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ANTI-NEPHRITIC POTENTIAL OF N-BUTANOLIC FRACTION OF *BUTEA MONOSPERMA* (LAM.) FLOWERS ON DOXORUBICIN INDUCED NEPHROTIC SYNDROME IN RATS

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ABSTRACT

The objective of the study was to investigate the possible benefits of n-butanolic fraction (NBF) of *Butea monosperma* (Lam.) flowers in doxorubicin (DOX) induced nephrotic syndrome in rats. NBF (100 and 200 mg/kg) was given orally for 28 days starting 2 days after single intravenous administration of DOX (7 mg/kg). Serum cholesterol, triglycerides, albumin, total protein, creatinine, blood urea nitrogen and 24-h urinary protein concentration evaluated as the markers of nephrotic syndrome. Renal catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) activities were determined using standard spectrophotometric methods. In-vitro antioxidant and anti-inflammatory activities of NBF were determined by DPPH assay and lipoxygenase inhibition method. NBF significantly reduced proteinuria, hypoalbuminemia, dyslipidemia and restored renal antioxidant enzyme activities like SOD, CAT and GSH. Histopathological studies have supported biochemical findings with no evidence of vacuolization and reduction of glomerular sclerosis in NBF treated rats. The NBF exhibited strong in vitro antioxidant and anti-inflammatory activity. The antioxidant and anti-inflammatory potential of NBF could be ascribed to the presence of butrin and isobutrin as its major constituents. NBF exhibited significant antinephritic activity in DOX induced nephritis model which can be related to, at least in part, to its antioxidant and anti-inflammatory activity.

Keywords: *Butea monosperma*, nephrotic syndrome, doxorubicin, lipoxygenase inhibition, oxidative stress, glomerular sclerosis.

INTRODUCTION

The incidences of idiopathic focal segmental glomerulosclerosis (FSGS) have progressively increased and FSGS now constitutes the most frequent diagnosis in native adult kidneys¹. Doxorubicin (DOX) induced nephrotic syndrome has become the classical experimental model of human primary FSGS and is characterized by heavy proteinuria, edema, hypoalbuminemia, and hypercholesterolemia². Although the exact mechanism of DOX-induced FSGS remains unknown; it is believed to be mediated through reactive oxygen species (ROS) and iron-dependent oxidative damage of biological macromolecules³. ROS may directly damage lipid membranes, alter metabolism of prostaglandins and may also mediate the activation of genes for some pro-inflammatory cytokines through the stimulation of transcription factor NF- κ B⁴ (Fig. 1). India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy⁵. Ayurvedic medicine, has utilized flowers, bark, leaves, gum and even the seeds of *Butea monosperma* (BM) to prepare herbal remedies⁶. Numerous scientific reports validate the traditional uses of BM flowers such as antioxidant⁷, anti-diabetic⁸, anti-inflammatory⁹, anti-cancer¹⁰ and hepatoprotective activity¹¹. The important active constituents of BM flowers are butrin, butein, butin, isobutrin, palasitrin,

coreopsin, isocoreopsin, chalcones and aurones¹². Our preliminary studies suggest that the n-butanolic fraction of *Butea monosperma* (Lam.) flowers (NBF) have potent free radical scavenging and anti-inflammatory activity. On the basis of these findings, it was hypothesized that NBF might play an important role as an antiproteinuric agent in DOX induced nephrotic syndrome; as its pathophysiology is mainly due to generation of ROS.

MATERIALS AND METHODS

Extraction

The dried flowers of *Butea monosperma* (Lam.) (Fabaceae) used in the study were procured from a local Ayurvedic dealer Goragandhi Ltd, Pydhonie in Mumbai and were authenticated by a plant taxonomist from Department of Life Science, Ramnarain Ruia College, Mumbai, India. The dried coarse powder of the flowers of *Butea monosperma* (100 g) was extracted with methanol (1L) in soxhlet extractor for 24 hrs at 40 °C. The extract was concentrated in a rotary vacuum evaporator (Buchi, Switzerland) at temperature 40°C to get methanolic extract. The crude methanolic extract (50 g) was suspended in distilled water (100 mL). The mixture was then transferred to a separating funnel and fractionated with n-butanol (3 x 100 mL). The resulting fractions were then concentrated on a rotary evaporator under reduced pressure to get n-butanol fraction (NBF).

