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Research Article

RENAL PROTECTION BY ISOLATED PHYTOCOMPOUNDS FROM *TERMINALIA ARJUNA* BARK FRACTION ON DEHYDARATION INDUCED UREMIA, OXIDATIVE STRESS AND KIDNEY DISEASE RATS

Koushik Das[1]., Suchismita Roy[1]., Shreya Mandal[1]., Shrabani Pradhan[1]., Sukumar Debnath[2]., Debidas Ghosh[3] and Dilip Kumar Nandi[1]*

[1]Department of Nutrition and Physiology, Raja N. L. Khans Women's College, Midnapore, Pin-721102, West Bengal. India
[2]Department of Traditional Foods and Sensory sciences, Central Food Technological Research Institute (CFTRI), Mysore
[3]Department of Biomedical Laboratory Science and Management, (UGC Innovative Programme Funded Department), Vidyasagar University, Paschm Medinipur-721102, West Bengal. India

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ABSTRACT

Background: *Terminalia arjuna* (TA) is a native plant of India and South East Asia and has been traditionally used as a cardio protective agent included in Charka Samhita and Astang Hridayam. The bark of this plant had used in many ancient Indian medicinal literature including claiming treating diabetes, obesity, potent lipid lowering and antioxidant activity.

***Aims of the study*:** The present study was evaluated the protective effect of bark of TA on dehydration induced uremia and oxidative stress in male Wister strain albino rats. The study has been designed mainly to search out which was the most effective dose of methanol fraction from bark of TA could reduce oxidative stress and uremia of said experimental rats.

***Materials and Methods*:** Thirty six male Wister strain albino rats (n=6 rats/group) were administered 4 ml water / 24 hrs interval for 15 days experimental period. Out of 6 groups, 1 control and 5 dehydration group (1 group without treatment and another 4 group's treatment with 10, 20, 40 and 80 mg / kg / day of methanol fraction bark of said plant part). Thin Layer Chromatography (TLC) and High Performances Liquid Chromatography (HPLC) were performed to identify the methanol fraction of bark of TA.

***Results*:** Water deprivation for 15 days showed a significant elevation in the level of blood urinary nitrogen (BUN-urea and creatinine), increased the levels of free radicals like malondialdehyde (MDA) and conjugated dienes (CD) along with significant diminution in the activities of superoxide dismutase (SOD) catalase (CAT) and peroxidase (Px) in kidney tissues which were protected significantly after coadministration daily orally 10-80 mg / kg / day of methanol fraction bark of said plant and were also corrected the level of blood nitrogenous end products to normal level. Beside this methanolic extract was played the most crucial role to correct the uremia and oxidative stress. By the phytocompounds analysis using TLC and HPLC, identified the purified fractions of bark of *Terminalia arjuna* contains three major phytocompounds, which have capability to control uremia and oxidative stress.

Conclusion: The results suggested that dehydration induced oxidative stress and uremia on male rats might be protected by using above mentioned medicinal plant methanol fraction and also had no toxic effect on blood and kidney by the measurement of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities.

INTRODUCTION

Now a day, kidney diseases possess the leading cause of death in the world and people are affecting from this silent killer disease by mainly hypertension and diabetes. Epidemiological surveys have been shown that the prevalence of hypertension and diabetes has raised day-by-day and kidney diseases also raised competitively. There is great urgency for a

*Corresponding author: *Dilip Kumar Nandi*
Department of Nutrition and Physiology, Raja N. L. Khans Women's College, Midnapore, Pin-721102, West Bengal. India

nonconventional, affordable therapy for patients who cannot afford expensive dialysis or kidney transplant to keep them alive. In this connection, we have been searching the different anti-uremic and nephroprotective phytocompound from different plant parts such as root of *Asparagus racemosus* (Roy *et al*., 2013), bark of *Terminalia arjuna* (Das *et al*., 2010a), root of *Withania somnifera* (Das *et al*., 2010b) which had been effectively played as medicine on experimentally induced uremic and oxidative stress rats. Beside this in our laboratory, we also established an alternative therapy like alpha lipoic acid (Pradhan *et al*., 2013) and probiotic (Mandal *et al*., 2013) had been shown excellence nephroprotective activity against acetaminophen induced renal failure male rats. In continuation of research in our laboratory on the antiuremic and antioxidative activity (Das *et al*., 2010a, 2011) of *Terminalia arjuna* (TA) (Family- Combretaceae) is a deciduous large-sized fluted tree to 30 m tall and 2-2.5 m, with an often buttressed trunk. This tree is usually an evergreen tree with new leaves appearing in the hot season (February to April) before leaf fall. It is one of the most versatile medicinal plants having a wide spectrum of biological activity. It is an important medicinal plant widely used in the preparation of ayurvedic formulations for over three centuries primarily as a cardiac tonic in India and worldwide (Dwivedi *et al*., 1997). Clinical evaluation of this plant indicates that it can be of benefit in the treatment of coronary artery diseases, heart failure and possibly hypercholesterolemia and renal disease (Mukerji, 1953; Dwivedi and Udupa, 1989; Kumar and Prabhakar, 1987; Bharani *et al*., 1995; Dwivedi *et al*., 1997; Sumitra *et al*., 2001). Ancient Indian physicians used the powdered tree bark of *Terminalia arjuna* (TA) Wight & Arn. for alleviating "hritshool" (angina) and other cardiovascular conditions. TA (Rox b.) W. & A. (Family: Combretacae) is a large tree distribution throughout India, its bark is extensively used in ayurvedic medicine (Pawar and Bhutani, 2005). The aqueous extract of the bark of TA could protect the liver and kidney tissues against CCl4-induced oxidative stress probably by increasing antioxidative defense activities (Manna *et al*., 2006). Aqueous extract of TA protects kidney against CCl_4 induced renal damage (Raghavan and Krishna, 2006). The aqueous extract of the bark of TA showed optimum protective activity against NaF-induced oxidative damages in kidney (Sinha *et al*., 2007). Ethanol extract of TA exhibited renal protection (Adeneye, 2008), used for treatment of coronary artery diseases, heart failure and possibly hypercholesterolemia and renal disease (Das *et al*., 2010a). In our earlier work, aqueous bark extract have been shown antiuremic and antioxidative properties (Das *et al*., 2010b), but methanol bark extract of TA showed better protection than these solvent water, chloroform, ethyl acetate crude extracts (Das *et al*., 2011). So, the present study was aimed to detect effective dose of methanol fraction(s) from bark of TA, which control oxidative stress and uremia in rats. Best fraction(s) would be purposed for characterization and compound identification by GC MS and NMR study in future in our laboratory.

