ORIGINAL ARTICLE

Cisplatin-induced Nephrotoxicity and the Antioxidant properties of *Ficus religiosa* and *Ficus bengalensis* in mice

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ABSTRACT

Cisplatin is an important anti-neoplastic agent against solid tumors. However, using the effective dosage of Cisplatin has been limited due to the major side effects such as nephrotoxicity because the abnormal accumulation of Cisplatin, could induce oxidative stress in nephrons. Ficus religiosa (FR) and Ficus bengalensis (FB) are two of the most important ingredients of Indian folk medicine due to their pharmacological attributes. This study investigated and compared the photochemical profiles and antioxidant properties of leaf and fruit extracts from FR and FB in order to discover new treatments against cisplatin nephrotoxicity. The highest level of phenolics and flavonoids component was observed in aqueous and methanolic leaf extract of FR (FRLM, FRLA) followed by methanolic fruit extract of FB, (FBFM). FRLM also exhibited the highest value of Hydrogen peroxide, Superoxide anion, Hydroxyl radical and ABTS radical scavenging activity. Cis-induced nephrotoxicity by increasing the serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkalin phosphatase (ALP), Blood Urea Nitrogen (BUN) and Creatinine (Cr). In addition, Cisplatin induced oxidative damage by decline activities of superoxide dismutase (SOD), reduced glutathione (GSH) and Catalase (CAT) in kidneys. Supplementation of FRLM, FRLA and FBFM (500 mg/kg BW) significantly ameliorated the Cisplatin toxicity by improving the of antioxidants status of kidneys and lowered the kidney toxicity markers. Therefore, the results of this study suggested that different extracts from of FR leaf and FB fruit can be proposed to protect kidneys against Cisplatin induced oxidative damage. This protective effect might be correlated with its antioxidant and free radical scavenging potential of FR and FB plants.

Index terms: Cisplatin, anti-neoplastic agent, methanolic

INTRODUCTION

Cisplatin is an important antineoplastic agent against solid tumors such as testis, bladder, head and neck cance [1] . However, using the effective dosage of cisplatin has been limited due to the major side effects such as nephrotoxicity [2]. The abnormal accumulation of cisplatin, could induce oxidative stress in both the proximal and distal nephrons. Tubular oxidative damage by cisplatin is associated with lipid peroxidation [3]. Mechanisms of cisplatin nephrotoxicity involved depletion of antioxidant enzymes [4], and enhanced generation of reactive oxygen species (ROS). Superoxide radical and hydroxyl radical are playing the important role in mechanism of cisplatin nephrotoxicity [5].

In the last few decades, some studies have been done to evaluate the antioxidant properties of medical plats against side effects induced by cisplatin. Administration of natural antioxidant sources has been shown to ameliorate cisplatin-induced nephrotoxicity in animals [6].

The medicinal plants selected for the current study, FR and FB Linn., are two of the most important ingredients of Indian folk medicine due to their pharmacological attributes. According to ayurveda medicine system, different parts of FR are used for disease related to infectious disorders, respiratory, and reproductive systems [7]; and different parts of FB are lessens inflammations, aphrodisiac, tonic and in piles [8]. Furthermore, preliminary studies on different parts of current plants have reported a large number of bioactive molecules such as polyphenols and alkaloids, and biological capabilities of them in both *in vitro* and *in vivo* experiments [9], [10]. Despite the possible nephroprotective potential of FR and FB against Cisplatin has not been reported so far. Therefore, we aimed to evaluate the anti-oxidative potential of FR and FB by using the Cisplatin induced oxidative injury model in mice.

MATERIALS AND METHODS

Reagents and laboratory wares

All reagents used in present study are analytical grade. Water used in this study was doubly distilled. Cisplatin purchased from Sigma–Aldrich Chemical Company, USA. All the kits were purchased from Crest Biosystems Company, India and all chemicals were purchased from Sigma Chemical Co., (St. Louis, MO, USA).

Plant material

Leaf and fruit samples of FR and FB were collected from botanical garden of University of Pune, Pune, India in JAN-2013 and identified by a botanist. Plant samples were washed with distilled water, cut into small pieces and oven dried at 45°C for four days. Dried samples were grounded into a moderately coarse powder in domestic electric grinder.

Preparation of the aqueous extract

One part of the ground powder (Leaf and fruit) was boiled with 15 parts of distilled water for a period of 15 min, filtered hot through three layer of muslin cloth and then centrifuged at 1000 rpm in room temperature for 10 min. Supernatant was then evaporated under reduced pressure in Rotary evaporator at 40°C to dryness. Finally the extract powder weighed and stored in -20°C for further use.

