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# Phytochemical Assessment, *in vivo* Hepatoprotective and Nephroprotective Evaluation of *Aerva javanica* Crude Methanolic Extract

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors Wisal, JNA and WK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MFB, YK, MA, MII and IIJ managed the analyses of the study. Authors UM and FA managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The current study was carried out to assess the effect of the crude methanolic extract of *Aerva javanica* for hepatoprotective and nephroprotective assessment along with their antioxidant potential against 2,2-azinobis(3-ethylenbenzthiazolin)-6sulfonic acid (ABTS) and 2,2diphenyle-1-picrylhyzyl

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(DPPH) using a standard protocol. Result of the crude methanolic extract of *Aerva javanica* showed 65.55, 59.37, 53.86, 47.49 and 34.76% inhibition against DPPH while against ABTS it showed 60.12, 48.45, 41.36, 37.99 and 31.89% inhibition at the concentrations 1000, 500, 250, 125 and 62.5 µg/mL. Ascorbic acid was used as a positive control and displayed a dose-dependent response. Results of Total phenolic content (TPC) and total flavonoid content (TFC) have shown 52% and 67%.The crude methanolic extract of *Aerva javanica*was tested at two different doses for hepatoprotective and nephroprotective effect (150 and 300 mg/kg) in rabbits model. In comparison with the standard drug and normal saline, the test sample showed a significant hepatoprotective and nephroprotective effect. The effects of all the analyzed biomarkers of the liver (AST, ALT, ALP, serum triglyceride, serum cholesterol, and serum bilirubin) and Urea (Blood serum, Blood sugar, and Serum creatinine) showed significant effects both at 150 and 300 mg/kg. Both doses were found hepatoprotective (150 mg/kg and 300 mg/kg). Whereas, some liver biomarkers (ALT, ALP, serum triglyceride) of the methanolic extract at a dose of 300mg/kg showed significant hepatoprotective while some biomarkers (AST, serum cholesterol and serum bilirubin) was found effective at a dose of 150 mg/kg rather than the higher dose. The nephroprotective effect of the plant also increases in a dose-dependent manner i.e it is more effective at a dose of 300 mg/kg as compared to 150 mg/kg body weight. On the bases of these biomarkers, the plant extract was found an effective hepatoprotective at both the test doses.

*Keywords: DPPH; ABTS; Ascorbic acid; silymarin; liver biomarkers; kidney biomarkers.*

## 1. INTRODUCTION

Medicinal plants have been used in traditional medicine practices since prehistoric times. A medicinal plant is used to maintain health, to be directed for a specific condition, or both, whether in medicine or traditional medicine [1,2]. The Food and Agriculture Organization projected that in 2002 over 50,000 medicinal plants are used across the world [3]. In the traditional healing system, wild resources of medicinal plants have been used by man. Indigenous folks have adopted different modes of application and use to exploit this natural resource [4] in the process of drug developmentfrom the plant the insufficient financing, poor practices, and weak scientific evidence blunted the use of medicinal plants [2].In different countries, plants are a potential source of antimicrobial agents and are rich in a variety of phytochemicals including terpenoids, alkaloids, tannins,and flavonoids which have been found *in vitro* to have antimicrobial properties [5-7]. Although the mode of action and effectiveness of these herbal extracts in most cases is still needed to be authenticated scientifically, these preparations mediate important host responses [8,9]. Throughout the world medicinal plant are considered a source of natural antioxidants and are used for the treatment of diseases [10]. Some of theseproperties are antimicrobial [11], anti-cancer [12], anti-diabetic [13], anti-atherosclerosis [7], immunomodulatory [14] and even reno-protection or hepatoprotective effects. [15].

### 1.1 Plant Description

*Aerva javanica* belongs to the Amaranthaceae family, Amaranthaceae locally called "Sparai" whereas desert cotton is its English name, counting about 169 genera and 2300 species. The plants of genus *Aerva* (Amaranthaceae) are perennial herb under shrubs and found in the North Temperate Zone, especially in the Asia and Mediterranean region. In Pakistan 20 species of Genus, *Aerva* belongs to family Amaranthaceae may be found [16]. The plant comprises numerous important biomolecules, which comprise tannins, alkaloids, saponins, sulfates, flavonoids, lipids, and carbohydrates. It is grown in tropical and subtropical dry areas. It is both erect and spreading, grows up to a height of 1.5 m high, and is covered with densely matted hairs on stems and leaves. *Aerva javanica* is much-branched, with vigorous round stems that are woody at the base, and a dark stout taproot. It has numerous leaves, ovate to lanceolate, 10-20 x 40-75 mm, alternate and white to grey. The flowers are small and whitish and arranged in dense, woolly terminal panicles. The aim of this study is to assess the Phytochemical, In Vivo Hepatoprotective and Nephroprotective Evaluation of *Aerva javanica* Crude Methanolic Extract.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plants