MATERIALS AND METHODS

Reagents

Major biochemical parameters were measured by Semiautoanalyser (Mearck, Microlab 150), so, diagnostic kits like urea, creatinine, total cholesterol, HDL cholesterol, triglycerides, SGOT, SGPT, ALP supplied by Merck LTd., Mumbai, India. Flavonoids like Querecetin and sodium galate were purchased from Sigma-Aldrich, India. All other chemicals were purchased from SRL, India and MERCK, India. sd FINE-CHEM Limited, India, HiMedia Laboratories Pvt. Ltd. Mumbai. India and Crest Biosystems Goa. India.

Instruments

Biochemical parameters were measured by Semiautoanalyser (Mearck, Microlab 150), others biochemical parameters were measured by absorbance UV-VIS Spectrophotometer (Systronics, India), Plant material were extracted by using Electrical Blender (Jaipan, India), Sox let apparatus (Yoma, India), Rotary evaporator with Chiller (Yoma, India), Microcentrufuge (Remi, India), BOD incubator, Incubator with shaker (Indian Instruments Ltd.), Freeze dryer-Lypolyser (Indian Instruments Ltd.). All materials weighted by Digital weight balance (Accuracy-0.1mg) Adhair Dutta and Sons. PH was measured by pH Meter (Mettler, India). Blood Sodium and potassium were measured by Electrolyte Analyser (Systronics India). Thin Layer Chromatography analysis was done by TLC plates (Merck, 20 cm x 20 cm) and High Performance Liquid Chromatography was performed by Perkin Elmer, Switzerland).

Chemicals

Bark of TA was collected from university road, Vidyasagar University, Midnapore, Paschim Medinipur, district of West Bengal. The material was identified by the taxonomist of the Botany Department at the Raja N. L. Khan Women's College, Midnapore. The voucher specimens were deposited in the Department of Botany, Raja N. L. Khan Women's College, voucher specimens were deposited in the Herbarium of Botany. The methanol bark crude extract of TA was prepared by the method of our earlier work (Das *et al*., 2011) and 25 g methanol extract was dissolved in 500 ml diethyl ether for 2 hours in a sox let apparatus. The extract was filtered through Whatman No.1 filter paper and the resulting filtrate was dried in the air. In the preparative TLC of the ether soluble part does not give any spot in chromatogram, therefore, the ether solid extract was dissolved in 300 ml acetone for 1 hour in a sox let apparatus. Then the extract was filtered through Whatman No.1 filter paper and the resulting filtrate was dried under reduced pressure at 40 λ C on a rotary evaporator. The acetone solid extract was further dissolved in 200 ml methanol and was dried in the air. The methanol fraction was subjected to TLC, three different bands were obtained and the Rf value of the bands were also determined (Singh *et al*., 2010). The methanol fraction was also subjected to HPLC analysis by the method of Singh *et al*., 2010. Finally, the methanol fraction was formulated named as MFTA (methanol fraction of bark of *Terminalia arjuna*) stored in desiccators.

Animal model and administration of methanol fraction

The study was conducted on 46 healthy, adult, male (41) and female (5) albino rats of Wister strain (Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata 54) having a body weight of 100 ±15 g. They were acclimatized to laboratory condition for 2 weeks prior to experimentation.

Animals were housed single rat/cage in a temperature-controlled room (22± 2^0C) with 12–12 h dark–light cycles (8.00–20.00 h light, 20.00– 8.00 h dark) at a humidity of 50 ±10%. They were provided with standard food and water *ad libitum* throughout the experimental period. Animal care was provided according to the Guiding Principle for the Care and Use of Animals (Olfert *et al*., 1993). This experiment was approved by our Institutional Animal Ethical Committee (**IAEC/05/2013 dt.20.06.2013**), guidelines followed by CPCSEA.

