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To study the safety of aqueous extract of *Emblica officinalis* on biochemical markers of liver and kidney function and oxidative stress indices in albino rats

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Abstract
The present study was performed in three groups of rats, consisting six rats in each group. The rats of group I were served as control. However, VI and VII were treated with aqueous extract of *Emblica officinalis* @ 200 mg/kg b. wt. and aqueous extract of *Emblica officinalis* @ 400 mg/kg b. wt., respectively. All the groups received medication orally, once daily for 28 days. *Emblica officinalis* did not alter the concentration of biochemical markers of liver function *viz*. ALT, AST, GGT, ALP, albumin and bilirubin as compared to control treated group. The concentration of biochemical markers of kidney function *viz*. BUN and creatinine was not alter after the administration of aqueous extract of *Emblica officinalis* as compared to control group. The safety profile of aqueous extract of *Emblica officinalis* was evaluated. The results of the present study indicated that the aqueous extract of *Emblica officinalis* @ 200 mg/kg b. wt. and 400 mg/kg b. wt. orally for 28 days did not alter the oxidative stress indices and biochemical markers of liver and kidney function as compared to control.

Keywords: *Emblica officinalis*, albino rats, oxidative stress

Introduction
Herbal products have a special place in the world of pharmaceuticals. Interests in the medicine of plant origin are spreading world-wide because of their safety, efficacy and cost effectiveness and negligible side effects. A number of plants have been mentioned in ayurveda for curing hepatic and renal diseases. The world health organization found that 80 percent of the world population depends on medicinal plant for their heath care needs, and more than 30 percent of the pharmaceutical preparations are based on plants (Shinwari and Khan, 1998) [16]. *Emblica officinalis*, commonly known as Indian gooseberry or Amla, belonging to family Euphorbiaceae, is a main herbal drug utilized in unani and ayurvedic system of medicine (Bhandari and Kamdod, 2012) [3]. The biologically and pharmacologically active chemical constituents isolated from fruits of *Emblica officinalis* are alkaloids (emblicanin A, emblicanin B), flavonoids (quercetin), gallic acid, tannoids *etc*. (Krishnaveni *et al.*, 2010) [10]. The fruits of *Emblica officinalis* contain higher amount of vitamin C and most of essential minerals and amino acids (Patel and Goyal, 2012) [12]. *Emblica officinalis* is reported to have hepatoprotective action against carbon tetrachloride induced hepatotoxicity (Deori *et al.*, 2017) [6] and nephroprotective action against cisplatin induced nephrotoxicity (Kalra *et al.*, 2017) [9]. It is also having antioxidant action against enrofloxacin induced oxidative stress (Rawal *et al*., 2014) [14]. *Emblica officinalis* have also been claimed to possess many other medicinal properties, such as anti-inflammatory, cardio protective, diuretic, laxative, stomachic, restorative, antipyretic and rejuvenating properties (Baliga and Dsouza, 2011) [2].

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 acclimatisation, 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 Department of Botany, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P).

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) [17]. 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
Eighteen rats were randomly divided into three groups with six rats in each group. The safety study of aqueous extract of *Emblica officinalis* was evaluated in two groups of rats that is group II and III. The experiment was conducted for 28 days.

Table 1: Design of experiment

Group	Treatment
I	Control
II	Aqueous extract of *Emblica officinalis* @ 200 mg/kg b. wt., once daily, orally for 28 days.
III	Aqueous extract of *Emblica officinalis* @ 400 mg/kg b. wt. once daily, orally for 28 days.

Collection of blood sample
Blood was collected on day 0 and day 28 from the retro-orbital plexus with the help of capillary tube as described by Archer and Riley (1981) [1]. Blood was collected in heparinised vials and used for biochemical and oxidative stress parameter study.

Biochemical Studies
Plasma was separated from heparinised blood samples and refrigerated at 4˚C for biochemical studies. The following biochemical markers of liver and kidney function were estimated by using Semi-auto analyzer with respective commercially available kits of ERBA, manufactured by Transasia Bio-Medicals Ltd., Daman.