Determination of total phenolic content

1 mL of appropriately diluted samples of NBF and a standard solution of gallic acid were added to a 25 mL volumetric flask containing 9 mL of ddH₂O. One milliliter of Folin- Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of a 7% Na₂CO₃ solution was added with mixing. The solution was then immediately diluted to a volume of 25 mL with ddH₂O and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance was measured at 750 nm using a spectrophotometer. The total phenolic contents of the samples are expressed in milligrams per serving of gallic acid equivalents (GAE)¹³. All samples were prepared in three replications.

Determination of total flavonoids

1 mL of appropriately diluted samples of NBF was added to a 10 mL volumetric flask containing 4 mL of ddH₂O. At time zero, 0.3 mL of 5% NaNO₂ was added to each volumetric flask; at 5 min, 0.3 mL of 10% AlCl₃ was added; at 6 min, 2 mL of 1 M NaOH was added. Each reaction flask was then immediately diluted with 2.4 mL of ddH₂O and mixed. After development of pink colour, absorbance was determined at 510 nm relative to a prepared blank. The total flavonoid contents of the samples are expressed in milligrams per serving of quercetin equivalents (QE)¹⁴. All samples were prepared in three replications.

UV-visible spectroscopy

The NBF (1mg/ml) was subjected to UV-visible analysis (UV spectrophotometer, Jasco, Japan), for detecting major constituents.

HPTLC fingerprint

NBF (5 mg/ml) was prepared in HPLC grade methanol. 5 µl of the sample solution was loaded as 5 mm band on the silica gel F254 precoated HPTLC plates, 10x10 cm (Merck, Darmstadt, Germany) using Hamilton syringe and CAMAG LINOMAT instrument. Plates were run in pre-saturated TLC chamber consisting of ethyl acetate: methanol: water: formic acid (4:0.8:0.2:0.2) as the mobile phase¹⁵. The solvent was allowed to move a distance of 80 mm. The plate was dried and scanned at 254. Finally, the area under curve and R_f values were determined.

Free radical scavenging potential by DPPH assay

1.5 ml DPPH (200 µM) of a solution of DPPH in ethanol were mixed with 1.5 ml of various concentrations extract (10-100 µg/ml) and the mixture was shaken gently and allow to stand at room temperature in the dark for 20 min. Thereafter, the absorbance was measured at 517 nm¹⁶. The violet colour developed was proportional to the free radical generation. Measurements were performed in triplicates. The percentage of DPPH radical scavenging

activity was calculated according to the following equation:

$$\% \text{ inhibition} = \frac{A(\text{control}) - A(\text{test})}{A(\text{control})} \times 100$$

Where A control indicates absorbance of control; A test indicates absorbance of test sample

Lipoxygenase inhibition activity

The substrate solution (2 mL) was mixed with 0.9 mL of 2M borate buffer, pH 9. NBF (50 µL), to obtain final concentrations of 10-100 µg/mL, and was preincubated with the 50 µL enzyme (10,000 units/mL) for 5 minutes. Lipoxygenation was started by addition of the enzyme solution to the substrate solution and change in absorbance was determined per minute at 234 nm using spectrophotometer for a time period of 5 minutes¹⁷. Measurements were performed in triplicates. The LOX inhibition assay was calculated using the formula as for DPPH radical scavenging activity.

Induction of experimental nephrotic syndrome

Female Sprague–Dawley rats (200 ± 15 g) were obtained from Glenmark Pharmaceuticals, Mumbai. All animals were housed in standard polypropylene cages and maintained under standard conditions of temperature 22 ± 3°C and relative humidity 55 ± 10% with 12:12 hrs light: dark cycle during the experimental period. Animals were fed with commercially available standard rodent pellet diet and tap water ad libitum. The animal experimentation protocol was approved by the Institutional Animal Ethics Committee (CPCSEA-BCP/2010/21). The animal experiments were performed in accordance with the CPCSEA guidelines. After one week of acclimatization, female Sprague dawley rats were divided in 5 groups (6 animals/groups): Group 1 (Vehicle control), Group 2 (Positive control), Group 3 (DOX + prednisolone 3 mg/kg), Group 4 (DOX + NBF 100 mg/kg) and Group 5 (DOX + NBF 200 mg/kg). All above groups except Group 1 received single dose of DOX (7 mg/kg) through a tail vein to induced nephritis, whereas Group 1 received saline only (1ml/kg, i.v.). After 2 days from DOX administration, Group 1 and Group 2 were received Tween 80 (2% v/v) in distilled water (1ml/kg, p.o), while Group 3, Group 4 and Group 5 received Prednisolone (3 mg/kg), NBF (100 mg/kg) and NBF (200 mg/kg) respectively suspended in Tween 80 (2% v/v) by oral gavage method once a day for twenty eight days.