Preparation of the ethanol and methanol extract

10g of ground powder (leaf and fruit) were subjected to 100ml of methanol or ethanol for 24 hrs at room temperature, filtered through three layer of muslin cloth and centrifuged at 1000 rpm for 10 min. Supernatant was then evaporated under reduced pressure in Rotary evaporator at 40°C to dryness. Methanol extracts stored at -20°C for further use.

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteau colourimetric method [11]. Briefly, 100 µl of plant extracts (0.2 mg/ml) was added to 400 µl deionized water and 100 µl Folin-Ciocalteu Reagent (FCR), and incubated in room temperature for 10 minutes; Then 1 µl 7% sodium carbonate and 0.8 µl deionized water was added, mixed well and incubated for extra 90 minutes at room temperature. The absorption at 415 nm was read with a spectrophotometer and the phenolic content was calculated as calibration curve of Gallic acid (GA) as the standard solution per gram (g) extract.

Total phenolic content (mg GA/g extract) = [(control absorbance - sample absorbance) / control absorbance] × 100

Determination of total Flavonoids

Total flavonoids content were determined[12]. Briefly, 100 µl of plant extracts (1mg/ml) was mixed with 100 µl of 2% aluminum trichloride, 100 µl of 5% $NaNO_2$, and diluted with 500 µl of NaOH. The absorption at 415 nm was read after 30 min against blank which contain all reagents without the samples. The flavonoid content was calculated as calibration curve of Quercetin (QUE) as the standard solution equivalent (equ) per 100g extract.

Total flavonoïde content (mmol QUE.equ / 100g) =
[(control absorbance - sample absorbance) / control absorbance] × 100

Hydrogen peroxide scavenging capacity

The ability of the extracts to inhibition the hydrogen peroxide activity was determined [13]. Briefly, 1 ml of sample (0.2 mg/ml) in distilled water (0 to 50µg/ml) was added to a hydrogen peroxide solution (0.6 ml, 40mM). The absorption at 230 nm was read after 10 min against blank containing the phosphate buffer. Ascorbic acid was used as standard and the percentage of hydrogen peroxide scavenging of extracts and standard compounds were calculated by current method:

Hydrogen peroxide scavenging capacity (%) = 1- (A blank – A sample) / A blank x 100
Where: A= Absorbance

Superoxide anion scavenging assay

Superoxide anion radical scavenging activity was based on riboflavin Nitro Blue-Tetrazolium (NBT) assay [14] . Briefly, 0.5 ml of plant extracts was taken at different concentrations (10 to 50 µg/ml) and mixed with 0.25 ml of phosphate buffer (50 mM, pH 7.6), 0.15 ml riboflavin (50 mM), 0.15 ml phenazine methosulfate (PMS, 20 mM), and 0.5 ml NBT (0.5 mM). Reaction was started by the reaction of mixture using a fluorescent lamp for 20 min. After the incubation time, the absorbance was measured at 560 nm. Ascorbic acid was used as standard and the Superoxide anion scavenging ability of the plant extract was determined by the following method:

Scavenging ability % = (1 -absorbance of sample / absorbance of control) ×100

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by the ability of the plant extracts to scavenge the hydroxyl radicals generated by the Fe^{3+}_ascorbate_EDTA_H_2O_2 system (Known as Fenton reaction [12].

The reaction mixture of 1000 µl of 2-deoxyribose (2.8 mM) in phosphate buffer (50 mM, pH 7.4), 400 µl of ferric chloride (100 mM) and EDTA (Ethylenediaminetetraacetic acid, 100 mM) solution (1:1; v/v), 200 µl of H_2O_2 (200 mM) with or without the extract solution (200 µl) was prepared. The reaction triggered by adding 100 µl of 300 mM ascorbate and incubated for 1 h at 37°C. 1 ml of the reaction mixture was added to 2 ml aqueous solution of TCA (Trichloroacetic acid 2.8%; w/v), then 1 ml of 1% TBA (Thiobarbituric acid), were added to the reaction mixture. The mixture was incubated in water bath for 20 min at 60°C. Finally the absorbance of cooled mixture was noted against a blank, with spectrophotometer at 532 nm.
Hydroxyl radical scavenging capacity % =
[(control absorbance - sample absorbance) / control absorbance] × 100

ABTS radical scavenging activity
The 2, 20-azinobis (3-ethylbenzthiazoline-6-sulphonic acid), commonly called ABTS cation scavenging activity was performed [15]. Briefly, 7 mM of ABTS solution was reacted with potassium per sulfate (2.45 mM) solution and kept for overnight in the dark to yield a dark coloured solution containing ABTS radical cautions. Prior to use in the assay, the ABTS radical cation was diluted with 50% methanol for an initial absorbance of about 0.70±0.02 at 745 nm, with temperature control set at 30°C. Free radical scavenging activity was assessed by mixing 500 µl of test sample with 5.0 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly one minute after mixing the solution, then up to 5 min. The percentage inhibition was calculated according to the formula:
Scavenging effect (%):
[(control absorbance - sample absorbance)/ control absorbance]×100