*Aerva javanica* leaves were collected from the hilly areas of Village Ramora (Gorha gut mountain) Chakdara lower Dir in 2019 and identified with the help of specimen (voucher no.675) placed in the herbarium lab Department of Botany, Bacha Khan University Charsadda KP, Pakistan.

### 2.2 Chemicals and Solvents

DPPH (Sigma-Aldrich, USA) and ABTS (Sigma-Aldrich, Germany) were used for antioxidant assays. Ascorbic acid (Sigma-Aldrich, UK) used as a standard in antioxidant activity.

### 2.3 Extract Preparation

The aerial parts of *Aerva javanica* were dried under shade at room temperature. The leaves were chopped through mortar and pestle into optimum size pieces. The chopped plants' materials were then grounded into powder form through an electric blender. The powdered fractions were transferred into separate closed containers for soaking.

### 2.4 Soaking

Two kg of powdered air-dried plant materials were soaked separately in 80% Methanol and 20% of distal water in the closed Containers and they were regularly shacked for maximum extraction. After one week, the extracts were filtered by using filter paper. The debris and un-dissolved material remained on filter paper. The filtrate was collected in a beaker and then shifted to the round bottom flask of the rotary evaporator for concentrating the extracts and separation of the solvents.

### 2.5 Rotary Evaporation

The extracts solutions were evaporated under reduced pressure at a temperature of 45°C by using a vacuum pump with the rotary evaporator. After the rotary evaporator, the pastes were obtained, which contained some water contents, which were further dried on a water bath at $60^0$C. The thick pastes obtained are known as the crude extract of plant materials. These extracts were kept in sterile bottles for refrigeration until use.

### 2.6 Fractionation

The crude methanolic extract was subjected to fractionation using a solvent-solvent extraction process. The crude methanolic extract was suspended in 500 mL of distilled water in a separating funnel.

### 2.7 Antioxidant (Free Radical Scavenging) Activity

#### 2.7.1 Preparation of 2,2-diphenyl-1-picrylhydrazyl(DPPH) solution for the antioxidant activity

0.039 g of DPPH was precisely weighted with the help of digital balance and dissolve in the distilled methanol to give an appropriate solution of 100 ml (0.039 gm/100 ml) of required concentration. The solution prepared was then covered with aluminum foil and kept in dark place to protect it from light.

#### 2.7.2 Preparation of *Aerva javanica* solution for antioxidant activity

0.01 g of methanolic extract (leaves) was accurately weighed with the help of digital balance and dissolved separately in the distilled methanol to prepare the required solutions of 10 ml and to get the required concentrations. These solutions were stored as *Aerva javanica* extract stock solutions. A stock solution of*AervaJavanica* extract was diluted to obtain the required concentration of 1000, 500, 250, 125, and 62.5 µg/mL. Control solutions were also prepared for extract/fraction which contains 5ml pure distilled methanol plus 0 ml *Aerva javanica* extract/fraction solution. Then 1ml of stock solution of DPPH was added to each of the control solutions and the diluted solution of fruit extract and all these solutions were kept in dark place for 30 minutes. Then their absorbance reading was taken at 517 nm of maximum wavelength with the help of a spectrophotometer in the laboratory [17]. The percent DPPH scavenging potential was figured out by using the following formula;

$$\%\ DPPH\ Scavenging\ activity = \frac{\text{absorption of control} - \text{absorption of test sample}}{\text{absorption of control}} \times 100$$

#### 2.7.3 Preparation of 2, 2-azinobis [3-ethylebenzthiazoline]-6-sulfonic acid (ABTS) solution for antioxidant activity

The 2,2-azinobis [3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) of free radicals scavenging action was examine followed standard procedure of (Ullah F and Ayaz M, et al. 2015). ABTS solution is prepared by taking 7 mM of ABTS and 2.45 Mm of potassium persulfate solution and mixed thoroughly. The solution prepared was put in dark overnight for the production of free radicals. After incubation time the absorbance of the solution was adjusted at 745 nm to 0.7 by the addition of 50% methanol. Test samples having a volume of 300 µl was taken in a test tube and 3 mL ABTS solution was added to it. The solution was transferred to the cuvette and absorbance values were taken for six minutes using a double beam spectrophotometer and percent ABTS radical scavenging. The potential was figured out using the following formula;

$$\%\ ABTS scavenging activity = \frac{\text{absorption of control} - \text{absorption of test sample}}{\text{absorption of control}} \times 100$$