Blood and Urine biochemical assay

Twenty eight days after treatments, animals were placed in metabolic cages for 24 hour urine collection. During urine collection animals had free access to food and water. After urine collection, rats were anesthetized with light ether and blood samples were obtained by puncturing the retro orbital plexus of the animals to measure serum cholesterol, triglycerides, albumin, total protein, creatinine and blood urea nitrogen. Uric protein and serum biochemical parameters were measured using

the commercial available Diagnostics kits (Erba Diagnostics Mannheim GmbH).

Tissue biochemical assay

At the end of experiment, animals were sacrificed in carbon dioxide chamber and dissected to remove kidneys. Each kidney was weighed and subsequently minced and homogenized with ice cold phosphate buffer (pH 7). Each homogenate was centrifuged and supernatant was used for the analysis of lipid peroxides (MDA)¹⁸, superoxide dismutase (SOD)¹⁹, catalase (CAT)²⁰ and reduced glutathione (GSH)²¹ using standard spectrophotometric methods.

Histopathological studies

Renal tissues were fixed in 10 % buffered formalin. After a week the tissues were washed thoroughly with 70% alcohol and then dehydrated in ascending grades of alcohol (70-100%). After dehydration, the tissues were cleaned in xylene and embedded in paraffin wax. Processed tissues were sectioned (at 5 µm) and stained by hematoxylin and eosin dyes. Stained slides were examined and photograph under Olympus BX10 optical microscope attracted with Olympus DP12 camera (Olympus, Japan).

Acute toxicity study

Acute toxicity study was performed as described in the “Guide-lines for Testing of Chemicals – Acute Oral Toxicity – Fixed Dose Procedure”²². Five female Sprague dawley rats were treated orally with a single dose of 2000 mg/kg of NBF suspended in 2% Tween 80 in distilled water.

Statistical analysis

All Values are means ± S.D. Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test: *: $p < 0.05$ when compared with positive control group and #: $p < 0.05$ when compared with vehicle control group were considered significant. Statistics was applied using Graph pad Prism 5 software (Graph pad Software Inc).

RESULTS

Extraction yield

The yield of the n-butanolic fraction of *Butea monosperma* (Lam.) flowers was found to be 24.9 % w/w.

Determination of total phenolic content

The calibration function for gallic acid [absorbance vs. concentration] was found linear over the working range of 10-100 µg with 6-point calibration. The content of phenolic compounds in NBF was determined using regression equation of calibration curve ($y: 0.012x + 0.029$, $r^2: 0.996$) and expressed in gallic acid equivalents

(GAE). The total phenol content in NBF was found to be 12.58 µg (GAE)/g of sample.

Determination of total flavonoids contents

The calibration function for quercetin [absorbance vs. concentration] was found linear over the working range of 10-100 µg with 6-point calibration. The content of total flavonoids in NBF was determined using regression equation of calibration curve ($y: y = 0.054 x + 0.014$, $r^2: 0.991$) and expressed as micrograms of quercetin equivalents (QE). The total flavonoids content in NBF was found to be 1.09 µg (QE)/g of sample.

UV visible spectroscopy

As shown in Figure 2, NBF exhibited four peaks 3 at 207nm, 268nm and 371nm respectively in UV/visible region. This indicates the presence of flavonoids which exhibited two major bands in the UV/visible wavelength, band I in the 310-560 nm range and band II in the 240-285 nm range.

