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AMELIORATIVE EFFECT OF *EMBLICA OFFICINALIS* (AMLA) ON ALTERED BIOCHEMICAL MARKERS AND OXIDATIVE STRESS INDICES AFTER SUB-ACUTE ENROFLOXACIN TREATMENT IN ALBINO RATS

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Enrofloxacin, *Emblica officinalis*, Albino rats, Oxidative stress

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ABSTRACT: Background: The current study was conducted at the department of veterinary pharmacology and toxicology to assess the impact of *Emblica officinalis* on oxidative stress produced by sub-acute enrofloxacin exposure in albino rats. **Methodology:** Albino rats weighing 200-300 grams were given enrofloxacin and *Emblica officinalis* orally through gavage needle. Blood samples were taken via capillary tube on the 28th day of the experiment and analyzed using laboratory procedures. **Result:** Sub-acute treatment of enrofloxacin at doses of 5 mg/kg b.wt. and 10 mg/kg b.wt. generated oxidative stress in rats, as evidenced by substantial changes in oxidative parameters, and affected liver and kidney functions, as seen by changes in biochemical markers of liver and kidney function. *Emblica officinalis* aqueous extract (200 mg/kg b. wt.) effectively reduces oxidative stress and changes in hepatic and renal function caused by enrofloxacin. **Conclusion:** *Emblica officinalis* (Amla) has antioxidant properties and can reduce oxidative stress caused by enrofloxacin in albino rats following sub-acute treatment.

INTRODUCTION: Enrofloxacin, a 6-fluoroquinolone, is one of the most widely used antimicrobials in veterinary practice. It is a bactericidal drug with a broad spectrum antibacterial activity against various bacteria, including strains resistant to other antimicrobial agents. The mechanism of action of enrofloxacin consists of inhibition of bacterial DNA gyrase, which plays basic role in the process of DNA replication, leading to inhibition of synthesis of bacterial proteins.

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The metabolism of enrofloxacin takes place in the liver through the N-oxidation, N-dealkylation, and de-ethylation processes. Enrofloxacin is mainly excreted through urine and bile. Fluoroquinolone antibiotics may cause adverse effects such as chondrotoxicity, renal damage, retinal damage, dysglycemia, cardiac arrhythmia, and even tendon rupture. These side effects of fluoroquinolones are associated with reduced collagen synthesis and induced oxidative stress [12].

Induction of oxidative stress has been reported with the administration of ofloxacin, ciprofloxacin, levofloxacin, gatifloxacin, and enrofloxacin [19]. There is evidence that free radical formation plays an important role in fluoroquinolone-induced cartilage defect and phototoxicity [17]. Herbal products have a special place in the world of pharmaceuticals. Interests in medicine of plant

origin are spreading worldwide because of their safety, efficacy, cost-effectiveness, and negligible side effects. Several plants have been mentioned in Ayurveda for curing hepatic and renal diseases. The world health organization found that 80 percent of the world's population depends on the medicinal plant for their health care needs, and more than 30% of the pharmaceutical preparations are based on plants [40]. *Emblica officinalis,* commonly known as Indian gooseberry or Amla, belonging to the family Euphorbiaceae, is a main herbal drug utilized in the unani and ayurvedic medicine [6].

MATERIAL AND METHOD: The suggested study was carried out on healthy albino rats weighing 150-200 g in the Department of Veterinary Pharmacology and Toxicology. The Institutional Animal Ethical Committee (IAEC) of the College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur, gave its approval to the study. For acclimatization, the rats were maintained in laboratory conditions for 7 days prior to the start of the experiment. The rats were kept in colony cages under standard management and given standard meal and water ad -libitum in order to maintain good sanitary conditions.

Drugs: Fresh fruit of *Emblica officinalis* (Amla) was collected from the Department of Botany, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P). Enrofloxacin was administered orally for subacute exposure.

***Emblica officinalis* Aqueous Extract Preparation:** To make powder, the fruits of *Emblica officinalis* were dried and crushed in a combination and grinder. Cold extraction was used to make the aqueous extract of *Emblica officinalis* (Shukla, 2006) [41]. The needed amount of *Emblica officinalis* fruit powder was weighed, steeped in distilled water, and kept at room temperature overnight. Filtration with filter paper yielded the cold aqueous extract.