## 2.8 Total Phenolic Contents (TPC)

Extract of *Aerva javanica* was checked for total phenolic content by reagent Folin-Ciocalteu, this protocol represented by [18] with small modifications. Initially, dilutions of crude extract were done about 50 times with water (deionized) prior to study. 1 ml of diluted F-C reagent (diluted with deionized water 10 times) was mixed with 1 ml of extract (diluted). After this, incubation of the mixture was done for 5 minutes at room temperature. 0.8 ml of 7.5% NaCO (anhydrous) was added into that mixture 3 followed by immediate mixing for 10 s on vortex mixture and finally incubated for 2 hours in a dark environment. A blank sample was assessed with the same procedure having deionized water instead of *Aerva javanica*. Absorbance was determined by comparison with blank at 765 nm under ultraviolet light spectrophotometer. Standard curve calibration was done through Gallic acid. Triplicate random crude extract samples were analyzed and the result was mentioned in mg Gallic acid equivalent/g of sample dry weight.

## 2.9 Total Flavonoid Contents (TFC)

According to Ozsoy et al. (2007) crude extract and other fractions of *Aerva javanica*were checked for total flavonoid content (TFC). Extract of *Zizyphusoxyphylla* was taken 0.25 ml and initially mixed with 1.25 ml of water (deionized), after which 75 µl of 5% sodium nitrate and after 6 minutes 150 µl of 10% Aluminium chloride was added and the mixture was permitted to stand for 5 minutes at room temperature. Afterward, 0.5 ml of 1 molar NaOH was added into the same mixture. Now 275 µl of water (deionized) was added and the mixture was subjected to vortex mixer for 10 s. The absorbance of the extract was recorded spectrophotometrically at 510 nm with UV light. Blank was run using deionized water instead of *Aerva javanica* extract. Standard curve calibration was done with Quercetin. Triplicate samples of the plant were analyzed and results were recognized mg of Quercetin equivalent/g of sample dry weight.

## 2.10 Nephroprotective and Hepato-protective Activities

#### 2.10.1 Animals used

Thirty domestic local mature rabbits (*Oryctolaguscuniculus*) of both sexes were purchased from the local market. They were kept in a well-ventilated and wide chambered animal house at the University of Malakand, Pakistan. The rabbits were fed on chaw pellets along with fresh green vegetables and grasses and free access to fresh water at *libitum*. The animals were acclimatized for two weeks in such conditions, before the experimentation.

#### 2.10.2 Animals grouping and dosing

The rabbits were arranged into four groups for eight days protocol [19,20]. One rabbit was kept in each group. Two doses low (150 mg/kg) and high doses (300mg/kg) were tested for each extract and fraction. Each rabbit was tagged separately for identification. Rabbit 1, administered with normal saline, served as normal control, rabbit 2 as the paracetamol control group, received paracetamol only, rabbit 3 served as the standard control group, received paracetamol on day zero followed by silymarin, a well-known standard drug of the hepatoprotective group, groups 4 received paracetamol followed by crude hydro-methanolic extract 150 and 300mg/kg body weight. The dosing detail was: paracetamol 1 g /kg body weight [21], silymarin 50 mg/kg body weight [22].

#### 2.10.3 Chemicals used

The serum levels of ALT, AST, and ALP were estimated by using commercially available kits

(purchased from AMP Diagnostics, Austria) on anUltraviolet-visible light spectrophotometer (Agilent 8453) and Silymarin.

#### 2.10.4 Mounting of coverslip

Slides were cleaned properly after completion of the staining process. For clear and neat slides DPX (Scharlau) was poured and covered with coverslips in such a manner to avoid the formation of bubbles.

### 2.11 Hematological and Serological Profile of Infected Rabbits

From clinically positive rabbits blood samples were collected. Blood was collected in clean EDTA tubes. For serology 3 ml blood were collected in the tubes and were allowed to clot and serum was harvested through centrifugation of tubes at 3000 rpm for 10 min. The serum was collected in 1ml Eppendorf tubes and was kept at 4°C until further use.