1. Aspartate aminotransferase (AST) (IU/L)
2. Alanine transaminase (ALT) (IU/L)
3. Alkaline phosphatase (ALP) (IU/L)
4. Bilirubin (mg/dl)
5. GGT – Gamma glutamyl transpeptidase (U/L)
6. Albumin (g/dl)
7. Creatinine (mg/dl)
8. Blood urea nitrogen (BUN) (mg/dl)

Assessment of oxidative stress indices
After blood collection the samples were centrifuged at 2000 rpm for 15 min 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 per cent) in phosphate buffer saline (mM: NaCl, 136.9, KCl, 2.68; KH2·PO4, 1.47; and Na2·HPO4, 6.62; pH 7.4) and kept at 4 °C until further analysis. The 33 per cent packed erythrocytes were used for the estimation of LPO, GSH, Glutathione reductase, Catalase and Superoxide dismutase activity by using Helios double beam spectrophotometer. LPO and GSH were measured on the day of blood collection (Prins and Loos, 1969) [13].

Statistical Analysis
Means and standard error were obtained as per standard procedure. Parameters were analyzed by using the method of complete randomized design with seven treatments allotted to groups of six animals each. The difference between treatments was tested statistically for their significance (Snedecor and Cochran, 1994) [18].
Safety of *Emblica officinalis* was studied by evaluating its effect on biochemical markers of liver and kidney function and oxidative stress indices in albino rats. Safety profile study of *Emblica officinalis* was carried out by administering aqueous extract of *Emblica officinalis* in two doses i.e. 200 mg/kg b.wt. and 400 mg/kg b.wt. orally, for 28 days.

Biochemical Studies
In the present research work, biochemical study was carried out to study the alterations in biochemical markers of liver and kidney function on sub-acute exposure of *Emblica officinalis* in albino rats.

Biochemical markers of liver function
The mean values of biochemical markers of liver function *Viz.* Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma-glutamiltransferase (GGT), alkaline phosphatase (ALP), albumin and bilirubin in rats treated with *Emblica officinalis* have been presented in table (1).

ALT (SGPT)
The effect of *Emblica officinalis* on Alanine aminotransferase was calculated in terms of mg/dl of blood on day 28 of experiment in albino rats. The concentration of ALT in control was 79.75 ± 7.50 mg/dl of blood. The concentration of ALT in *Emblica officinalis* treated groups was 76.13 ± 0.10 mg/dl of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 73.0 ± 1.70 mg/dl of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of ALT in blood, as the values were non significant in comparison to control.

AST (SGOT)
The effect of *Emblica officinalis* on Aspartate aminotransferase was calculated in terms of mg/dl of blood on day 28 of experiment in albino rats. The concentration of AST in control was 159.98 ± 6.27 mg/dl of blood. The concentration of AST in *Emblica officinalis* treated groups was 161.67 ± 3.80 mg/dl of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 161.82 ± 3.37 mg/dl of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of AST in blood, as the values were non-significant in comparison to control.

GGT (Gamma Glutamyl Transferase)
The effect of *Emblica officinalis* on gamma glutamyl transferase was calculated in terms of IU/L of blood on day 28 of experiment in albino rats. The concentration of GGT in control was 7.07 ± 0.03 IU/L of blood. The concentration

of GGT in *Emblica officinalis* treated groups was 7.01± 0.3 IU/L of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 6.9 ± 0.07 IU/L of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of GGT in blood, as the values were non significant in comparison to control.

ALP (Alkaline Phosphatase)

The effect of *Emblica officinalis* on alkaline phosphatase was calculated in terms of IU/L of blood on day 28 of experiment in albino rats. The concentration of ALP in control was 449.50 ± 2.54 IU/L of blood. The concentration of ALP in *Emblica officinalis* treated groups was 448.67± 2.56 IU/L of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 448.67 ± 2.56 IU/L of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of ALP in blood, as the values were non significant in comparison to control.

Albumin

The effect of *Emblica officinalis* on albumin was calculated in terms of g/dl of blood on day 28 of experiment in albino rats. The concentration of albumin in control was 4.05 ± 0.03 g/dl of blood. The concentration of albumin in *Emblica officinalis* treated groups was 4.03± 0.03 g/dl of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 3.90 ± 0.08 g/dl of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of albumin in blood, as the values were non significant in comparison to control.