Acute toxicity study of MFTA

An acute toxicity of MFTA was conducted using acute toxic class method as per Organization of Economic Co-operation and Development (OECD) guidelines 425 **(OECD, 2001)** where the limit dose of 2000 mg/kg body weight was used. Ten healthy Wister strain rats (n=5) of either sex selected by random sampling technique were employed in this study. Wellness parameters of animals were made and recorded systematically 30 min, 4 hour, 24 hour and 48 hour after dose administration for skin and fur, eyes, mucus membrane, behavioral pattern changes, tremor, convulsions, salivations, diarrhea, lethargy, sleep and mortality.

Dehydration protocol

Animals were randomly placed in 1 rat /cage with free access with dry food (pellet diet) and adequate water and daily measured water intake /rat. Dehydration was achieved by withdrawing the drinking water bottle for 24 hrs and by providing two ml water to each rat after interval of 24 hrs for 30 days dehydration period of experimentation (Das *et al*., 2010a, 2010b, 2011).

Dose dependent study of MFTA

The main test was conducted on 36 healthy male albino rats (n=6). The rats were randomized in to 6 groups (each group had 6 rats): untreated control, untreated dehydration and 4 dehydration treated with MFTA on the basis of different doses: high, medium, sub-medium and low dose groups:

Untreated control group (UCG): animals were subjected to feed dry food (pellet diet) and adequate amount of water. Rat of this group received 0.5 ml olive oil/ day for 15 days of experimentation through forceful oral route at 8.00 h. through gavages.

Untreated dehydration group (UDG): initially rats were supplied with normal diet and adequate amount of water for first 15 days of experimentation then these rats were induced to dehydration (according to dehydration protocol) for 15 days of experimentation and fed olive oil as same pattern of UDG. Dehydration and treated with low dose of MFTA (D MFTA 10): rats were subjected to dehydration and fed MFTA at the dose of 10mg / kg body weight / day / rat in 0.5 ml olive oil for 15 days. Dehydration and treated with sub-medium dose of MFTA (D MFTA 20): rats were subjected to same treatment with 20 mg MFTA. Dehydration and treated with medium dose of MFTA (D MFTA 40): rats were subjected to same treatment with 40 mg MFTA. Dehydration and treated with high dose of MFTA (D MFTA 80): rats were subjected to same treatment with 80 mg MFTA.

Thin layer chromatography analysis

About 2 µg of MFTA was loaded on TLC plates (Merck, 20 cm x 20 cm). The plates were developed in toluene: chloroform: methanol (4:4:1, v/v/v) to separate compounds of the extracts. The developed plate was air dried. Then anisaldehyde sulfuric acid was sprayed on the surface of the plate and incubated for 20 min at 100^{o}c. The present compound of this extract was detected as blue spot on developed TLC plate. The Rf values of the bands were also determined.

HPLC analysis

HPLC of phytocompound isolated from methanolic fraction of *Terminalia arjuna* bark run in HPLC chromatogram profile [C_{18}, su (250X 4.6 mm) reverse phase (like; Thermo ODS-2 250X 4.6, %um) UV Detector 205 (nm) Flow rate: 1.5 ml/ min]; Solvent system: anhydrous potassium dihydrogen orthophosphate with ortho-phosphoric acid (A) and Acetonitrile (B) in mobile phase.

Blood sodium, potassium and plasma uremic markers of renal damage

Rats of each group were individually housed in metabolic cages for 24 h and blood samples were collected on 16th day of treatment from overnight fasted animals through retro-orbital sinus puncture in ethylene diamine tetra acetic acid coated vials. Sodium and potassium was measured by direct blood sample using electrolyte analyser, systronics India by manufacturer instruction and plasma was separated by cold centrifugation of vial at 3000 rpm for 10 min. Urea and creatinine were assayed in plasma using commercially available kits using Merck micro lab L 150 semi-autoanalyser according to the instruction of the manufacturer.

Red blood cells and haemoglobin for hematological parameters of uremia

Blood was used to analyze of hematological parameters like total red blood cells by hemocytometer and hemoglobin by standard kit method (Merck, Japan).

Preparation of homogenate of renal tissue

Kidneys were excised and homogenized in chilled Tris buffer (10mM, pH 7.4) at concentration of 10 % (w/v) using Remi tissue homogenizer in a serrated pestle. Homogenates were then centrifuged at 10,000 rpm for 20 min in a high speed cooling centrifuge and supernatant were used for further biochemical estimations.

Measurement of Lipid Peroxidation from the levels of Malondialdehyde (MDA)

Measurement of malondialdehyde was done using thiobarbituric acid assay as per Ohkawa *et al*., 1979 The MDA in sample was calculated by using the extinction coefficient of 1.56x 105 M/cm and expressed in the unit of nM/mg of tissue or nM/ml of plasma.