Animal treatment
Swiss albino male mice weighing 30.0±0.5 g, prepared from the animal laboratory of department of Zoology, Pune University, Pune, India. Animal house was kept at a temperature of 25±5 °C with a 12 h light/dark cycle and a relative humidity of 50–60% with free access to food and water at all the time. Mice were housed six per cage in the plastic cages with wood shaving bedding. The animals were allowed to one week adaptation before commencing the experiments. The Institutional Animal Care and Ethics Committees in Pune University, Pune, India; approved the protocol of this experiment on mice.

Acute toxicity assay
To study of acute toxicity of extracts, animals were randomly divided into five groups (n = 6). The animals were fed with the leaf and fruit extract of FR and FB by forcible feeding (gavage) method at different doses (100, 250, 500, 1000 and 2000 mg/kg body weight). The animals were continuously observed for behavioral profiles and death occurred after 24 hour and 72 hour [16].

Animal and treatments
Sixty male albino mice weighing 30.0±2.0 g, prepared from the animal laboratory of Zoology department of Pune University. Animal room was kept at a temperature of 20±2 °C with a 12 h light/dark cycle and a relative humidity of 50–60% with Free access to food and water at all the time. Mice were housed six per cage in the plastic cages with wood shaving bedding. After the one week adaptation period, animals were divided into ten groups of six mice each. 500 mg/kg body weight of extracts was administered daily to animals by forcible feeding (gavage) for fifteen days and Cisplatin (5mg/kg body weight) was injected intra peritoneally (IP) in 3 days interval, as follows:
Control: Administrated with distilled water as the normal Control group
NCG: Administrated with Cisplatin only, as the negative control group
FRLA: Administrated aqueous leaf extract of *Ficus religiosa* + Cisplatin
FRLM: Administrated methanolic leaf extract of *Ficus religiosa* + Cisplatin
FRFA: Administrated aqueous fruit extract of *Ficus religiosa* + Cisplatin
FRFM: Administrated methanolic fruit extract of *Ficus religiosa* + Cisplatin
FBLA: Administrated aqueous leaf extract of *Ficus bengalensis* + Cisplatin
FBLM: Administrated methanolic leaf extract of *Ficus bengalensis* + Cisplatin
FBFA: Administrated aqueous fruit extract of *Ficus bengalensis* + Cisplatin
FBFM: Administrated methanolic fruit extract of *Ficus bengalensis* + Cisplatin
The Institutional Animal Care and Ethics Committees in Pune University, Pune, India approved the protocol of this animal experiment. The animals were sacrificed 24 h after the FR treatment using ether anesthesia.

Sample preparations
Blood was collected directly from heart and centrifuged at 1500 rpm for 15 mins; serum was collected and stored at 80 ºC for further studies.
The liver and Kidney were removed, washed thoroughly in ice-cold saline to remove the blood then homogenized (10 ml/g of tissue) with 0.05 M phosphate buffer (pH 7).

The suspension was centrifuged at 1500 rpm at 4ºC for 15 min, and clear supernatant was stored at 80 ºC for further determinations.

Liver and kidney toxicity markers

MDA activity was assayed according to the method of Jain, [17]. 10% homogenate tissue was centrifuged at 4 °C; 3500 rpm for 10 min. 0.2mL supernatant was mixed with 0.67% TBA and 20% TCA solution, and heated in a boiling water bath for 30 min. The pink-colored chromogen formed by the reaction of Thiobarbituric acid with MDA was measured at 532 nm.

Liver functions were evaluated by measuring the activity of serum transaminases (ALT, AST) and ALP by standard commercially kits (Crest Biosystems Company, India). Kidney functions BUN and Cr concentration were determined spectrophotometrically from serum samples using commercially available standard kits.

Protein content was determined [18], bovine serum was used as standard.

CAT activity assay

CAT activity was determined according to the method of Aebi, [17]. Briefly, two set of the mixtures of 0.1 M Potassium buffer (pH-7.0), 50 mM potassium phosphate buffer (pH 7.8), 0.01 M EDTA, 65mM L-methionine, 750μM NBT, 2mM Riboflavin and 100μl of enzyme extract were kept in dark and near light sources for 30 min. The intensity of the color, measured at 240nm. CAT activity calculated by following method:

CAT (U/ml)=$[A/t] \times [V \times 1000/ _0 \times d \times v]$

Where:

A/t = Slope

V = total volume of the assay mixture

$_0$ = 0.003942 lit mM^{-1} mm^{-1}

v = Volume of sample

d= Path length

SOD activity assay

SOD activity was determined according to the method of Offer, [19], based on the generation of superoxide anions by pyrogallol autoxidation. The enzyme extracts was centrifuged at 10,000 rpm for 15 minutes at 4°C. 0.25 ml of supernatant, 0.5 ml of tris cacodylic buffer, 0.1 ml of 16% triton x- 100 and 0.25 ml NBT were added. The reaction was started by the addition of 0.01 ml diluted pyrogallol. Incubation was maintained for 5 minutes at 37ºC. The reaction was stopped by the addition of 0.3 ml of 2M formic acid. The formazan color developed was determined spectrophotometrically at 450 nm.