### 2.12 Serology

Blood samples 3 ml were collected in clean tubes, centrifuged for 10 min at 3000 pm. 1 ml Eppendorf tubes were used for serum separation. Serum glutamic pyruvate transferase (SGPT), total serum proteins, albumin, and globulin were measured by using the Biochemistry analyzer (PS-520 SHENZHEN PROCAN ELECTRONICS, CHINA).

### 2.13 Serum Glutamic Pyruvate Transferase (SGPT)

Reagents R1 and R2 are two reagents in the kit (Reactivos, GPL Barcelona, Spain) for the estimation of serum glutamic pyruvate transferase (SGPT). As per manufacturer instruction solution was prepared by mixing 4 volumes of R1 and 1 volume of R2. 1 ml of the solution was then mixed with 100 µl of a serum sample, incubated at 37ºC for 1 min. In the Automatic Biochemistry analyzer, the SGPT activity sample was loaded.

### 2.14 Total Serum Protein (TP)

Estimation of total serum protein was done by using Kit (Reactivos, GPL Barcelona, Spain) which contains a calibrator and reagent (R). Reading was obtained by mixing a reagent (R) 1ml with 25 µl of the calibrator. Reagent (R) (1 ml) was then mixed with 25 µl of serum sample and incubated at 37ºC for 5 min. In the Automatic Biochemistry analyzer, the sample was loaded and the result was recorded.

### 2.15 Serum Albumin

Estimation of serum albumin was used by using Kit (Reactivos, GPL Barcelona, Spain) containing reagent (R) and a calibrator. Reading was obtained by using reagent (R) 1 ml mixed with 5 µl of the calibrator. By incubation at 37ºC for 5 min, 1 ml of reagent (R) was mixed with 5 µl of serum sample. In the Automatic Biochemistry analyzer, the sample was loaded and the result was recorded.

### 2.16 Serum Isolation and Assessment of Some Liver-related Serum Enzymes and Kidney Parameters

This is an 8 days protocol. On the $9^{th}$ day, the animals were dissected. Before twelve hours from dissection, food was withdrawn and then anesthetized by chloroform inhalation. Directly after dissection blood was directly drawn with 21 Gauge needle in 3 ml syringe from the heart chambers by cardiac puncture (Illahi I et al 2012) for hematological analysis the blood samples were collected into Ethylene Diamine Tetra-acetic Acid (EDTA)-coated tubes (K2-EDTA tubes), with a coagulant. These are kept for 1 hour at room temperature. Serum was separated (Eppendorf 5702R, Germany) through centrifugation at 3000 rpm for 5 min and stored at –20°C until analyzed. For hepatotoxicity, the biochemical markers analyzed were: aspartate aminotransferase (AST), enzymatic activities of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Serum urea and creatininetests were done for nephrotoxicity.

### 2.17 Determination of Glomerular Filtration Rate

The urea and creatinine clearance tests were used to estimate the glomerular filtration rate.

### 2.18 Urea Clearance

The following formula was used for the urea clearance test:

GFR x Serum urea = Urine urea x Urine volume
Or GFR = [Urine urea x Urine volume]/Serum urea. This formula is called the urea clearance.

### 2.19 Creatinine Clearance

The following formula was used for the creatinine clearance test:

GFR x Serum creatinine = Urine creatinine x Urine volume Or GFR = [Serum creatinine x Urine volume]/Serum creatinine. This formula is called the creatinine clearance.

### 2.20 Statistical Analysis

Data are presented as means ± standard deviation (SD). To compare means the data were subjected to Tukey Test of Post Hoc Multiple Comparisons in One Way Anova for all these analysis computer software SPSS 16.0 was used.

### 2.21 Collection and Analysis of Urine

On a ninth day, all the animals for the collection of urine samples were kept in individual cages. 24hrs. urine samples were collected. During this period the animals had free access to drinking water. In graduated cylinder 24 hrs, total urine volume in ml of each rabbit was measured. The sample is analyzed for urinary creatinine and urinary urea after storage at 4°C for one day. The parameters were estimated through COBAS chemistry automation using Roche Diagnostic kits.

## 3. RESULTS AND DISCUSSION

### 3.1 Antioxidant activity

#### 3.1.1 DPPH scavenging assay

The antioxidant activity of methanolic extract of *Aerva javanica* leaves against DPPH free radicals showed a dose-dependent response. The methanolic extract of leaves showed 65.55, 59.37, 53.86, 47.49, and 34.76% inhibition at the concentrations 1000, 500, 250, 125 and 62.5 µg/mL (Table 1 and Fig. 1). The percent DPPH inhibition of methanolic extract was compared with the positive control (Ascorbic acid). Ascorbic acid displayed 89.13, 83.25, 78.46, 70.68 and 65.71% inhibition at 1000, 500, 250, 125 and 62.5 µg/mL against DPPH.