Measurement of renal antioxidants

Catalase activity was measured biochemically in tissue supernatant by the method of Beers and Sizer, 1952 using spectrophotometer and reading of absorbance was noted at 240 nm. Biochemical assay of superoxide dismutase (SOD) using supernatant by measuring the percentage of inhibition of the

pyragallol (HIMEDIA, India) auto oxidation by SOD according to the Marklund and Marklund, 1974. One unit of SOD was defined as the enzyme activity that inhibited the autooxidation of pyragallol by 50 percent.

Statistical analysis

Data were expressed as mean ± SE (n=6). ANOVA followed by Bonferroni multiple two-tail t-test to detect inter group differences and bars with different superscripts (a, b, c) differ from each other significantly ($p< 0.05$) (Sokal *et al*., 1997).

RESULTS

TLC analysis for Methanol extract of TA bark

The plates TLC were developed in Chloroform: Toluene: Methanol (4:4:1) and sprayed with anisaldehyde sulfuric acid reagent. It gives three spots of the TA bark extract with Rf value of 0.25, 0.32, and 0.38 **(Fig 1)**. The eluted compounds showed blue color represents single compound. The 3 different spots were obtained and compounds were collected separately named as Fractioned-1, Fractioned-2 and Fractioned-3, and was stored in refrigerator for future investigation of their chemical composition.

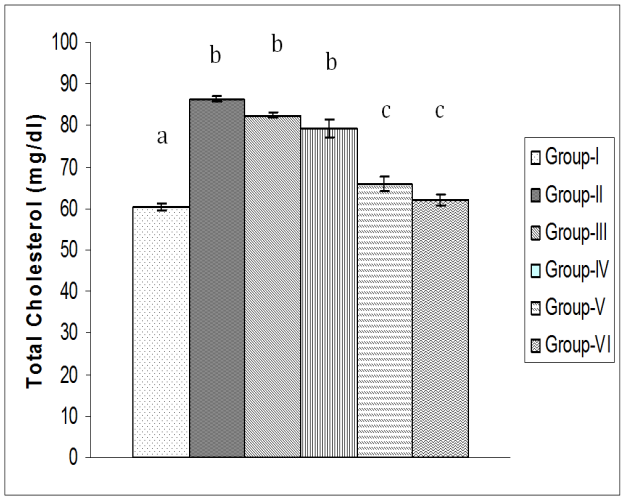

Fig 1 Effect of different doses of methanol fraction of *Terminalia arjuna* on total cholesterol (mg/dl) of dehydration induced oxidative stress and uremia condition in rats. Data are expressed as mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and bars with different superscripts (a, b, c) differ from each other significantly ($p< 0.05$).

UCG – Untreated Control Group,
UDG- Untreated Dehydration Group,
D MFTA 10- Dehydration with methanol fraction of *Terminalia arjuna* 10 mg/ kg body weight.
DMFTA 20- Dehydration with methanol fraction of *Terminalia arjuna* 20 mg/ kg body weight.
DMFTA 40- Dehydration with methanol fraction of *Terminalia arjuna* 40 mg/ kg body weight.
DMFTA 80- Dehydration with methanol fraction of *Terminalia arjuna* 80 mg/ kg body weight.

HPLC analysis for Methanol extract of TA bark

Quantification and isolation of phytocompounds in methanolic fraction of *Terminalia arjuna* (TA) bark through HPLC technique. In this result **(Fig 2)** the calibration curve of concentration versus detector response (peak area) develops a highest peak, number 7 (Height 57.265%) and the retention time is 24.919 min, where 1-6 and 8-12 peaks may not the active compounds and required more purification steps. The highest peak indicated that the phytocompound is present in this area in huge concentration and this compound has more protective effect to reduced uremia and oxidative stress.

The analysis of the phytocompounds were identified from purified fractions of bark of *Terminalia arjuna* and showed that in TLC, 3 major bands and in HPLC, 1 major peak and 9 minor peaks depending on the concentration of phytocompounds.

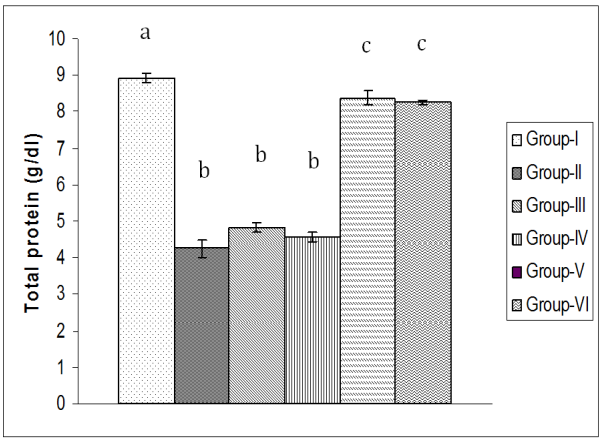

Fig: 2 Effect of different doses of methanol fraction of *Terminalia arjuna* on total protein (g/dl) of dehydration induced oxidative stress and uremia condition in rats. Data are expressed as mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and bars with different superscripts (a, b, c) differ from each other significantly ($p< 0.05$).

UCG – Untreated Control Group,
UDG- Untreated Dehydration Group,
DMFTA 10- Dehydration with methanol fraction of *Terminalia arjuna* 10 mg/ kg body weight.
DMFTA 20- Dehydration with methanol fraction of *Terminalia arjuna* 20 mg/ kg body weight.
DMFTA 40- Dehydration with methanol fraction of *Terminalia arjuna* 40 mg/ kg body weight.
DMFTA 80- Dehydration with methanol fraction of *Terminalia arjuna* 80 mg/ kg body weight.