HPTLC fingerprint

As shown in Table 1 and Figure 3, HPTLC fingerprinting for NBF exhibited 4 peaks at the R_f values 0.29, 0.37, 0.74, 0.77, indicating the occurrence of at least 4 different components. It is also clear that out of 4 components, the component with R_f values 0.29 and 0.37 at 254nm were found to be more predominant as the percentage area is more with 46.68 % and 45.71% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 5.0%. As shown in Figure 4, it was observed that peaks 1 and 2 may be due to presence of butrin and isobutrin which matches exactly with reported λ_{max} of butrin (λ_{max} at 279 nm and 313 nm) and isobutrin (264 nm and 373 nm)²³.

Evaluation of NBF for antioxidant and anti-inflammatory potential

The DPPH radical scavenging activity and lipoxxygenase inhibitory effect of NBF are presented in Figure 5. The NBF exhibited strong antioxidant activity with an EC₅₀ of 82.2 µg/ml and lipoxxygenase inhibitory activity with an EC₅₀ of 75.72 µg/ml.

Effect of NBF on urine biochemical parameters

As seen in Figure 6, after a single i.v. injection of DOX (7mg/kg), the urinary protein excretion in positive control group markedly increased as compared with vehicle control group (325.4 ± 17.1 mg/24 vs. 14.4 ± 1.34 mg/24h, $p < 0.05$). However, after treatment with prednisolone (3mg/kg) and NBF (200mg/kg), proteinuria was significantly inhibited compared to positive control (234.3 ± 17.8, 237.8 ± 18.6 mg/dl vs. 325.4 ± 17.1 mg/24h, respectively, $p < 0.05$). On other hand, decreased in 24h urine volume was observed in positive control

group as compared with vehicle control group (8.17 ± 1.2 ml/24h vs. 14.3 ± 1.3 ml/24h, $p < 0.05$). Treatment with prednisolone and NBF inhibited decrease in the urine volume when compared with positive control group but this effect was not statistically significant.

Effect of NBF on serum lipid profile

Serum cholesterol (Figure 7A) and triglycerides (Figure 7B) levels were markedly increased in the DOX treated nephritic rats compared to vehicle control group (271.4 ± 21.7 mg/dl, 469.2 ± 38.7 mg/dl vs. 57.8 ± 7.56 , 137 ± 17.4 mg/dl, respectively, $p < 0.05$). Treatment with prednisolone (3mg/kg) and NBF (200 mg/kg) significantly inhibited the rise in serum cholesterol level (215.5 ± 17.9 mg/dl, 189.9 ± 17.4 vs. 271.4 ± 21.7 mg/dl respectively, $p < 0.05$) and triglyceride level (362.7 ± 28.1 mg/dl, 343.7 ± 29.7 mg/dl vs. 469.2 ± 38.7 respectively, $p < 0.05$) as compared with positive control group.

Effect of NBF on serum total protein and albumin

Serum total protein (Figure 7C) and albumin (Figure 7D) levels were significantly decreased in the DOX treated nephritic rats compared to vehicle control group (5.62 ± 0.14 g/dl, 2.36 ± 0.17 g/dl vs. 7.88 ± 0.19 g/dl, 3.73 ± 0.21 g/dl, respectively). Treatment with prednisolone (3mg/kg) and NBF (200 mg/kg) significantly prevented decrease in serum total protein level (6.62 ± 0.21 g/dl, 6.98 ± 0.15 vs. 5.62 ± 0.14 g/dl respectively, $p < 0.05$) and albumin level (3.04 ± 0.19 g/dl, 2.95 ± 0.23 g/dl vs. 2.36 ± 0.17 respectively, $p < 0.05$) compared with positive control group.

Effect of NBF on serum nephrotoxicity markers

Serum creatinine (Figure 7E) and blood urea nitrogen (Figure 7F) levels were significantly increased in the DOX treated nephritic rats compared to vehicle control group (0.76 ± 0.042 mg/dl, 42.1 ± 3.78 mg/dl vs. 0.65 ± 0.035 mg/dl, 30.9 ± 2.87 mg/dl, respectively, $p < 0.05$). Treatment with prednisolone (3mg/kg) and NBF (200 mg/kg) significantly prevented increase in serum creatinine level (0.65 ± 0.029 mg/dl, 0.66 ± 0.020 mg/dl vs. 0.76 ± 0.042 mg/dl respectively, $p < 0.05$) and BUN level (32.9 ± 3.6 mg/dl, 30.8 ± 2.9 mg/dl vs. 42.1 ± 3.78 mg/dl, respectively, $p < 0.05$) as compared with positive control group.

