. 2020;168(1):91–104. https://doi.org/10.1111/eea.12870
3. Gowthami R, Sharma N, Pandey R, Agrawal A. Status and consolidated list of threatened medicinal plants of India. *Genet Resour Crop Evol*. 2021;68:2235–2263. https://doi.org/10.1007/s10722-021-01199-0
4. Yang LL, Yen KY, Kiso Y, Hikino H. Antihepatotoxic actions of formosan plant drugs. *J Ethnopharmacol*. 1987;19(1):103–110. https://doi.org/10.1016/0378-8741(87)90142-5
5. Ramírez G, Zavala M, Pérez J, Zamilpa A. In vitro screening of medicinal plants used in Mexico as antidiabetics with glucosidase and lipase inhibitory activities. *Evid Based Complement Alternat Med*. 2012;2012:701261. https://doi.org/10.1155/2012/701261
6. Morales D, Ramirez G, Herrera-Arellano A, Tortoriello J, Zavala M, Zamilpa A. Identification of digestive enzyme inhibitors from Ludwigia octovalvis (Jacq.) P.H.Raven. *Evid Based Complement Alternat Med*. 2018;2018:8781352. https://doi.org/10.1155/2018/8781352
7. Lin WS, Lo JH, Yang JH, et al. Ludwigia octovalvis extract improves glycemic control and memory performance in diabetic mice. *J Ethnopharmacol*. 2017;207:211–219. https://doi.org/10.1016/j.jep.2017.06.044
8. Wu SJ, Ng LT, Wang GH, Huang YJ, Chen JL, Sun FM. Chlorophyll a, an active anti-proliferative compound of Ludwigia octovalvis, activates the CD95 (APO-1/CD95) system and AMPK pathway in 3T3-L1 cells. *Food Chem Toxicol*. 2010;48(2):716–721. https://doi.org/10.1016/j.fct.2009.12.001
9. Bork PM, Schmitz ML, Weimann C, Kist M, Heinrich M. Nahua Indian medicinal plants (Mexico): inhibitory activity on NF-kB as an anti-inflammatory model and antibacterial effects. *Phytomedicine*. 1996;3(3):263–269. https://doi.org/10.1016/S0944-7113(96)80064-X
10. Yakob HK, Uyub AM, Sulaiman SF. Toxicological evaluation of 80% methanol extract of Ludwigia octovalvis (Jacq.) P.H. Raven leaves (Onagraceae) in BALB/c mice. *J Ethnopharmacol*. 2012;142(3):663–668. https://doi.org/10.1016/j.jep.2012.05.035
11. Chen CP, Lin CC, Tsuneo N. Screening of Taiwanese crude drugs for antibacterial activity against Streptococcus mutans. *J Ethnopharmacol*. 1989;27(3):285–295. https://doi.org/10.1016/0378-8741(89)90003-2
12. Silva ACO, Santana EF, Saraiva AM, et al. Which approach is more effective in the selection of plants with antimicrobial activity? *Evid Based Complement Alternat Med*. 2013;2013:308980. https://doi.org/10.1155/2013/308980
13. Kannan DS, Mahboob S, Al-Ghanim KA, Venkatachalam P. Antibacterial, antibiofilm and photocatalytic activities of biogenic silver nanoparticles from Ludwigia octovalvis. *J Clust Sci*. 2021;32:255–264. https://doi.org/10.1007/s10876-020-01784-w
14. Ramasamy K, Dhavamani S, Natesan G, et al. A potential role of green engineered TiO2 nanocatalyst towards enhanced photocatalytic and biomedical applications. *Environ Sci Pollut Res Int*. 2021;28(30):41207–41223. https://doi.org/10.1007/s11356-021-13530-4
15. Lin WS, Chen JY, Wang JC, et al. The anti-aging effects of Ludwigia octovalvis on Drosophila melanogaster and SAMP8 mice. *Age* (*Dordr*). 2014;36(2):689–703. https://doi.org/10.1007/s11357-013-9606-z