GSH activity assay

GSH activity was assayed according to the method of Beutler [20]. 50μL of tissue sample was mixed with 0.32 mol/L disodium hydrogen phosphate and 0.04% DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)] solution. The yellow-colored substance formed by the reaction of GSH and DTNB was measured at 412 nm.

Statistical Analysis

All data were represented as mean ± SD. Data were statistically analyzed by using one-way analysis of variance (ANOVA) followed by the Student's t-test. P values less than 0.05 were considered as significant using SPSS 19 software.

RESULTS

Total phenol and flavonoids content

The results obtained from the analysis of total phenol (Figure 1) and flavonoids (Figure 2) content were reported. FRLM (73.4 ± 3.8 mg GA/g) and FRLA (65.2 ± 2.4 mg GA/g) presented the highest level of phenolics followed by the highest level of flavonoids (FRLM: 69.3 ± 3.6 mg GA/g mmol que.equi/100g and FRLA: 66.8 ± 4.8 mg GA/g mmol que.equi/100g).

In vitro study

Hydrogen peroxide scavenging capacity

The scavenging capacity of different plant extracts on hydrogen peroxide was reported in Figure 3. FRLM displayed the highest concentration-dependent scavenging activity (50.2±2.1μg/ml) followed by FRLA (55.7±3.4 μg/ml) at 50 μg/ml.

Superoxide anion scavenging capacity

The Superoxide anion scavenging capacity of plant extracts were showed in Figure 4. FRLM showed the highest capacity to scavenging of superoxide anions (44.7±1.3 μg/ml) followed by FRLA (40.5±2.1 μg/ml) at 50 μg/ml.

Hydroxyl radical scavenging capacity

FRLM also exhibited concentration dependent increase in its ability to scavenge hydroxyl radical (Figure 5) with maximum ability of 69.4±3.7 µg/ml at a concentration of 50 µg/ml.

ABTS radical scavenging activity

All the fractions of plants scavenged ABTS radical in a concentration-dependent way (0 - 50 µg/ml) (Figure 6). The results showed that the ABTS radical scavenging ability of FRLM sample is highest (85.7±3.4 µg/ml) compared to another fractions.

In vitro study

Acute toxicity study

The study of acute toxicity were suggested the non-toxic nature of plant samples. Until the end of the acute toxicity study (72h) the extracts did not show any mortality. However, weight loss was observed in animals which were treated with high concentration of extracts (2000 mg/kg BW).

BUN and Cr

As shown in figures 7 and 8, Cisplatin administration in NCG, resulted in a significant increase in the BUN and Cr levels ($p < 0.05$), respectively; compared to control group. FRLA, FRLM reduced the elevated levels of BUN and Cr. However, fruit and leaf extracts of FB, did not made any changes on decreased levels of, nephrotoxicity markers, compared to normal control group ($p < 0.05$).

MDA

As shown in Figure 9 the Cisplatin treatment significantly ($P<0.05$) increased the MDA level of kidney, ($P<0.05$). FRLA and FRLM treatment for 15 days significantly ($P<0.05$) decreased the elevated MDA in kidney.

ALT, AST and ALP

Serum levels of transaminase (ALT and AST) and ALP were given in Table 1. Cisplatin (5mg/kg) significantly elevated the liver toxicity markers when compared to normal control animals. Pre and post treatment of FRLA, FRLM significantly protected the increased level of transaminase (ALT and AST) and ALP compared to NCG. However, Oral administration of another extracts, did not caused any significant changes on the levels of serum transaminases as well as ALP.

SOD, CAT and GSH

The level of antioxidant enzymes of kidney had shown in Table 2. In NCG, Cisplatin decreased the level of SOD, GSH, and CAT. The level of antioxidant enzyme SOD, CAT and GSH were significantly ($P < 0.05$) increased in FRLA, FRLM which were treated with aqueous and methanolic leaf extract of FR compared to NCG.

DISCUSSION AND CONCLUSION

The medicinal plants selected for the present study, have long been used in the folk medicine due to their pharmacological properties, which are mainly due to the presence of antioxidant constituents such as phenolic and flavonoid components [9], [10].