Experimental Design: 30 rats were randomly divided into five groups, each with six rats. The effects of sub-acute enrofloxacin exposure on oxidative stress and organ damage, as well as the ameliorative potential of *Emblica officinalis* against these effects, were studied in five groups of rats, ranging from group I to group V. The trial took place over 28 days.

Group	Treatment
I	Control
II	Enrofloxacin @ 5 mg/kg b. wt. once daily, orally for 28 days
III	Enrofloxacin @ 10 mg/kg b. wt. once daily, orally for 28 days
IV	Enrofloxacin @ 5 mg/kg b. wt. along with aqueous extract of *Emblica officinalis*@ 200mg/kg b .wt. once daily, orally for 28 days
V	Enrofloxacin @ 10 mg/kg b. wt. along with aqueous extract of *Emblica officinalis* @ 200 mg/kg b. wt. once daily, orally for 28 days

Collection of Blood Sample: Blood was taken from the retro-orbital plexus using a capillary tube on days 0 and 28 as reported by Archar and Riley (1981) [4]. Biochemical and oxidative stress parameters were studied using blood obtained in heparinized vials.

Biochemical Studies: For biochemical analysis, plasma was extracted from heparinized blood samples and kept at 4°C. The biochemical indicators of liver and kidney function were determined using a semi-automated analyzer and commercially available ERBA kits from Transasia Bio-Medicals Ltd., Daman.

Oxidative Stress Indicators Evaluation: The samples were centrifuged at 2000 rpm for 15 minutes after blood collection to separate plasma. The layer of white blood cells above the packed erythrocytes was discarded. Erythrocyte pellet was washed three times with 0.15 M NaCl, diluted (33 %) in phosphate buffer saline (mM: NaCl, 136.9, KCl, 2.68; $KH_2 \cdot PO_4$, 1.47; and $Na_2 \cdot HPO_4$, 6.62; pH 7.4) and kept at 4°C until further analysis. The 33% packed erythrocytes were used to estimate LPO, GSH, Glutathione reductase, Catalase and Superoxide dismutase activity using Helios double beam spectrophotometer. LPO and GSH were measured on the day of blood collection (Prins and Loos, 1969) [34].

Lipid Peroxidation: Lipid peroxidation (LPO) was estimated in 33 % packed cell erythrocytes prepared in phosphate buffer saline of pH 7.4. Membrane peroxidative damage in erythrocytes was determined in terms of malondialdehyde

(MDA) production as method suggested by Rehman (1984) [37].

Reduced Glutathione (GSH): 200 µL whole blood was used for the estimation of blood glutathione (GSH). GSH was estimated by the 5, 5' dithiobis (2- nitrobenzoic acid) (DNTB) method suggested by (Habig *et al.,* 1974) [18].

Superoxide Dismutase: Superoxide dismutase activity (SOD) in the blood supernatant was measured using the Madesh and Balasubramanian technique (1998). The suppression of superoxide dependent reduction of tetrazolium dye (MTT) [3-(4-5 dimethyl thiazol- 2-4) 2, 5-diphenyl tetrazolium bromide] in a calorimetric experiment. One unit of SOD was responsible for a 50% drop in MTT reduction rate. **(**Marklund and Marklund, 1974) [27].

Catalase: Catalase activity in erythrocytes was assayed by the spectrophotometric method of Aebi (1983) [1].

Reagents: Phosphate buffer saline**,** Haemolysate (10 per cent)**,** Hydrogen peroxide (10 mM)

Statistical Analysis: Means and standard error were obtained as per standard procedure. Parameters were analyzed using the complete randomized design method with seven treatments allotted to groups of six animals each. The difference between treatments was tested statistically for their significance [43].