16. Yakob HK, Uyub AM, Sulaiman SF. Immune-stimulating properties of 80% methanolic extract of Ludwigia octovalvis against Shiga toxin-producing E. coli O157:H7 in Balb/c mice following experimental infection. *J Ethnopharmacol*. 2015;172:30–37. https://doi.org/10.1016/j.jep.2015.06.006
17. Chang CI, Kuo CC, Chang JY, Kuo YH. Three new oleanane-type triterpenes from Ludwigia octovalvis with cytotoxic activity against two human cancer cell lines. *J Nat Prod*. 2004;67(1):91–93. https://doi.org/10.1021/np030267m
18. Chang CI, Kuo YH. Oleanane-type triterpenes from Ludwigia octovalvis. *J Asian Nat Prod Res*. 2007;9(1):67–72. https://doi.org/10.1080/10286020500382769
19. Yan J, Yang XW. Studies on the chemical constituents in herb of Ludwigia octovalvis. *Chin J Chin Mater Med*. 2005;30(24):1923–1926. (Chinese) https://d.wanfangdata.com.cn/periodical/zgzyzz200524012
20. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care*. 2001;24(6):1069–1078. https://doi.org/10.2337/diacare.24.6.1069
21. Réus GZ, Carlessi AS, Silva RH, Ceretta LB, Quevedo J. Relationship of oxidative stress as a link between diabetes mellitus and major depressive disorder. *Oxid Med Cell Longev*. 2019;2019:8637970. https://doi.org/10.1155/2019/8637970
22. de Morais H, de Souza CP, da Silva LM, et al. Increased oxidative stress in prefrontal cortex and hippocampus is related to depressive-like behavior in streptozotocin-diabetic rats. *Behav Brain Res*. 2014;258:52-64. https://doi.org/10.1016/j.bbr.2013.10.011
23. Rahman MM, Islam MB, Biswas M, Alam AHMK. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC Res Notes*. 2015;8:621. https://doi.org/10.1186/s13104-015-1618-6
24. Rani R, Dahiya S, Dhingra D, et al. Antidiabetic activity enhancement in streptozotocin + nicotinamide-induced diabetic rats through combinational polymeric nanoformulation. *Int J Nanomedicine*. 2019;14:4383–4395. https://doi.org/10.2147/IJN.S205319
25. Planchez B, Surget A, Belzung C. Animal models of major depression: drawbacks and challenges. *J Neural Transm* (*Vienna*). 2019;126(11):1383–1408. https://doi.org/10.1007/s00702-019-02084-y
26. Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The forced swim test as a model of depressive-like behavior. *J Vis Exp*. 2015;(97):52587. https://doi.org/10.3791/52587
27. Rodríguez-Landa JF, Vicente-Serna J, Rodríguez-Blanco LA, Rovirosa-Hernández Mde J, García-Orduña F, Carro-Juárez M. Montanoa frutescens and Montanoa grandiflora extracts reduce anxiety-like behavior during the metestrus-diestrus phase of the ovarian cycle in Wistar rats. *Biomed Res Int*. 2014;2014:938060. https://doi.org/10.1155/2014/938060
28. Kohler I, Meier R, Busato A, Neiger-Aeschbacher G, Schatzmann U. Is carbon dioxide (CO_2) a useful short acting anaesthetic for small laboratory animals? *Lab Anim*. 1999;33(2):155–161.

https://doi.org/10.1258/002367799780578390
29. Wang YF, Zhang YC, Hu W, et al. Rapid alteration of protein phosphorylation during postmortem: implication in the study of protein phosphorylation. *Sci Rep*. 2015;5:15709. https://doi.org/10.1038/srep15709
30. Patlolla AK, Barnes C, Yedjou C, Velma VR, Tchounwou PB. Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. *Environ Toxicol*. 2009;24(1):66–73. https://doi.org/10.1002/tox.20395