Acute toxicity study purified fractions from TA

The high doses purified fractions (2000mg/kg bodyweight of rat) did not produce any sign of toxicity and mortality. At the doses used, the plant extract did not significantly affect the body weights of treated rats or weight of the kidneys relative to body weights. It revealed that the extract at the doses caused no adverse effects on feed intake or metabolism. Therefore, the approximate LD_{50} should be above 2000mg/kg bodyweight of rat. Healthy Wister strain rats (n=6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only.

Table 1 Effect of different doses of methanol fraction of *Terminalia arjuna* on body weight of dehydration induced oxidative stress and uremia condition in rats. Data are expressed as mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b) in a specific vertical column differ from each other significantly ($p< 0.05$).

Groups	Initial body weight (g)	Final body weight (g)	Elevation/diminution in body growth (g%)
UCG	109.3±5.5^a	143.7±6.0^a	34.4
UDG	108.4±2.3^a	122.4±4.7^b	14.0
DMFTA 10	108.4±2.3^a	122.4±4.7^b	14.0
DMFTA 20	107.3±1.3^a	120.2±5.3^b	12.9
DMFTA 40	109.8 ±4.2^a	142.5 ±3.5^a	32.7
DMFTA 80	106.2±4.1^a	136.7±7.1^a	30.5

Untreated control group (UCG), Untreated dehydration group (UDG),
DMFTA10- Dehydration with methanol fraction of *Terminalia arjuna* 10mg/ kg body weight.
DMFTA20-Dehydration with methanol fraction of *Terminalia arjuna* 20mg/ kg body weight.
DMFTA40-- Dehydration with methanol fraction of *Terminalia arjuna* 40mg/ kg body weight.
DMFTA80-- Dehydration with methanol fraction of *Terminalia arjuna* 80mg/ kg body weight.

The dose up to 2000 mg/kg was well tolerated without producing any alteration in gross behaviour, signs of toxicity and mortality. Thus all the doses of TA were found to be non-toxic. Four doses (10, 20, 40 and 80 mg/kg) of TA were selected for further studies.

Dose dependent study

Body Weight

Body weight was significantly decreased at the end of experiment in only dehydrated animals (UDG) in comparison to control groups (UCG) (Table: 1). Co administration of extracts at four different doses to dehydrated animals for 30 days result a significant increase body weight towards control groups (Table 1).

RBC and Hemoglobin

In this present study it was observed that dehydrated animals (UDG) for 15 days significantly decreased Hb level (21.72%) and RBC count (36.18%) compared with UCG. After co-administration of MFTA extract at the different doses (like 100mg, 200mg and 400mg and 800mg/kg body weight /day) for 15 days, Hb levels were significantly higher as .53%, 11.92%, 20.89% and 26.33% compared with UDG. Total RBC counts were also improved with MFTA extract at the different doses (like 10mg, 20mg and 40mg and 80mg/100g body weight /day) for 15 days like. 60%, 30.26% and 19.03% and 9.01% respectively compared with UDG (Table 2).

MFTA 40) showed better result in comparison to other doses (D MFTA10, D MFTA 20 and D MFTA 80) (Table 2).

Urea and Creatinine Level

Urea and creatinine increased in blood of UDG rats (66%) compared to UCG rats (Table. 2). In dehydrated rats, 30 days of dehydration was resulted in a significant elevation in the values of both the parameters in blood. The levels of above uremic parameters like Urea decreased significantly (2.12%, 22.47%, 60.37% and 62.63%) and creatinine (6.89%, 30.17%, 73.27% and 63.79%) in co administration of MFTA extracts in D MFTA10, D MFTA 20, D MFTA 40 and D MFTA 80 in respect to dehydrating animals (UDG) respectively. It was also revealed that the levels of above blood constituents decreased significantly in D MFTA 400 group rats in comparison to other extracts treated groups (Table 2).

Electrolytes Alteration

Electrolytes mainly sodium and potassium altered in different groups. Na is significantly increased (47.22%) and K decreased significantly (58.96%) in UDG rats compared with UCG group and this values are resettled in the group D MFTA10, D MFTA 20, D MFTA 40 and DMFTA 80 but the highest activities showed in the DMFTA 40 group rats (Table 2).

Table 2 Effect of four different doses of isolated fractions from the bark of *Terminalia arjuna* on blood electrolytes, plasma uremic markers, hematological parameters, Lipid Per oxidation, antioxidative enzymes and kidney tissue toxicity of dehydration induced oxidative stress and uremic rats. Data are expressed as mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b,c and d) in a specific vertical column differ from each other significantly ($p< 0.05$).