#### 3.1.2 ABTS scavenging assay

The results of % ABTS inhibition potential of methanolic extractof *Aerva javanica* leaves are shown in (Table 1 and Fig. 2). The % inhibition of methanolic extract of leaves were 60.12, 48.45, 41.36, 37.99 and 31.89% at concentrations 1000, 500, 250, 125 and 62.5µg/mL and displayed a dose-dependent response. Percent ABTS inhibition of methanolic extract was compared with the positive control (Ascorbic acid). Ascorbic acid has shown 87.12% inhibitions at 1000 µg/mL against ABTS.

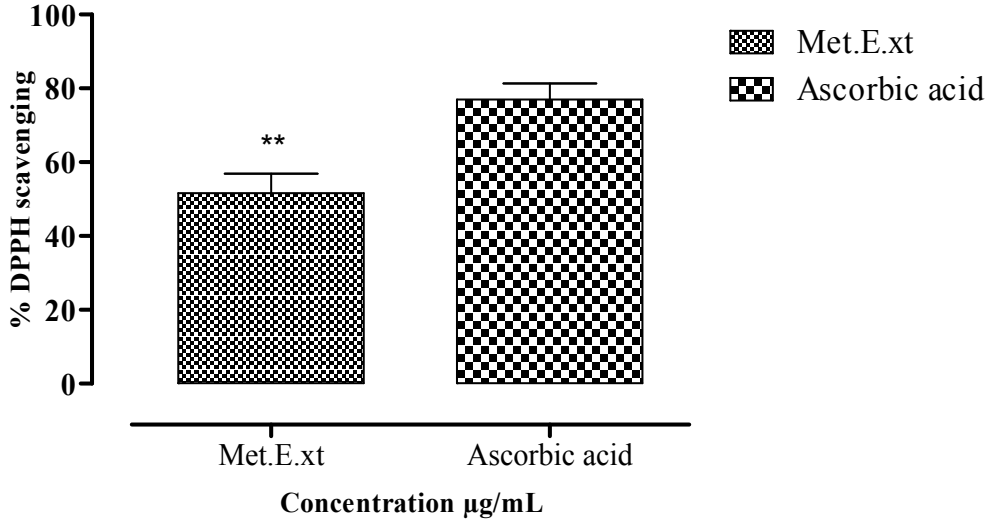


**Fig. 1. DPPH scavenging percentage**

**Table 1. Antioxidant activity of methanol extract of *Aerva javanica* against DPPH and ABTS free radicals assay**

| S.No | Sample | Concentration (µg/mL) | % DPPH scavenging | % ABTS scavenging |
|---|---|---|---|---|
| | | | Mean ± SEM | Mean ± SEM |
| 1 | Met.Ext | 1000 | 65± 0.55 | 60± 0.12 |
| | | 500 | 59± 0.37 | 48± 0.45 |
| | | 250 | 53± 0.86 | 41± 0.36 |
| | | 125 | 47± 0.49 | 37± 0.99 |
| | | 62.5 | 34± 0.76 | 31± 0.89 |
| 2 | Ascorbic acid | 1000 | 89± 0.13 | 87± 0.12 |
| | | 500 | 83± 0.25 | 80± 0.24 |
| | | 250 | 78± 0.46 | 76± 0.45 |
| | | 125 | 70± 0.68 | 69± 0.56 |
| | | 62.5 | 65± 0.71 | 62± 0.68 |