In the current study the leaf extract of *Ficus religiosa* exhibited the highest content of phenolics which was along with highest flavonoids content, compared to other fractions. Plant extracts rich in phenolic components are being used in the natural medicine systems because of their potential to oxidative degradation of lipids [21]. Flavonoids have been shown to be effective scavengers of free radicals such as superoxide and hydrogen peroxide implicated in several diseases.

In the present study, several techniques have been done to study the ability of extracts to scavenge a variety of free radicals. The reducing power was assayed to study the primary anti oxidative potential of extracts. Whereas hydrogen peroxide, superoxide and hydroxyl radical scavenging capacity was assayed to study the potential of extracts to scavenging of free radicals.

Table 1: Effect of FR and FB extracts on serum levels of ALT, AST and ALP in Cisplatin intoxicated-mice.

Treatments	Parameters		
	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal	25.5±1.2	32.7±1.1	65.2±2.1
NCG	69.2±3.6*	96.1±6.1*	134.4±5.9*
FRLA	33.7±1.5**	44.4±2.4**	62.4±1.8**
FRLM	28.5±2.4**	35.3±2.6**	69.6±3.6**
FRFA	62.8±4.8	81.4±4.2	129.1±5.3
FRFM	68.3±3.9	94.9±5.1	123.3±4.8
FBLA	68.2±4.1	79.9±3.2	122.7±6.2
FBLM	55.4±4.6	75.7±5.8	144.6±6.3
FBFA	66.3±2.7	86.4±3.2	133.1±5.8
FBFM	64.6±1.9	95.1±4.7	127.6±6.9

Each value in the table is represented as mean ± SD (n = 6).
* Indicate significance compared to control group (p< 0.05).
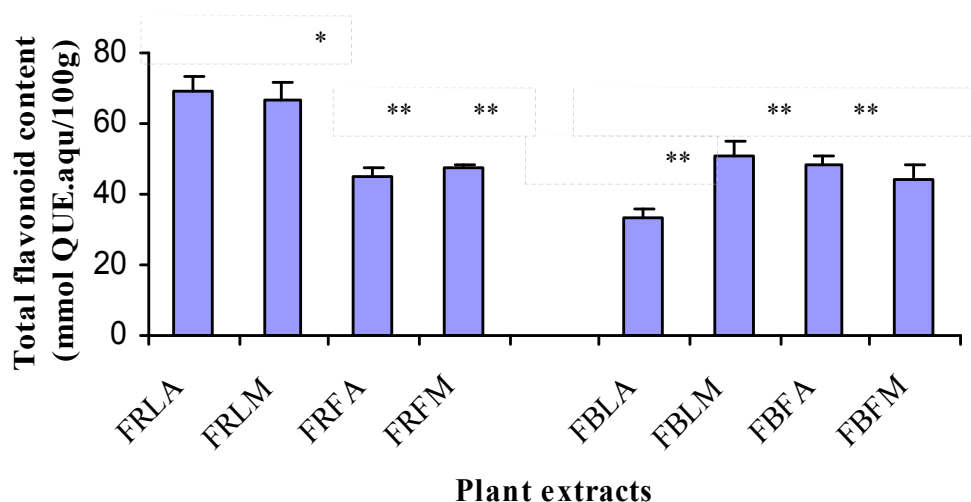** Indicate significance compared to NCG group (p< 0.05).

Table 2: Effect of FR and FB extracts on antioxidants level of liver in Cisplatin intoxicated-mice.

Treatments	Parameters		
	CAT (U/mg Pr)	SOD (U/mg Pr)	GSH (U/mg Pr)
Normal	177.5 ± 14.5	19.4 ± 2.1	4.1 ± 0.3
NCG	131.6 ± 7.4*	8.3 ± 0.3*	2.3 ± 0.1*
FRLA	165.2 ± 13.7**	17.2 ± 2.3**	3.7 ± 0.2**
FRLM	176.4 ± 12.1**	16.7 ± 1.1**	3.55 ± 0.1**
FRFA	138. 8 ± 1.3	8.5 ± 0.3	2.4 ± 0.2
FRFM	135.4 ± 2.7	8.4 ± 0.4	3.2 ± 0. 2
FBLA	139. 4 ± 12.6	9.6 ± 0.2	2.8 ± 0.3
FBLM	125. 7 ± 21.4	8.4 ± 0.7	2.2 ± 0.1
FBFA	146.9 ± 12.6	8.1 ± 0.3	2.5 ± 0.3
FBFM	132.8 ± 17.8	9.5 ± 0.6	2.3 ± 0.1

Each value in the table is represented as mean ± SD (n = 6).
* Indicate significance compared to control group (p< 0.05).
** Indicate significance compared to NCG group (p< 0.05).