Parameters	Groups					
	UCG	UDG	DMFTA 10	DMFTA 20	DMFTA 40	DMFTA 80
RBC /cumm×1000000**.**	7.82 ± 0.53^a	4.99 ± 0.84^b	4.96 ± 0.75^b	6.50 ± 0.88^c	5.94 ± 0.93^c	5.44 ± 0.63^c
Hemoglobin (g%)	7.18 ± 0.25^a	5.62 ± 0.56^b	5.59 ± 0.82^b	6.29 ± 0.9^c	7.12 ±1.12^a	7.10 ± 0.54^a
Catalase (mmol of H_2O_2 consumption/ mg of tissue/min)	0.58±0.02^a	0.22±0.07^b	0.23±0.08^b	0.19±0.10^b	0.52±0.06^a	0.56±0.04^a
SOD (mmol of H_2O_2consumption/ mg of tissue/min)	1.40±0.08^a	0.49±0.08^b	0.50±0.07^b	0.43±0.11^b	1.38±0.05^a	0.58±0.07^c
Urea (mg/dl of plasma)	25.4±0.7^a	75.2±0.8^b	73.6±0.7^b	58.3±1.2^c	29.8±0.5^a	28.1±1.93^a
Creatinine (mg/dl of plasma)	0.29±0.2^a	1.16±0.6^b	1.08±0.5^c	0.81±0.22^d	0.31±0.4^a	0.42±0.13^e
Sodium (mmol/l of plasma)	122.68±6.27^a	180.61±10.3^b	178.53±12.5^b	173.44±16.24^b	143.25±11.25^c	138.53±13.5^c
Potassium (mmol/l of plasma)	4.46±0.36^a	1.83±0.32^b	1.82±0.13^b	2.58±0.63^c	3.92±0.57^d	4.62±0.45^a
GOT (U/ mg of tissue)	23.6±0.2^a	12.9±0.1^b	14.3±0.6^b	13.2±0.5^b	15.3±0.1^b	14.3±0.4^b
GPT (U/ mg of tissue)	23.5±0.2^a	25.1±0.5^a	24.6±0.7^a	24.2±0.2^a	28.4±0.6^a	24.5±0.8^a
MDA (nmol/ mg of tissue)	70.22±5.04^a	120.13±6.34^b	118.24±9.53^b	115.08±9.63^b	83.23±8.86^c	74.52±11.93^a
CD (nmol H_2O_2 / mg of tissue)	290.48±9.05^a	419.25±12.54^b	422.63±10.43^b	389.16±10.32^c	323.53±9.97^d	284.34±8.23^a
ALP (U/l of plasma)	113.27±7.34^a	375.53±13.67^b	372.18±12.26^b	371.42±14.34^b	231.28±7.38^c	286.44±9.74^d

Untreated control group (UCG), Untreated dehydration group (UDG), Dehydration and treated with low dose of MFTA (D MFTA 10), Dehydration and treated with sub-medium dose of MFTA (D MFTA 20), Dehydration and treated with medium dose of MFTA (D MFTA 40), Dehydration and treated with high dose of MFTA (D MFTA 80). D: Dehydration, MFTA: Methanol fractions of *Terminalia arjuna.*10, 20, 40, and 80 indicates the dose of MFTA fractions per kg body weight of rat.

Activities of Catalase and SOD

CAT and SOD activities were significant reduced in plasma (88.88% and 45.55%) and kidneys (62.06% and 65%) in UDG (dehydrated animals) compare to UCG. The activities of catalase and SOD in blood and kidneys were recovered significantly after co-administration of MFTA extract at different doses (10, 20, 40 and 80mg/kg body weight/day), but MFTA extract at the dose of 40mg/kg body weight/day (D

Activities of SGOT and SGPT

In serum and kidneys, the GOT levels were slightly changed in UDG group compared to UCG rats and extract treated groups (Table 2). After co-administration of extracts, a slight recovery was noted in above parameters in case of the control levels.

Quantification of MDA and CD

Quantity of MDA increased (63.60% and 41.45%) in plasma and kidney of UDG groups compared to control Groups (UCG)

(Table.2). In dehydrated rats, 30 days of dehydration result a significant elevation in the values of both the parameters in blood and kidney. The quantities of above enzyme MDA decreased significantly (1.32%, 4.60%, 62.87% and 60.20%) in co administration of MFTA extracts in group D MFTA10, D MFTA 20, D MFTA 40 and D MFTA 80 in respect to dehydrating animals (UDG) res. It was also seen that quantities of above enzymes decreased significantly in D META 40 groups compare to other extracts treated groups (Table 2).

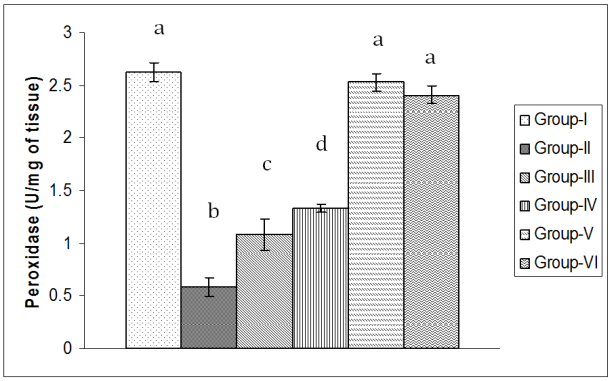

Fig 3 Effect of different doses of methanol fraction of *Terminalia arjuna* on peroxidase in kidney of dehydration induced oxidative stress and uremia condition in rats. Data are expressed as mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and bars with different superscripts (a, b, c, d) differ from each other significantly ($p< 0.05$).