Biochemical Studies:

ALT (SGPT) and AST (SGOT): The concentration of ALT in control was 79.75 ± 7.50IU/L. Enrofloxacin significantly increased the concentration of ALT, and this increase was 91.38 ± 0.22U/L at the dose rate of 5 mg/kg b.wt., orally for 28 days. In higher doses i.e. 10 mg/kg b.wt. orally for 28 days, enrofloxacin further increased the concentration of ALT to 106.32 ± 0.10 A significant amelioration by *E. officinalis* was observed in group IV, where rats were treated with enrofloxacin at the dose rate of 5 mg/kg b.wt. and *Emblica officinalis* 200 mg/kg b.wt., orally for 28 days. In this group the mean values of ALT were 78.55±.14 IU/L, which showed a non-significant difference from the control. This amelioration *of E. officinalis* was also observed when enrofloxacin was administered in higher doses i.e. 10 mg/kg b.wt. along with *Emblica officinalis* 200mg/kg b.wt., orally for 28 days. In this group the mean values of ALT were 71.92 ± 1.73IU/L, which showed a non-significant difference from the control. 54C4CC4

The concentration of AST in control was 159.98 ± 6.27 IU/L. Enrofloxacin, significantly increased the concentration of AST and this increase was 177.33 ± 0.49U/L at the dose rate of 5 mg/kg b.wt., orally for 28 days. In higher doses i.e.10 mg/kg b.wt., orally for 28 days, enrofloxacin further increased the concentration of AST to 180.53 ± 0.09U/L. A significant amelioration by *E. officinalis* was observed in group IV, where rats were treated with enrofloxacin at the dose rate of 5 mg/kg b.wt. and *Emblica officinalis* at the rate 200 mg/kg b.wt., orally for 28 days. In this group the mean values of AST were 165.33 ± 3.22 IU/L, which showed non-significant difference from control. This amelioration of *Emblica officinalis* was also observed, when enrofloxacin administered in higher doses i.e. 10 mg/kg b.wt. along with *Emblica officinalis* at the rate of 200mg/kg b.wt., orally for 28 days. In this group the mean values of AST were 165.00 ± 3.71 IU/L, which showed non-significant difference from control.

The current study found that following subacute enrofloxacin exposure, serum ALT and AST levels were raised, which agrees with [11-16] findings. The liver enzyme markers ALT and AST are utilised to detect enrofloxacin-induced hepatic alterations in albino rats [21]. In carbon tetrachloride-induced liver injury [15], *Emblica officinalis* demonstrated a substantial reduction in increased levels of SGPT and SGOT. These findings are consistent with the findings of the current study.

GGT (Gamma Glutamyl Transferase): The concentration of GGT (Gamma glutamyl transferase) in control was 7.07 ± 0.03 IU/L. Enrofloxacin, significantly increased the concentration of GGT and this increase was 7.40 ± 0.04 IU/L after the subacute exposure of enrofloxacin at the dose rate of 5 mg/kg b.wt., orally for 28 days. In higher doses, *i.e.*10 mg/kg b. wt., orally for 28 days, enrofloxacin further increased the concentration of GGT to 7.70 ± 0.05 IU/L. A significant amelioration by *Emblica*

officinalis was observed in group IV, where rats were treated with enrofloxacin at the dose rate of 5 mg/kg b.wt. and *Emblica officinalis* at the rate 200 mg/kg b.wt., orally for 28 days. In this group the mean values of GGT were 7.20 ± 0.04 IU/L, which showed a non-significant difference from the control. This amelioration of *E. officinalis* was also observed, when enrofloxacin administered in higher doses i.e. 10 mg/kg b.wt., along with *Emblica officinalis* at the rate of 200 mg/kg b.wt., orally for 28 days. In this group, the mean values of GGT were 7.28± 0.04 IU/L, which showed non-significant difference from control.

Serum GGT is a liver enzyme that is sensitive, specific, and suggestive of liver function. According to [44], enrofloxacin enhanced the amount of the enzyme GGT in blood following sub-acute dosing. Nagaraj *et al*. (2007) [30] found that an aqueous extract of *Emblica officinalis* (Amla) reduced the raised level of GGT in rat liver after exposure to hexachloro-cyclohexane-induced cytotoxicity.