Figure 1: Total flavonoids contents in the different extracts of FR and FB, leaf and fruit.
Each values represented in tables are means ± SD (N=6).
* Indicate significance compared to control group (p< 0.05).
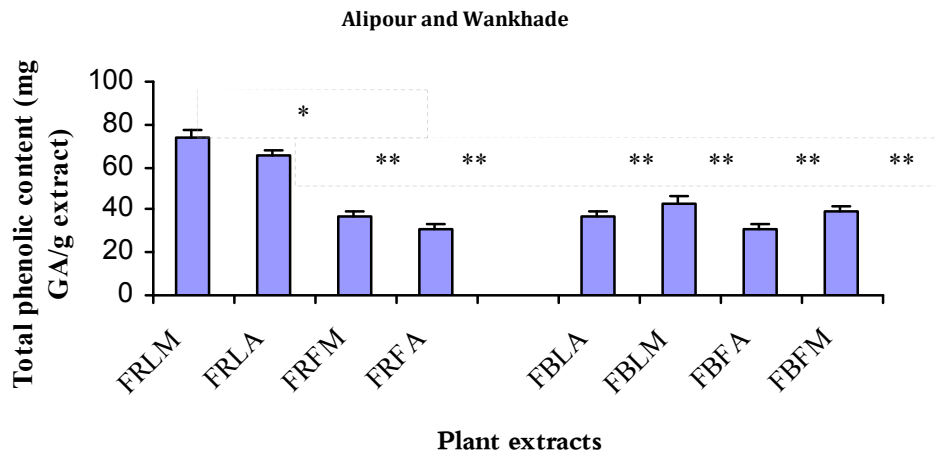** Indicate significance compared to NCG group (p< 0.05).

Figure 2: Total phenolic content in the different extracts of FR and FB, leaf and fruit.
Each values represented in tables are means ± SD (N=6).
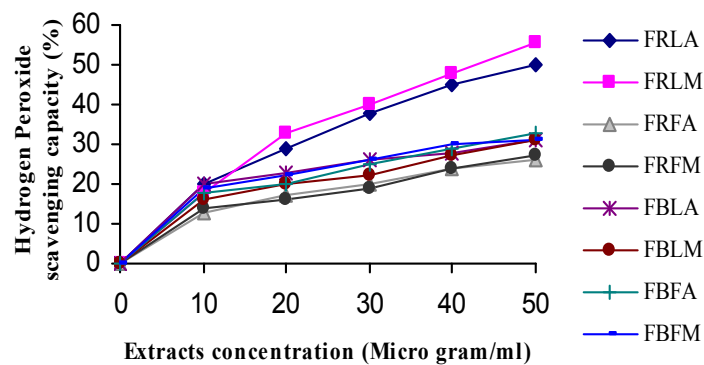* Indicates significance difference between the groups (p< 0.05).

Figure 3: Hydrogen peroxide scavenging capacity of different extracts of FR and FB, leaf and fruit, at different concentrations.
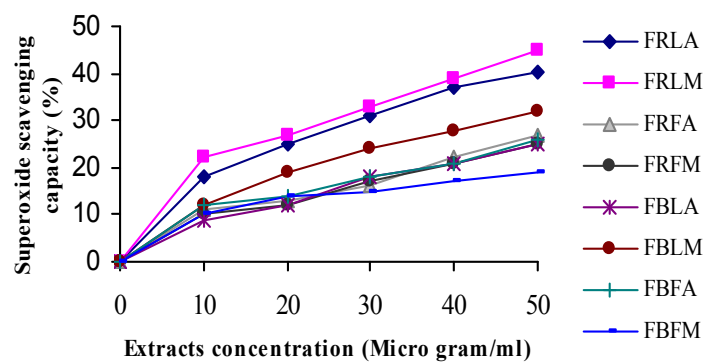

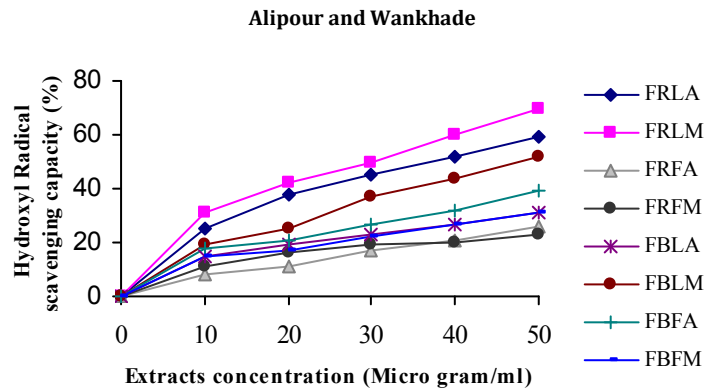**Figure 4:** Superoxide scavenging activity of different extracts of FR and FB, leaf and fruit, at different concentrations.