Effect of NBF on Body and kidney weight

As shown in Figure 8, the body weight (BW) was decreased in DOX treated positive control rats compared to vehicle control rats (223.3 ± 4.05 gm vs. 250.2 ± 5.17 gm). Although the absolute kidney weight (KW) was unchanged (1.427 ± 0.069 gm vs. 1.380 ± 0.063 gm) but the ratio of KW to BW was increased in DOX treated positive control rats (639.1 ± 35.5 , mg/100gm vs. $551.6 \pm$

22.4 , mg/100gm) compared to vehicle control rats. Treatment with prednisolone (3mg/kg) and NBF (200 mg/kg) significantly prevented the decrease in BW (256.5 ± 7.01 , 248.2 ± 6.41 vs. 223.3 ± 4.05) and increase in KW/BW ratio (556.9 ± 34.2 , 574.6 ± 25.5 vs. 639.1 ± 35.5), while there was no any significant change observed in KW (1.425 ± 0.071 , 1.425 ± 0.038 vs. 1.427 ± 0.069) compared to positive control rats.

Tissue biochemical assay

As shown in Figure 9, DOX significantly increased MDA level (17.4 ± 1.45 μ g/mg tissue protein vs. 6.68 ± 0.89 μ g/mg tissue proteins) compared to vehicle control group. Moreover, there was also decreased in activities of SOD (0.204 ± 0.07 UA/mg tissue protein vs. 0.547 ± 0.10 UA/mg tissue protein), CAT (11.2 ± 3.94 UA/mg tissue protein vs. 50.9 ± 11.9 UA/mg tissue protein) and GSH (6.28 ± 3.11 μ g/mg tissue protein vs. 23.9 ± 4.40 μ g/mg tissue proteins) was observed in positive control group compared to vehicle control group. NBF (200mg/kg) significantly decreased an MDA level (10.5 ± 4.17 μ g/mg tissue protein vs. 17.4 ± 1.45 μ g/mg tissue protein) compared to positive control group. Furthermore, treatment with NBF (200mg/kg) significantly prevent decreased in activities of CAT (35.9 ± 6.99 vs. 11.2 ± 3.94 UA/mg tissue protein), GSH (20.4 ± 4.86 vs. 6.28 ± 3.11 μ g/mg tissue protein) and SOD (0.504 ± 0.10 vs. 0.204 ± 0.07 UA/mg tissue protein) compared to positive control group. The effect of NBF (200mg/kg) was found to be comparable with prednisolone (3mg/kg).

Histopathological evaluation

The vehicle control group show morphologically normal glomeruli (GL) and tubules (Figure 10a). DOX treated positive control group showed moderate to severe grade tubular vacuolization (V), global glomerular sclerosis (GLS) and focal infiltration of inflammatory cells (I) (Figure 10b). However, treatment with prednisolone (3mg/kg) showed mild glomerular sclerosis and showed minimal degree infiltration of inflammatory cell with no evidence of vacuolization (Figure 10c). NBF-100 treated rats showed mild to moderate tubular vacuolization, glomerular sclerosis and infiltration of inflammatory cells (Fig.10d). NBF-200 treated rats exhibited minimal glomerular sclerosis and infiltration of inflammatory cells with no evidence of vacuolization (Figure 10e).

Acute toxicity

A single oral administration of 2000 mg/kg of NBF did not produce any sign of acute toxicity or mortality. Thus, NBF were found to be safe at the single oral dose of 2000 mg/kg. Hence 1/10th of the maximum dose administered (i.e., 200 and 100 mg/kg, p.o. respectively) were selected as the therapeutic dose in this present study.