ALP (Alkaline Phosphatase): The concentration of alkaline phosphatase in control was 449.50± 2.54. Enrofloxacin, significantly increased the concentration of ALP and this increase was 532.50 ± 0.96U/L after the subacute exposure of enrofloxacin at the dose rate of 5 mg/kg b.wt., orally for 28 days. In higher doses i.e. 10 mg/kg b.wt., orally for 28 days, enrofloxacin further increased the concentration of ALP to 545.17 ± 3.71 IU/L. A significant amelioration by *Emblica officinalis* was observed in group IV, where rats were treated with enrofloxacin at the dose rate of 5 mg/kg b.wt. and *Emblica officinalis* 200mg/kg b.wt., orally for 28 days. In this group, the mean values of ALP were 450.17 ± 2.69 IU/L, which showed a non-significant difference from the control. This amelioration of *E. officinalis* was also observed, when enrofloxacin was administered in higher doses i.e. 10 mg/kg b.wt. along with *Emblica officinalis* 200mg/kg b.wt., orally for 28 days. in this group the mean values of ALP were 461.33 ± 0.7 U/L, which showed non-significant difference from control. Alkaline phosphatase is used to detect liver disease and is also an indicative enzyme for liver function. Elkholy *et al.* (2009) [13] showed that administration of enrofloxacin @ 10 mg /kg body weight daily for five successive days caused elevation of serum ALP when given orally. Davoren and Mainstone (1993) [10] and Moustafa *et al.* (1998) [29] showed that oral administration of enrofloxacin for 15 consecutive days caused elevation in serum ALP level in dogs. The investigations by Reddy *et al*. (2009) [36], mentioned the protective role of *Emblica officinalis* on alcohol induced liver damage in respect of ALP level in albino rats.

Albumin and Bilirubin: The concentration of albumin in control was 4.05 ± 0.03. Enrofloxacin, significantly increased the concentration of albumin and this increase was 4.33 ± 0.03 g/dl after the administration of enrofloxacin at the dose rate of 5 mg/kg b.wt., orally for 28 days. In higher doses i.e.10 mg/kg b.wt., orally for 28 days, enrofloxacin further increased the concentration of albumin to 4.50 ± 0.05 g/dl. A significant amelioration by *Emblica officinalis* was observed in group IV, where rats were treated with enrofloxacin at the dose rate of 5 mg/kg b.wt. and *Emblica officinalis* 200 mg/kg b.wt., orally for 28 days. In this group, the mean albumin values were 4.10± 0.02 g/dl, which showed a non-significant difference from control. This amelioration of *Emblica officinalis* was also observed when enrofloxacin administered in higher doses i.e., 10 mg/kg b.wt. along with *Emblica officinalis* 200 mg/kg b.wt., orally for 28 days. In this group, the mean albumin values were 4.15± 0.03 g/dl, which showed a non-significant difference from the control. In the present study, the values of albumin were measured on day 28 of the experiment; the albumin concentration was significantly higher in the enrofloxacin treated group as compared to the control group.

The concentration of Bilirubin in control was 0.107± 0.003 mg/dl. Enrofloxacin, significantly increased the concentration of bilirubin, and this increase was 0.127± 0.003 mg/dl after the administration of enrofloxacin at the dose rate of 5 mg/kg b.wt., orally for 28 days. In higher doses *i.e.* 10 mg/kg b.wt., orally for 28 days, enrofloxacin further increased the concentration of bilirubin to 0.137± 0.002 mg/dl. A significant amelioration by *Emblica officinalis* was observed in group IV, where rats were treated with enrofloxacin at the dose rate of 5 mg/kg b.wt. and *Emblica officinalis* 200 mg/kg b.wt., orally for 28 days. In this group,

the mean values of bilirubin were 0.110±0.004 mg/dl, which showed a non-significant difference from the control. This amelioration of *E. officinalis* was also observed when enrofloxacin was administered in higher doses, *i.e.,* 10 mg/kg b.wt. along with *Emblica officinalis* 200 mg/kg b.wt., orally for 28 days. In this group, the mean values of bilirubin were 0.117 ± 0.002 mg/dl, which showed non-significant difference from the control. Enrofloxacin dosing resulted in a considerable rise in albumin and bilirubin in the current investigation. Enrofloxacin raised albumin and bilirubin levels following subacute treatment, according to Kock *et al*. (1987) [24, 25]. *Emblica officinalis* (Amla), rich in vitamin C, gallic acid, flavonoids, and tannins, protects against enrofloxacin-induced hepatotoxicity and lowers liver enzyme concentrations [36].