Figure 5: Hydroxyl radical scavenging activity of different extracts of FR and FB, leaf and fruit, at different concentrations.
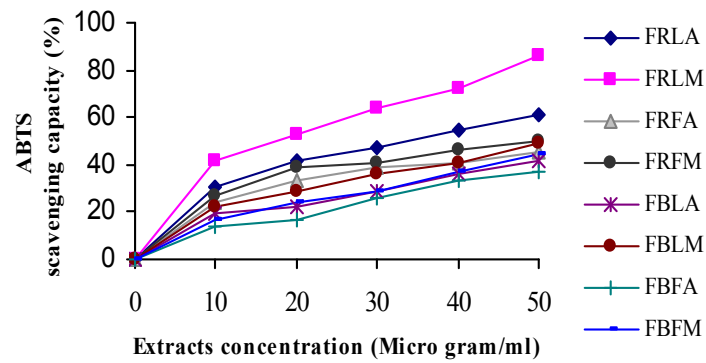

Figure 6: ABTS radical scavenging activity of different extracts of FR and FB, leaf and fruit, at different concentrations.
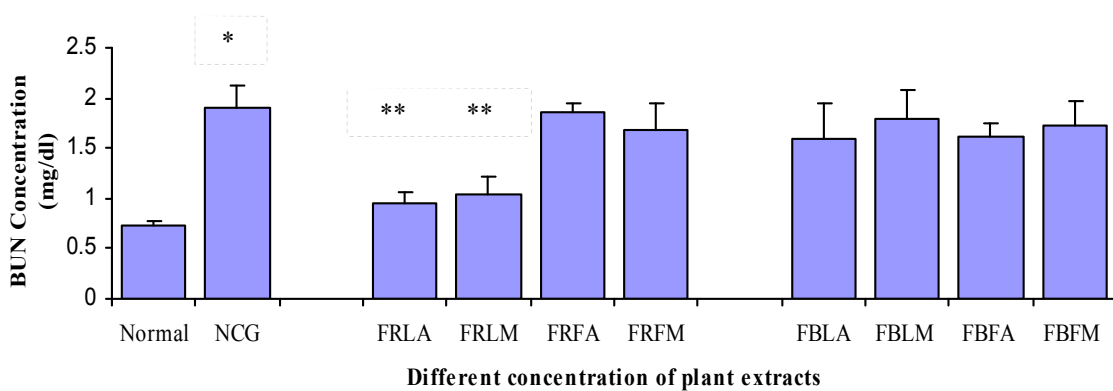

Figure 7: Effect of FR and FB extracts on the serum levels of BUN in Cisplatin intoxicated-mice.
Each values represented in tables are means ± SD (N=6).
* Indicate significance compared to control group (p< 0.05).
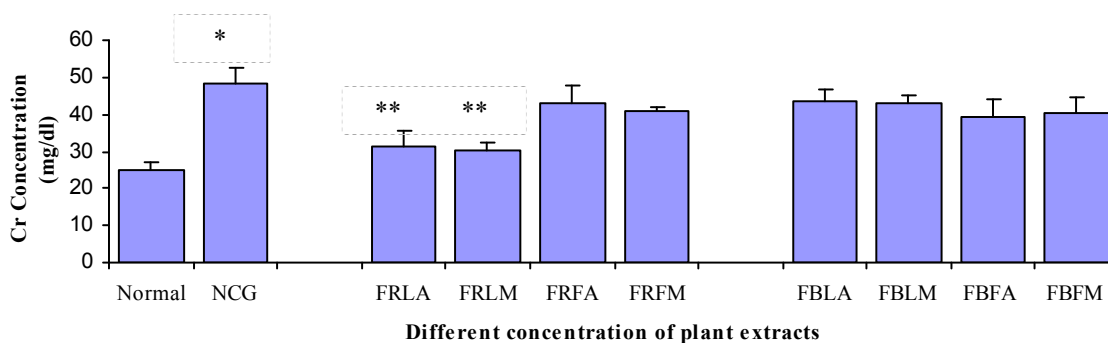** Indicate significance compared to NCG group (p< 0.05).