Table 2: Safety profile study of *Emblica officinalis* on biochemical markers of liver function in albino rats

Group	Treatment	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	ALP (IU/L)	Albumin (g/dl)	Bilirubin (mg/dl)
I	Control	79.75 ± 7.50	159.98±6.27	7.07± 0.03	449.50±2.54	4.05± 0.03	0.107 ±0.003
VI	Aqueous extract of *Emblica officinalis* @ 200 mg/kg b. wt., once daily, orally for 28 days.	76.13 ± 0.10	161.67 ± 3.80	7.01 ± 0.3	448.67± 2.56	4.03 ± 0.03	0.103 ±0.002
VII	Aqueous extract of *Emblica officinalis* @ 400 mg/kg b. wt. once daily, orally for 28 days.	73.0 ± 1.70	161.82 ± 3.37	6.9± 0.07	448.67 ±2.56	3.90 ± 0.08	0.100 ±0.003

Values are mean ±SE (n=6).
Values in rows are non- significant ($p< 0.01$)

Bilirubin

The effect of *Emblica officinalis* on bilirubin was calculated in terms of mg/dl of blood on day 28 of experiment in albino rats. The concentration of bilirubin in control was 0.107 ± 0.003 mg/dl of blood. The concentration of in *Emblica officinalis* treated groups was 0.103 ± 0.002 mg/dl of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 0.100 ± 0.003 mg/dl of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of bilirubin in blood, as the values were non significant in comparison to control.

Biochemical markers of kidney function

The mean values of biochemical markers of Kidney function *viz.* Creatinine and blood urea nitrogen in rats treated with enrofloxacin alone and combination of enrofloxacin with *Emblica officinalis* have been presented in table (03).

Creatinine

The effect of *Emblica officinalis* on creatinine was calculated in terms of mg/dl of blood on day 28 of experiment in albino rats. The concentration of creatinine in control was 0.640 ± 0.021 mg/dl of blood. The concentration of in *Emblica officinalis* treated groups was 0.680 ± 0.010 mg/dl of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 0 .650 ± 0.020 mg/dl of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of creatinine in blood, as the values were non significant in comparison to control.

BUN (Blood Urea Nitrogen)

The effect of *Emblica officinalis* on blood urea nitrogen was calculated in terms of mg/dl of blood on day 28 of experiment in albino rats. The concentration of blood urea nitrogen in control was 14.35 ± 0.23 mg/dl of blood. The concentration of in *Emblica officinalis* treated groups was 14.33 ± 0.22 mg/dl of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 14.33 ± 0.22 mg/dl of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of blood urea nitrogen in blood, as the values were non significant in comparison to control.

Table 3: Safety profile study of *Emblica officinalis* on biochemical markers of kidney function in albino rats

Group	Treatment	Creatinine (mg/dl)	BUN (mg/dl)
I	Control	0.640 ± 0.021	14.35± 0.23
VI	Aqueous extract of *Emblica officinalis* @ 200 mg/kg b. wt., once daily, orally for 28 days.	0.680 ± 0.010	14.33 ± 0.22
VII	Aqueous extract of *Emblica officinalis* @ 400 mg/kg b. wt. once daily, orally for 28 days.	0.650 ± 0.020	14.33 ± 0.22

Values are mean ±SE (n=6).
Values in rows are non- significant ($p< 0.01$)

During safety profile study, aqueous extract of *E. officinalis* @ 200 and 400 mg/kg, orally for 28 days did not cause any significant change in biochemical markers of liver and kidney function and there was no gross observational effect. Jeevraj *et al.* (2018) [8] reported that *Emblica officinalis* @ 600 mg/kg b.wt. produced no significant change in biochemical markers of liver and kidney function. Martin *et al.* (1981) [11] and Chakraverthy (1993) [4] found that high dose of *Emblica officinalis* (amla) at 1000 mg/kg b.wt. showed no significant changes in parameters of liver and kidney function after sub acute exposure in albino rats. Roe (1993) [15] did not found detrimental effects during the acute toxicity study of very high dosage of *Emblica officinalis*. These findings support the results of the present research.