Biochemical Markers of Kidney Function:

Creatinine and Blood Urea Nitrogen: The creatinine concentration in control was 0.640±0.021 mg/dl Enrofloxacin, significantly increased the creatinine concentration, and this increase was 0.847 ± 0.013 mg/dl after the administration of enrofloxacin at the dose rate of 5 mg/kg b.wt. In higher doses, i.e.10 mg/kg b.wt, enrofloxacin further increased the creatinine concentration to 1.052±0.023 mg/dl. In group IV, where rats were treated with enrofloxacin at the dose rate of 5 mg/kg b.wt., and *Emblica officinalis* at the rate of 200 mg/kg b.wt. orally for 28 days, the mean values of creatinine were 0.840 ±0.013 mg/dl, significantly lower than the enrofloxacin (10 mg/kg) treated group.

A significant amelioration by *Emblica officinalis* was observed, when enrofloxacin administered in higher doses i.e. 10 mg/kg b.wt. along with *Emblica officinalis* in group V. In this group, the mean values of creatinine were 0.913±0.005 mg/dl, which were significantly lower than enrofloxacin alone (10 mg/kg) treated group. Blood urea nitrogen concentration in control was 14.35± 0.23 mg/dl. At the dose rate of 5 mg/kg b.wt., Enrofloxacin orally for 28 days did not significantly affect the BUN level in serum, and the mean value was 14.62± 0.03. However, enrofloxacin in higher doses *i.e.,* 10 mg/kg b.wt., orally for 28 days, significantly increased the concentration of BUN to 23.84± 0.02 mg/dl.

In group IV, where rats were treated with enrofloxacin at the dose rate of 5 mg/kg b.wt. and *Emblica officinalis* 200 mg/kg b.wt., orally for 28 days, the mean value of BUN was 14.36± 0.02 mg/dl, which showed a non-significant difference from control. A significant amelioration by *E. officinalis* was observed in group V where rats were treated with enrofloxacin at the dose rate of 10 mg/kg b.wt. and *Emblica officinalis* @ 200 mg/kg b.wt., orally for 28 days. In this group, the mean value of BUN was 19.12± 0.01 mg/dl, significantly lower than the enrofloxacin (10 mg) treated group. According to [22,] sub-acute enrofloxacin exposure raised creatinine and BUN concentrations. In hyperammonemic patients [26], *Emblica officinalis* restored blood urea nitrogen and creatinine levels by boosting antioxidant status. The conclusions of this inquiry corroborate those of the mentioned researchers.

Oxidative Stress Indices:

Lipid Peroxidation (MDA): Lipid peroxidation was calculated in terms of nM MDA/gm of blood on day 28 of experiment in albino rats. The concentration of LPO in control was 4.63±0.02 nM MDA/gm of blood, However, enrofloxacin at a dose of 5 mg/kg b.wt and 10 mg/kg b.wt. significantly increased the concentration of LPO, and the mean values were 5.20 ±0.03 and 5.78±0.03 nM MDA /gm of blood, respectively. Significant amelioration of enrofloxacin-induced oxidative stress was shown by *Emblica officinalis* and was observed by reduced LPO concentration in groups IV and V with mean values 4.17 ±0.04 and 4.83±0.03, respectively.

Sarban *et al*. (2005) [39] discovered a rise in MDA levels in the plasma after taking enrofloxacin, as evidenced by the appearance of lipid hydroperoxide. In this study, oral administration of *Emblica officinalis* aqueous extract at the time of enrofloxacin exposure significantly reduced the degree of lipid peroxidation. This might be due to the existence of radical scavengers with antioxidant properties that can reduce the generation of peroxides, hydroxyl radicals, and superoxide radicals. Our findings are consistent with those of Bast *et al*. (1991) [5], Bhattacharya *et al*. (1999) [7], and Khopde *et al.* (2001) [23], who all found that aqueous extract of *Emblica officinalis* significantly

reduced LPO content in albino rats, indicating free radical scavenging action.