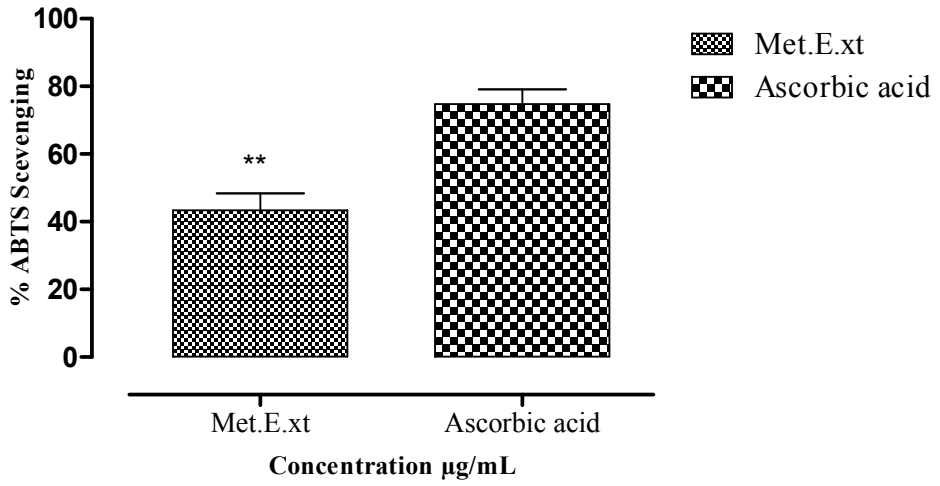


**Fig. 2. ABTS scavenging percentage**

#### 3.1.3 Total phenolic/flavonoid content (TPC)

Results of TPC and TFC of the crude methanolic extract of *Aerva javanica* are presented in (Fig. 3). The total phenolic contents of the crude methanolic extract are 52% while TFC is 67%. The extract showed the highest percentage of total flavonoid contents followed by total phenolic content.

### 3.2 *In-vivo* Biological Activities

#### 3.2.1 Acute toxicity

Plant crude extract tested for acute toxicity at various doses (1000, 1500 and 2000 mg/kg, i.p.) proved a safe herbal medicine. The Rabbits were safe and behaved normally when observed in the first 12 h and no death occurred after 24 h. Assessment bioassay period is represented in the (Table 2).

### 3.3 Hepatoprotective Effect of *Aerva javanica* Leaves Methanolic Extract

#### 3.3.1 Hepatoprotective effect

Table showing the hepatoprotective effects obtained from the different biomarkers (AST, ALT, ALP, serum triglyceride, serum cholesterol, and serum bilirubin) of the liver. It is clear from the results that the ALT, AST, ALP and Serum Triglyceride that the values of purely paracetamol treated groups have been increased in comparison with the normal controlled (normal saline) and standard hepatoprotective drug (Silymarin). Both doses were found hepatoprotective (150 mg/kg and 300mg/kg).

Whereas, comparatively methanolic extract at a dose of 150 mg/kg showed a more significant hepatoprotective effect rather than the 300mg/kg. Crude extract at a dose of 150 mg/kg showed pronounced effects. They have activity closer to the standard drug silymarin. Even the values of AST and ALP are found more significant than the standard drug, silymarin. From all the biomarkers' values except serum cholesterol, it is clear that the plant extract at a dose of 150mg/kg body weight has produced a strong hepatoprotective effect. So it is clear from the study thatthe methanolic extract of *Aerva javanica* leaves are more effective hepatoprotective drugs and can be used effectively for low doses.