Table 1: HPTLC fingerprinting of NBF at 254 nm

Peak #	Start		Max			End		Area	
	Rf	height	Rf	height	max %	Rf	height	area	% area
1	0.24	10.2	0.29	164.1	47.97	0.33	29.6	4879.4	46.68
2	0.33	29.6	0.37	147.8	43.19	0.43	2.8	4777.5	45.71
3	0.69	2.1	0.74	13.1	3.84	0.75	11.8	334.8	3.20
4	0.75	11.8	0.77	17.1	5.00	0.79	10.2	460.1	4.40

Figure 1: Mechanisms of NBF against doxorubicin induced nephrotic syndrome

Figure 2: UV-visible spectra of NBF

Figure 3: HPTLC chromatogram of NBF at 254 nm

Figure 4: HPTLC spectral scan of component 1 and component 2 at 200 nm to 800 nm

Figure 5: Free radical scavenging potential and lipoxigenase inhibition effect of NBF

Figure 6: Effect of NBF on urine protein excretion (A) and urine volume (B) in DOX treated rats.

After 2 days from DOX (7 mg/kg, i.v) administration, rats were treated with prednisolone (3mg/kg), NBF (100 or 200 mg/kg) by a gavage method once daily for 28 days. All Values are means $\pm$ S.D (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test: *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group

Figure 7: Effect of NBF on serum levels of cholesterol (A), triglyceride (B), total protein (C), albumin (D), creatinine (E) and BUN (F) in DOX treated rats.

After 2 days from DOX (7 mg/kg, i.v) administration, rats were treated with prednisolone (3mg/kg), NBF (100 or 200 mg/kg) by a gavage method once daily for 28 days. All Values are means $\pm$ S.D (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test: *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group

Figure 8: Effect of NBF on body weight (BW, A) kidney weight (KW, B) and the ratio of KW to BW (KW/BW, C) in DOX treated rats.

After 2 days from DOX (7 mg/kg, i.v) administration, rats were treated with prednisolone (3mg/kg), NBF (100 or 200 mg/kg) by a gavage method once daily for 28 days. All Values are means $\pm$ S.D (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test: *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group

Figure 9: Effect of NBF on lipid peroxides (MDA, A), reduced glutathione (GSH, B), superoxide dismutase (SOD, C), catalase (CAT, D) activities in DOX treated rats.

After 2 days from DOX (7 mg/kg, i.v) administration, rats were treated with prednisolone (3mg/kg), NBF (100 or 200 mg/kg) by a gavage method once daily for 28 days. At the end of experiment, the kidneys samples were collected and oxidant and antioxidant parameters in renal tissue were performed (a) Vehicle control, (b) Positive control, (c) Prednisolone (3mg/kg), (d) NBF-100 mg/kg, (e) NBF-200 mg/kg All Values are means $\pm$ S.D (n=6). Significance was determined by One-Way ANOVA followed by Bonferroni Multiple Comparisons Test: *: $p < 0.05$ when compared with positive control group. #: $p < 0.05$ when compared with vehicle control group

10a. Vehicle control

10b. Positive control

Figure 10: Effect of NBF on histopathology (X-100).

After 2 days from DOX (7 mg/kg, i.v) administration, rats were treated with prednisolone (3 mg/kg), NBF (100 or 200 mg/kg) by a gavage method once daily for 28 days. At the end of experiment, the kidney samples were collected and histopathological studies were performed (10a) Vehicle control, (10b) Positive control, (10c) Prednisolone (3 mg/kg), (10d) NBF-100 mg/kg, (10e) NBF-200 mg/kg

DISCUSSION

The anti-nephritic activity of *Butea monosperma* (BM) flowers was evaluated by using doxorubicin (DOX) induced nephrotic syndrome. DOX-induced nephrotic syndrome has proven to be a very reproducible model and this model also has been useful in testing the efficacy of various therapeutic strategies to prevent or retard progressive focal segmental glomerulosclerosis (FSGS)²⁴. DOX causes chronic nephrotoxicity via several mechanisms and oxidative stress has been suggested to play an important role in DOX nephropathy. It is reported that oxidative stress may induce the injury of glomerular filtration membranes through endothelial cell injury, destroying of electrostatic barrier, and podocyte injury²⁵. Reactive oxygen species may directly damage lipid membranes, altered metabolism of prostaglandins and may also mediate the activation of genes for some pro-inflammatory cytokines (TNF- α , IL-1) through the stimulation of transcription factor NF- κ B. Cumulative evidence suggests that some herbal plants, including *Astragali radix*, *Tripterygium wilfordii* and *Ligusticum wallichii* have a beneficial role in slowing the renal disease. The mechanisms are multiple and include anti-oxidant, anti-inflammatory and inhibition of TGF- β