UCG – Untreated Control Group,
UDG- Untreated Dehydration Group,
D MFTA 10- Dehydration with methanol fraction of *Terminalia arjuna* 10 mg/ kg body weight.
DMFTA 20- Dehydration with methanol fraction of *Terminalia arjuna* 20 mg/ kg body weight.
DMFTA 40- Dehydration with methanol fraction of *Terminalia arjuna* 40 mg/ kg body weight.
DMFTA 80- Dehydration with methanol fraction of *Terminalia arjuna* 80mg/ kg body weight.

Table 3 Identification of three major compounds present in ***Terminalia arjuna*** **bark methanolic fraction** by TLC.

Extract	Solvent System	Revealing reagent	No. of Spot	Rf Value	
Methanol	Chloroform: Toluene: Methanol (4:4:1,v/v/v)	anisaldehyde sulfuric acid	3	a.	0.25
				b.	0.32
				c.	0.38

Levels of ALP

The present study showed that the activities of ALP were increased with hepatotoxicity due to oxidative stress. The ALP was significantly higher as 69.73% dehydrated group compared with control group. Oral -administration of MFTA extracts in D MFTA10, D MFTA 20, D MFTA 40 and D MFTA 80 reduces the ALP levels at the different doses (like 10mg, 20mg and 40mg and 80 mg/kg/day) as 89%, 1.09% , 38.41 % and 23.72% respectively compared with dehydrated animals (UDG) (Table 2).

Phyto compound isolation by HPLC and TLC analysis

The present study was conducted Thin Layer Chromatography (TLC) and High Performances Liquid Chromatography (HPLC) of methanol fraction of bark of *Terminalia arjuna* to identify and quantify the phytocompounds. There were three fractions developed in TLC plate, but in HPLC analysis major quantitatively single compound present in Methanol Fractions of *Terminalia arjuna* (MFTA) (Fig: 4 and 5).

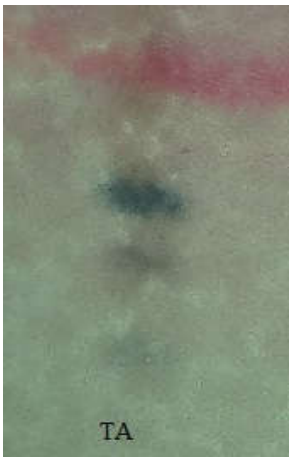

Fig 4 TLC analysis for Terminalia arjuna bark methanolic fraction.

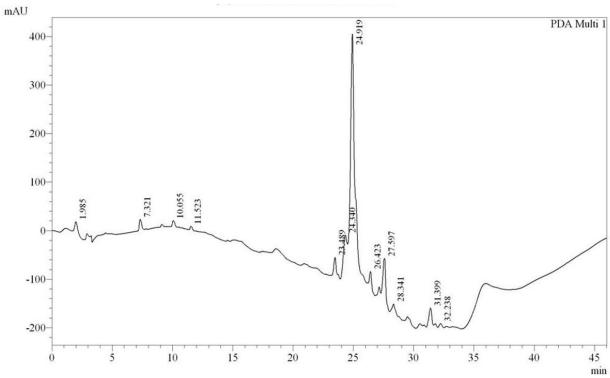

Fig 5 the chromatographic profile of MFTA. HPLC conditions: Reverse phase: **C_{18}, su (250X 4.6 mm); Mobile phase:** anhydrous potassium dihydrogen orthophosphate and ortho-phosphoric acid aqueous solution (A), and Acetonitrile (B) in gradient: flow rate: **20µl/ min; detective wavelength: 205nm.**

PDA Ch1 205nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.985	409700	24113	2.343	2.799
2	7.321	196578	17278	1.124	2.005
3	10.055	160917	12112	0.920	1.406
4	11.523	101216	7865	0.579	0.913
5	23.489	501350	37601	2.867	4.364
6	24.340	1872131	85261	10.705	9.897
7	24.919	11515948	493347	65.847	57.265
8	26.423	349762	31716	2.000	3.681
9	27.597	1323365	87718	7.567	10.182
10	28.341	366699	20323	2.097	2.359
11	31.399	588534	36627	3.365	4.251
12	32.238	102859	7554	0.588	0.877
Total		17489058	861516	100.000	100.000