SOD (Superoxide Dismutase): Superoxide dismutase was calculated in terms of U/g of Hb on day 28 of the experiment in albino rats. The concentration of SOD in control was 1.22±0.01 U/g of Hb; however enrofloxacin at dose of 5 mg/kg b.wt and 10 mg/kg b.wt. significantly decreased the concentration of SOD and the mean values were 0.88±0.01 and 0.84±0.02 U/g, respectively. Significant amelioration of enrofloxacin-induced oxidative stress was shown by *Emblica officinalis* and was observed by elevated SOD concentration in groups IV and V with mean values 1.12±0.03 and 1.14±0.04, respectively. SOD activity contributes to increasing the level of superoxide radicals, thus increased oxidative stress. Sureshkumar e*t al*. (2013) indicated that enrofloxacin administration reduced superoxide dismutase (SOD). The aqueous extract of *Emblica officinalis* (Amla) was found to increase the activity of antioxidant enzymes. Bhattacharya *et al*. (2002) [8] and Rajak *et al*. (2004) [35] reported that an aqueous extract of *Emblica officinalis* boosted the activity of the antioxidant enzyme SOD in rats these findings are consistent with those of the current study.

GSH (Reduced Glutathione): Reduced glutathione was calculated in terms of µmol /ml of blood on day 28 of the experiment in albino rats. The concentration of GSH in control was 340.67±0.21 µmol /ml of blood; however, enrofloxacin at dose of 5 mg/kg b.wt and 10 mg/kg b.wt. significantly decreased the concentration of GSH, and the mean values were 332.57±0.24 and 330.42±0.44 µmol /ml of blood, respectively. Significant amelioration of enrofloxacin-induced oxidative stress was shown by *Emblica officinalis* and was observed by increased GSH concentration in groups IV and V with mean values 334.33±0.17 and 335.42±0.35 µmol /ml of blood, respectively.

According to Cerreras *et al.*, (2005) [9], the metabolism of enrofloxacin residues generates free radicals and increases oxidative stress, resulting in cellular enzyme inhibition owing to glutathione peroxidase decrease (GSHPx). In the current study, GSH concentration was considerably lower following enrofloxacin therapy compared to the control group. In agreement with the fact that GSH concentration was significantly lower after enrofloxacin treatment compared to the control group in the current study (Sai ram *et al*., 2002 [38], Alia *et al*., 2006, [2, 3] Anila *et al*., 2006), *Emblica officinalis* (Amla) normalised glutathione peroxidase levels by increasing antioxidant status in the oxidative state. When the findings of the current inquiry are considered collectively, it is clear that they support the conclusions of the previous researchers.

Catalase: Catalase was calculated in terms of µmol H_2O_2 decomposed/min/gm Hb on day 28 of the experiment in albino rats. The concentration of CAT in control was 235.08±0.38 µmol H_2O_2 decomposed. However, enrofloxacin at dose of 5 mg/kg b.wt and 10 mg/kg b.wt. significantly decreased the concentration of CAT and the mean values were 157.83±0.48 and 131.57±0.20 µmol H_2O_2 decomposed, respectively. Significant amelioration of enrofloxacin-induced oxidative stress was shown by *Emblica officinalis* and was observed by increased CAT concentration in groups IV and V with mean values of 231.50±0.34 and 209.92±0.24, respectively.

According to Yazar and Tras (2001) [45], the metabolism of enrofloxacin residues generates free radicals and contributes to an increase in oxidative stress, resulting in cellular enzyme inhibition owing to a decrease in catalase (CAT). The current study's findings are consistent with Yazar and Tras (2001) [45], since CAT concentration was considerably lower in the enrofloxacin-treated group compared to the control group.

In arsenic-induced oxidative stress, Singh *et al.* (2014) [42] found that a crude aqueous extract of *Emblica officinalis* (Amla) enhanced catalase levels. When compared to data from rats treated with arsenic alone, amla co-treatment boosted activity by 77%, suggesting a protective effect against oxidative damage.

CONCLUSIONS: It is concluded that enrofloxacin at doses of 5 mg/kg body wt. and 10 mg/kg body wt., administered orally daily for 28 days in albino rats, induced liver and kidney impairment as indicated by increased concentrations of biochemical markers of liver and

kidney function, and at doses of 5 mg/kg body wt. and 10 mg/kg body wt., enrofloxacin significantly induced oxidative stress after subacute exposure. Aqueous extract of *Emblica officinalis* at 200 mg/kg b. wt., orally, daily for 28 days dramatically improved biochemical indicators of liver and kidney functioning, as well as oxidative stress indices.

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