Oxidative stress indices

The mean values of oxidative stress indices *viz*. Lipid peroxidation (MDA), Superoxide dismutase (SOD), reduced glutathione (GSH) and catalase in rats treated with *Emblica officinalis* have been presented in table (04).

Lipid Peroxidation (MDA)

The effect of *Emblica officinalis* on 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. The concentration of LPO in *Emblica officinalis* treated groups was 4.61 ±0.03 nM MDA/gm of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 4.56 ±0.03 nM MDA/gm of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of LPO in blood, as the values were non significant in comparison to control.

Table 4: Safety profile study of *Emblica officinalis* on oxidative stress indices in albino rats

Group	Treatment	Oxidative Stress Indices (Mean± SE)			
		MDA (nM MDA/gm)	SOD (U/g of Hb)	GSH (µmol /ml of blood)	Catalase (µmol H_20_2 decompose/min/gm Hb)
I	Control	4.63±0.02	1.22 ±0.01	340.67 ± 0.21	235.08 ± 0.38
VI	Aqueous extract of *Emblica officinalis* @ 200 mg/kg b. wt., once daily, orally for 28 days.	4.61 ±0.03	1.21 ±0.04	340.27 ± 0.16	234.83 ± 0.30
VII	Aqueous extract of *Emblica officinalis* @ 400 mg/kg b. wt. once daily, orally for 28 days.	4.56±0.03	1.20 ±0.04	340.08 ± 0.30	234.83 ± 0.30

Values are mean ±SE (n=6).

Values in rows are non- significant ($p< 0.01$)

SOD (Superoxide Dismutase)

The effect of *Emblica officinalis* on superoxide dismutase was calculated in terms of U/g of Hb in blood on day 28 of experiment in albino rats. The concentration of SOD in control was 1.22±0.01 U/g of Hb. The concentration of catalase in *Emblica officinalis* treated groups was 1.21 ±0.04 U/g of Hb *(E. officinalis* @ 200 mg/kg b.wt.) and 1.20 ±0.04 U/g (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of catalase in blood, as the values were non significant in comparison to control.

GSH (Reduced Glutathione)

The effect of *Emblica officinalis* on reduced glutathione was calculated in terms of µmol /ml of blood on day 28 of experiment in albino rats. The concentration of GSH in control was 340.67±0.21 µmol /ml of blood. The concentration of catalase in *Emblica officinalis* treated groups was 340.27± 0.16 µmol /ml of blood *(E. officinalis* @ 200 mg/kg b.wt.) and 340.08 ± 0.304 µmol /ml of blood (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of catalase in blood, as the values were non significant in comparison to control.

Catalase

The effect of *Emblica officinalis* on catalase was calculated in terms of µmol H_20_2 decomposed/min/gm Hb on day 28 of experiment in albino rats. The concentration of catalase in control was 235.08±0.384 µmol H_20_2 decomposed/min/gm Hb. The concentration of catalase in *Emblica officinalis* treated groups was 234.83 ± 0.304 µmol H_20_2 decompose/min/gm Hb *(E. officinalis* @ 200 mg/kg b.wt.) and 234.83 ± 0.304 µmol H_20_2 decomposed/min/gm Hb (*E. officinalis* @ 400 mg/kg b.wt.). *Emblica officinalis* in both the doses did not affect the concentration of catalase in blood, as the values were non significant in comparison to control.

During safety profile study, aqueous extract of *E. officinalis* @ 200 and 400 mg/kg, orally for 28 days did not cause any significant change in oxidative stress parameters (CAT, SOD, LPO, and GSH) and there was no gross observational effect. Golechha *et al*. (2014) [7] showed that the two doses of *E. officinalis* i.e. 200 mg/kg and 400 mg/kg were selected on the basis of an initial toxicity study where the rats appeared healthy without any visual signs or symptoms of illness for one month of administration. Swetha and Krishna (2014) [19] indicated that high doses of *Emblica officinalis* (Amla) caused no significant change in oxidative status of rat. Chatterjee *et al*. (1999) [5] suggested that high doses of *Emblica officinalis* for 30 days produced no toxic effects and no changes were found in oxidative stress indices. These findings are in agreement with the results of present research.