DISCUSSION

In the initial evaluation of toxicological effects, In acute study, a single administration of the MFTA at the doses of 2000 mg/kg exhibited no mortality with no signs and symptoms, so, lethal dose 50 of MFTA is higher than 2000 mg. Dehydration induced uremia and oxidative stress is characterized by elevated levels of urea, creatinine in plasma and diminished the levels of SOD, CAT and Px in renal tissues (Das *et al*., 2009). MFTA treatment to dehydration rats recorded decrement in the levels of urea and creatinine as well as increments of SOD, CAT and Px in renal tissues. These observations indicate at an improved renal function in form of

antioxidant activities of secondary metabolites in bark of *Terminalia arjuna* (Kumar and Prabhakar, 1987). The purified fractions of *Terminalia arjuna* also showed diminished role of lipid Peroxidation by reducing MDA level in kidney tissues (Rajani and Purnima, 2009). The natural product (fraction) from bark of *Terminalia arjuna* showed potent lipid lowering and antioxidant activity (Rajani and Purnima, 2009) More than twenty compounds, including glycosides with a modified steroidal ring have been isolated from *Terminalia arjuna* (Kumar and Prabhakar, 1987). The body posses several defense systems such as radical scavengers (Nasik, 2003). Dehydration is the risk factor at the point when urine production declines and finally results in no urine output (anuria). Here, dehydration is only used for causing oxidative stress, elevation of blood urea and creatinine levels (Mayne., 1994). Without urinary excretion of waste products, dangerous levels of urea accumulate in the blood. Decreased blood volume occurs with deficient fluid intake, which causes a reduced blood flow in kidney resulting in a decreased glomerular filtration rate (GFR) (Das *et al*., 2009a). This can consequently lead to acute renal failure (ARF) (Antia and Abraham *et al*., 2007). Urea and creatinine increased in plasma of UDG group (66%) compared to control groups (UCG). But co-administration of MFTA in group D MFTA10, D MFTA 20, D MFTA 40 and D MFTA 80 significantly decreased both these parameters and it is resettled near to the UCG group level. In this present study it was observed that dehydration induced for 15 days significantly decreased Hb level and RBC count compared with untreated control group (UCG). The decreased in Hb was supposed to be due to destruction of RBCs and lowered total RBC counts were due to hemolytic anemia or suppressive inflammation. After administration of MFTA in group D MFTA10, D MFTA 20, D MFTA 40 and D MFTA 80 at the different doses (like 10, 20, 40 and 80mg/kg/day) for fifteen days, Hb levels were significantly higher as compared with dehydration induced groups (UDG) (Table 2). Dehydration induced oxidative stress in plasma and kidney has been established in this study to cause low activities of SOD and CAT, are important antioxidant enzymes (William and Juneja, 2008; Venkateswaran and Pari, 2003). The decrease in the activity of antioxidant enzymes, as a result of dehydration, might be due to their use against the free radicals destruction and their inhibition by free radical species (Debnath and Mandal, 2000). Both these anti-oxidant enzyme levels are significantly ($p<0.005$) reduced in UDG group in compared with UCG group. The activities of catalase and SOD in plasma and kidneys were recovered significantly after co-administration of MFTA at different doses (10, 20, 40 and 80 mg/kg body weight/day), but MFTA at the dose of 40 mg/kg body weight/day (D MFTA 40) showed better result in comparison to other doses (group D MFTA10, D MFTA 20, and D MFTA 80) (Table 2). Increase in the levels of oxidative stress products like MDA and CD in blood and kidney in the dehydrating group, again indicated the low level of antioxidant enzymes activities which causes progression of lipid peroxidation. Other possibilities for such elevation in MDA and CD could be the ischemia-reperfusion phenomenon (Freeman BA and Crapo JD, 1982; Jewett et., 1989). In the present study quantity of MDA increased (63.60% and 41.45%) in plasma and kidney of UDG compared to UCG groups (Table 2). The quantities of above enzyme MDA decreased significantly in co administration of MFTA in group D MFTA10, D MFTA 20, D MFTA 40 and D MFTA 80 in respect to dehydrating animals (UDG) respectively. It was also seen that quantities of above enzymes decreased significantly in Group D MFTA 40 compare to other MFTA treated groups (Table 2). The extract had no general and metabolic toxic effect, as reflected from the insignificant variation in body growth and activities of SGOT and SGPT in plasma and kidney tissues. This may be disseminated to the renal disease patients as a beneficial outcome.

By the phytocompound analysis using TLC and identified the purified fraction contains three major phytocompounds, which have capability to control uremia and oxidative stress. From the previous experiments on MFTA at the dose of 40 mg/kg body weight showed potential therapeutic effect to reduce uremia and oxidative stress (Fig 1). From the literature study, phenolic compound present in bark of *Terminalia arjuna* have been reported to possess powerful antioxidant activity (Nasik, 2003). Flavanoids are major class of phenolic compounds present in bark of *Terminalia arjuna* and are found to have a potential role in prevention of various diseases through their antioxidant activity (Sharma *et al*., 2010). So, it was assumed that the major compound identified from bark of *Terminalia arjuna* by the TLC and HPLC analysis was most probably polyphenolic flavonoid class of compound(s). *Terminalia arjuna* bark consist of many useful compound such as flavonoids, tannins, phenols, phytosterols, saponins and alkaloids. Its antioxidant activity is largely due to flavonoids (Fig 1 and 2). Higher amount of flavonoid constituents are present of this fraction. So the results further supported the view that the bark of *T. arjuna* is promising source of natural antioxidants and useful therapeutic agents. Therefore, extract from this plant could be seen as a good source for useful as traditional medicine practice that further work should be carried out to the effect of MFTA on proteomic and genomic kidney injury markers and the role of MFTA on low molecular weight protein in urine of uremic rats in future work.

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Conflict of interest statement

We declare that we have no conflict of interest.

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