Figure 8: Effect of FR and FB extracts on the serum levels of Cr in Cisplatin intoxicated-mice.
Each values represented in tables are means ± SD (N=6).
* Indicate significance compared to control group (p< 0.05).
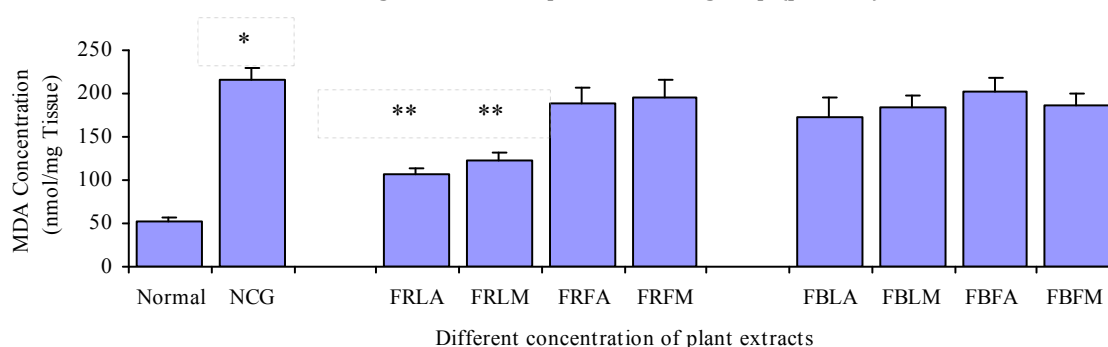** Indicate significance compared to NCG group (p< 0.05).

Figure 9: Effect of FR and FB extracts on the serum levels of MDA in Cisplatin intoxicated-mice.
Each values represented in tables are means ± SD (N=6).
* Indicate significance compared to control group (p< 0.05).
** Indicate significance compared to NCG group (p< 0.05).

In the present study, the leaf extract of *Ficus religiosa* demonstrated the higher anti oxidative potential compared to other extracts in *in vitro* condition. This potential could be attributed to the ability of the extracts to act as a strong donor of electrons or hydrogen atoms [22].Our results on pharmacological properties of *Ficus religiosa* could provide a pragmatic support for its special role in traditional medicine systems. The pervious primary studies on phytochemestry of *Ficus religiosa* had led to the detection of so many of antioxidant components such as phytosterols, tannins, steroids, alkaloids, flavonoids, phenolic components and few other classes of secondary metabolites [10], and the study by Hanef has suggested the apoptosis inducing potential of this plant against cancerous cells [23]. However the majority of other studies has been focused on the basic studies on anti-inflammatory [7], anti-ulcer [24], anti-diabetic properties of this plant [25].

A variety of previous studies have suggested that unwanted formation of ROS may be directly related to the ability of cisplatin to induce renal toxicity [3]. In this study, liver and kidneys became unable to protect the cell membrane against lipid peroxidation, because of shortage of antioxidant enzymes. This was evident with the significant elevation of BUN, Cr, MDA, serum teransaaminases and ALP.

BUN and Cr are reliable markers as a measure of renal function status. The increased levels of BUN and Cr resulting from cisplatin in this study (Table 2), is in agreement with a previous study done by Sahu, [26]. High values of BUN and Cr indicate renal damage [27] and this may be correlated with the increased level of MDA in NCG group (Table 1).

From this study, pretreatment with FRLA, FRLM at 500mg/kg could prevent Cisplatin induced renal damage as shown by the decreasing levels of BUN and Cr. These parameters were almost significantly normalized by the administration of FRLA, FRLM, extracts (Table 2). This result is consistent with many previous studies done using other natural antioxidants, which is strongly attributed to the ability of antioxidants to scavenging free radicals and reduced lipid peroxidation.

In case of oxidative stress, the various inherent defense mechanisms such as antioxidant defense system that includes superoxide dismutases, catalase, glutathione become significantly impaired and insufficient

[28]. This is because the body has used its antioxidant defense mechanism to minimize the process of lipid peroxidation using these antioxidant enzymes. In the current study, when the oxidative stress was happened, the enzymes become decreased and unable to fight against the free radicals. This was evident with the elevation in lipid peroxidation level in NCG.

In this study, the primary phytochemical study on FR and FB extracts suggested the FRLA and FRLM the higher phenolics and flavonoid contents. In addition, it was followed by the ability of extracts to scavenge a variety of free radicals. This was evident in the elevated levels of antioxidant enzymes (CAT, SOD, and GSH), as well as the decreased in MDA level in the FRLA and FRLM-treated groups. This is due to the antioxidant properties of *Ficus religiosa*, which may account for the nephroprotective potential of it against Cisplatin-induced nephrotoxicity.

The results of this study demonstrated that FRL treatment prevented Cisplatin induced nephrotoxicity in mice, referenced to increased levels of antioxidant enzymes and protected the kidney against lipid peroxidation. In this study, FRL possess radical scavenging activity, probably due to higher concentration of polyphenols. This was evident in the improved the antioxidant status of kidney as well as the decreased in lipid peroxidation level in the FRL treated groups. However, this is due to the antioxidant properties of the plant extract, which may account for the nephroprotective potential of FR against Cisplatin-intoxicated mice.

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