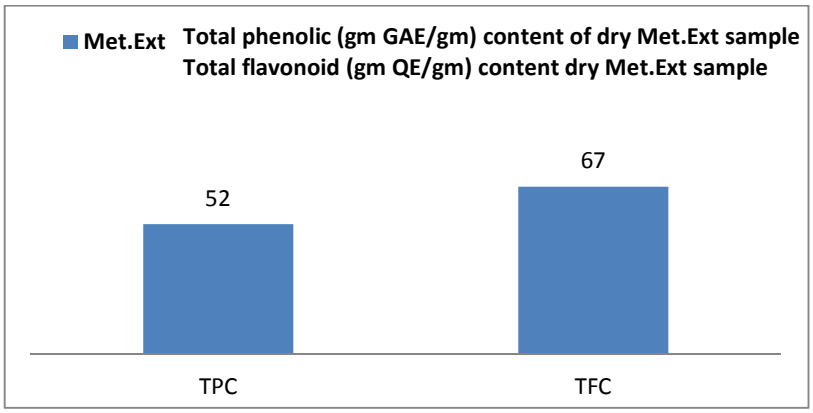


**Fig. 3. Total phenolic/flavonoid contents of *Aerva javanica***

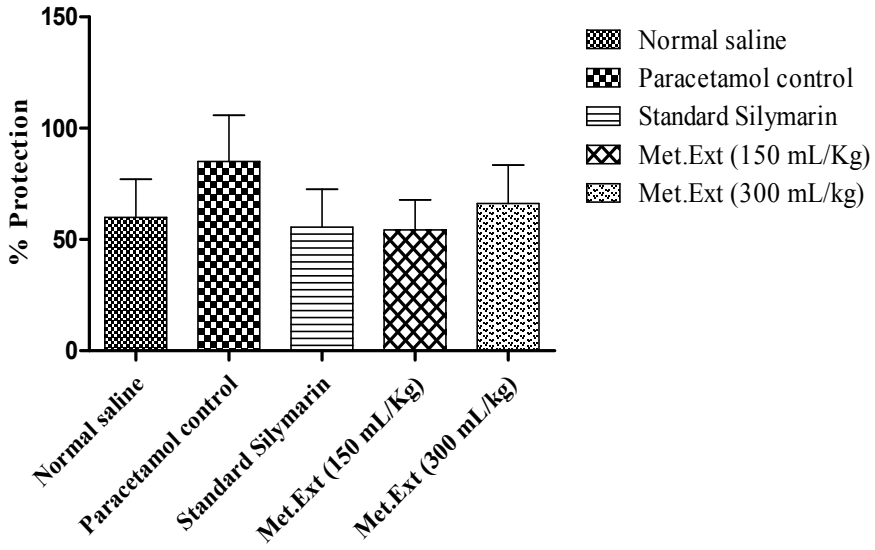


**Fig. 4. Effects of the crude extracts of *Aerva javanica* leaves on the liver related parameters (AST, ALT, and ALP) in the rabbits models**

**Table 2. Acute toxicity of the crude extract of *Aerva javanica***

| Extract/Fraction | Doses (mg/kg) | Mortality rate after 12h | Mortality rate after24h |
|---|---|---|---|
| Crude extract | 1000 | Alive | Alive |
| | 1500 | Alive | Alive |
| | 2000 | Alive | Alive |

### 3.3.2 Nephroprotective effect

The (Table 4) shows the nephroprotective effects of the crude methanolic extractobserved from the different blood parameters (Blood urea, Blood sugar and serum creatinine). The crude methanolic extract of *AervaJavanica*Leaveswas tested in two different doses for the nephroprotective effect (150 and 300 mg/kg) on the rabbits model. In comparison with the standard drug and normal saline, the test sample showed a significant effect both at 150 and 300 mg/kg. The effects ofall the three analyzed biomarkers of the kidneys i.e Blood urea, Blood sugar, and Serum creatinine showed significant effects. In comparison with the values of paracetamol, the values of the test sample showed a significant nephroprotective effect. The nephroprotective effect of the plant also increases in a dose-dependent manner i.e it is more effective at a dose of 300 mg/kg as compared to 150 mg/kg body weight except for the value of Serum creatinine which has been increased with increased dose. But the overall nephroprotective effect is dose dependent. The present study has already been done on this side but on a very small scale, based on antioxidant activity and in-vivo activity of the methanol*ic extract*/fraction of *Aerva javanica* plant leaves. A similar result is reported by [23] that drugs openly of either their metabolites come in contact with liver or kidneys they are effected deficiently or may result in their toxicity. Acute centrilobular necrosis and Centrizonalheamorrhagic are the effects of the commonly used analgesic and antipyretic drug [24,25]. Most of its metabolites are produced in the liver which is eliminated from the body through kidneys [26,27]. Five percent of the therapeutic drugs and most of the toxic paracetamol dose is converted into N-acetyl-p-benzoquineimine (NAPQI), an intermediate metabolite of cytochrome P450 which is very reactive [28]. The strong bonding of liver membrane lipids or protein with N-acetyl-p-benzoquineimine results in peroxidation of lipid and finally causes kidneys and liver intoxication [29]. Increased values of kidneys and liver biomarkers indicate the toxicity of both liver and kidneys by treatment with toxic paracetamol dose [30]. In the present study, the test doses of the test sample significantly reduced the values of the kidneys and liver biomarkers. This clarifies the regeneration of the intoxicated liver and kidneys by the use of the plant extract, *Aerva javanica* leaves**.** Different likely mechanisms may be involved/ give in the protective effect of the plant. Free radical scavenging effect [31] or compounds of phenol in the plants may be responsible for the significant defensive effects of the liver and kidneys. There is a direct proportionality between the compounds of phenol in a plant and antioxidant activities [30] as oxidative degradation of cellular components is not permitted by them [32]. It has also been reported that few flavonoids are responsible for the nephroprotective and hepatoprotective activities [31]. A current study of certain phenolic contents, antioxidants, and flavonoids could be the reason behind its hepatoprotective and nephroprotective effects against the paracetamol-induced hepatotoxicity and nephrotoxicity.