overproduction²⁶. The results obtained from our studies have shown antioxidant and anti-inflammatory activity of NBF is because of its potential to scavenge stable DPPH radicals and lipoxygenase enzyme inhibitory activity. This indicates that the NBF is more useful in the treatment of various inflammatory diseases. The UV visible spectroscopy and HPTLC fingerprinting for NBF suggested the presence of butrin and isobutrin as a major active constituents. The antioxidant and anti-inflammatory potential of NBF could be assigned to the presence of butrin and isobutrin. Proteinuria is the primary clinical manifestation of FSGS. It has been reported that increase in 24h urinary protein excretion and decrease in 24h urine output was observed in DOX treated rats²⁷. Similarly, in our study we have found that DOX (7 mg/kg i.v.) treatment markedly increased 24 h protein excretion, accompanied by a decrease in urine volume. Treatment with NBF (200mg/kg) significantly inhibited the degree of proteinuria as compared to positive control group. This effect was found to be comparable with standard drug prednisolone (3mg/kg). The selection of prednisolone and its dose was based on the protective effect of prednisolone against the DOX induced nephropathy²⁸ and nephrotoxic serum nephritis in the rat²⁹. NBF prevented decrease in the urine volume when

compared with positive control group but this effect was not statistically significant. The mechanism by which NBF provides significant protection against proteinuria is unclear. In agreement with early reports, NF- κ B plays a central role in the DOX induced nephrotic syndrome³⁰. Butrin and isobutrin present in NBF inhibits the NF- κ B activation and down regulates the production of TNF- α , IL-6 and IL-8³¹. Therefore, decrease in severity of pathogenesis of glomerulonephritis in the NBF treated groups may be attributed to the presence of butrin and isobutrin as major constituents. Moreover, our result showed that serum total protein and albumin levels were significantly decreased in DOX treated rats. Treatment with NBF caused significant increased serum total protein and albumin levels compared to positive control group. Dyslipidemia is also one of the main phenotypes in DOX-induced nephrotic syndrome in the rats. Proteinuria decreases tissue lipoprotein receptor levels resulting in altered lipoprotein structure and increasing lipid levels³². Consistent with previous reports, our present study confirmed that DOX increased serum cholesterol and triglycerides levels. In the present study, treatment with NBF improved DOX induced hypercholesterolemia and hypertriglyceridemia. The results of this study indicate the ability NBF to inhibit proteinuria could have additional beneficial effects in ameliorating hyperlipidemia. DOX causes impaired renal function with significant increase in blood urea nitrogen (BUN) and serum creatinine levels. Our results showed that nephrotoxicity markers like serum BUN and creatinine levels were significantly elevated in DOX treated nephritic rats compared to vehicle control rats. In the present study, NBF significantly inhibited the rise in serum BUN and creatinine levels. Long-term nephrotic syndrome may induce changes in body weight and kidneys weight. In this study untreated nephritic rats shows significant loss in body weight where as significant increase in body weight was observed in NBF and prednisolone treated group. A number of experiments have suggested that renal antioxidant enzyme activities are decreased after DOX injection in rat. In our study, we reported that the activities of antioxidant enzymes (CAT, SOD, and GSH) were significantly decreased and MDA levels increased in DOX treated rats, suggesting an increased oxidative stress. However, treatment with NBF significantly elevated the levels of antioxidant enzymes and decreased renal MDA levels. Histopathological evaluation revealed moderate to severe grade tubular vacuolization and glomerular sclerosis in DOX treated positive control group. Marked reduction in glomerular sclerosis with no evidence of vacuolization was observed with NBF treated rats. In conclusion, results revealed that NBF can bring about a wide range of improvements in the pathological conditions related to nephrotic syndrome, and can exert antinephritic activity. Further relevant studies are necessary to determine the mechanism by which NBF exhibit antinephritic potential. Results of our present studies support to the hypothesis that flowers of *Butea monosperma* Lam. (Fabaceae) could be of value in the treatment of glomerulonephritis, either as alternatives or as adjuncts to conventional therapy.

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