References

1. Archar RK, Riley J. Standardized method of bleeding rats. Laboratory Animals. 1981;15:25-28.
2. Baliga MS, Dsouza JJ. Amla (*Emblica officinalis* Gaertn), a wonder berry in the treatment and prevention of cancer. European Journal of Cancer Prevention, 2011;20:225-39.
3. Bhandari PR, Kamdod MA. *Emblica officinalis* (Amla): a review of potential therapeutic applications. International Journal of Green Pharmacy. 2012;6(4):257-269.
4. Chakravarthy BK. Herbal medicines safety and efficacy guidelines. Journal of Regular Affairs. 1993;4:699-701.
5. Chatterjee A, Ghosal S, Bhattacharya SK. Antioxidant activity of tannoids principles of Emblica officinalis (Amla). Indian Journal of Experimental Biology. 1999;38:877-880.
6. Deori C, Das S, Bordoloi SK. Study of hepatoprotective activity of Emblica officinalis (amla) in albino rats. Journal of Evidence Based Medicine and Healthcare. 2017;4(54):3298-3301.
7. Golechha M, Sarangal V, Ojha S, Bhatia J, Arya DS. Anti-inflammatory effect of *Emblica officinalis* in rodent models of acute and chronic inflammation: Involvement of possible mechanisms. International Journal of Inflammation. 2014;21(3):178-408.
8. Jeevraj K, Kalaivani A, Shanmugapriya P, Madhavan R. Toxicological Profile on Ayapodielagam - A Siddha Herbomineral Formulation in Wister Albino Rats.

International Journal of Ayurveda and Pharmacological Research. 2018;6(12):8-16.
9. Kalra P, Karwasra R, Nag TC, Gupta YK, Singh S. Protective effect of *Emblica officinalis* fruit extract on cisplatin induced nephrotoxicity in female rats. Bulletin of faculty of pharmacy. 2017;4(1):1-9.
10. Krishnaveni M, Swathi A, Mirunalini S. Therapeutic potential of Phyllanthus emblica (amla): the ayurvedic wonder. Journal of Basic Clinical Physiology and Pharmacology. 2010;21:93-105.
11. Martin DW, Mayes PA, Rodwell YW. Transaminase, in harpers review of biochemistry, 18th edition, Lange Medical, CA, 1981, 61pp.
12. Patel SS, Goyal RK. *Emblica officinalis* Geart: A Comprehensive Review on Phytochemistry, Pharmacology and Ethnomedicinal Uses. Research Journal of Medicinal Plant. 2012;6:6-16.
13. Prins HK, Loos. Biochemical methods in red cell genetics. Yunis, J.J. Glutathion. Publ. Academic press. NY, 1969, 115-137pp.
14. Rawal S, Singh P, Gupta A, Mohanty S. Dietary intake of *Curcuma longa* and *Emblica officinalis* increases life span in Drosophila melanogaster. Journal of Biomedical Research International. 2014;14(2):1-7.
15. Roe FJ. Influence of animal species, strain, age, hormonal, and nutritional status, in Experimental Toxicology, The Basic Issues, 2nd Edition, Anderson D and Conning D (Editors) (Cambridge: The Royal Society of Chemistry), 1993, 23-34pp.
16. Shinwari MI, Khan P. Indigenous use of medicinal trees and shrubs of Margalla Hills National Park, Islamabad. Pakistani Journal of Forest. 1998;48:63-90.
17. Shukla D. Studies on pharmacological action of *Boerhaavia diffusa* with special reference to its antibacterial activity in rats. M.V.Sc. & A.H. thesis (Pharmacology and Toxicology), Nanaji Deshmukh Veterinary Science University, Jabalpur, 2006.
18. Snedecor GW, Cochran WG. Statistical Methods, 8th Edn., The lowa State College Press, Inc. USA, 1994, 950pp.
19. Swetha D, Krishna T. Current Trends in the Research of Emblica officinalis (Amla): A Pharmacological Perspective, International Journal of Pharmaceutical Science. 2014;24(2):150-159.