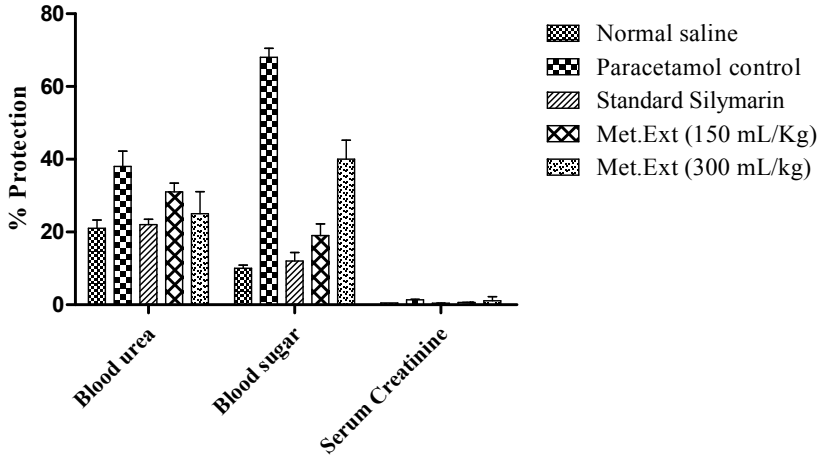


**Fig. 5. Effect of the crude extract *Aerva javanica* leaves on the kidney's function in the rabbits models**

**Table 3. Effects of the crude extracts of *Aerva javanica* leaves on the liver related parameters (AST, ALT, and ALP) in the rabbits models**

| Groups | Dose mg/kg | Liver-related parameters with % change values | | | | | |
|---|---|---|---|---|---|---|---|
| | | ALT | AST | ALP | Serum triglyceride | Serum Cholesterol | Serum Bilirubin |
| Normal saline | 1 mL/kg | 66 ±5.1*** | 89 ±2.4*** | 120 ±1.4*** | 49 ±3.1*** | 35 ±3.2** | 0.6 ±0.01$^{ns}$ |
| Paracetamol control | 1000 | 99 ±6.2 | 115 ±4.5 | 145 ±4.2 | 98 ±6.4 | 52 ±7.2 | 1.5 ±0.1 |
| Standard Silymarin | 50 | 49 ±3.2*** | 87 ±2.5*** | 119 ±2.1*** | 44 ±4.1*** | 34 ±2.5** | 0.7 ±0.01$^{ns}$ |
| Met. Ext | 150 | 52 ±2.1*** | 76 ±2.4*** | 99 ±1.5*** | 54 ±3.2*** | 44 ±4.2$^{ns}$ | 0.6 ±0.01$^{ns}$ |
| | 300 | 74 ±4.5*** | 92 ±3.5*** | 121 ±4.2*** | 74 ±5.1*** | 35 ±4.3** | 0.8 ±0.1$^{ns}$ |

**Table 4. Effect of the crude extract of *Aerva javanica* leaves on the kidney's function in the rabbits models**

| Groups | Dose mg/kg | Kidney related parameters with % change values | | |
|---|---|---|---|---|
| | | Blood urea | Blood sugar | Serum Creatinine |
| Normal Saline | 1 mL/kg | 21±2.3*** | 10±0.9*** | 0.5±0.01$^{ns}$ |
| Paracetamol control | 1000 | 38±4.2 | 68±2.5 | 1.4±0.1 |
| Standard Silymarin | | 22±1.5** | 12±2.4*** | 0.4±0.01$^{ns}$ |
| Met. Ext | 150 | 31±2.4$^{ns}$ | 19±3.2*** | 0.6±0.01$^{ns}$ |
| | 300 | 25±6.1** | 40±5.2*** | 1.1±1.1$^{ns}$ |

**P<0.05, **P<0.01 ***P<0.001. Data were interpreted by using Mean±SEM and by using two way ANOVA followed by Bonferroni Post Test*

## 4. CONCLUSION AND RECOMMENDATION

This study concludes from the current investigation that the methanolcrude extracts showing the highest % free radical scavenging potential against DPPH and ABTS at the concentration range of 1000, 500, 250, 125, and 62.5 µg/mL. The hepatoprotective and nephroprotective effects showed a significant effect on the plant. The nephroprotective effect of the plant also increases in a dose-dependent manner. The *Aerva javanica* leaves extract showed biological activities, which required further work to explore novel and safe bioactive compounds for the treatment of oxidative stress-related disorders.

## ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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