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ANTIOXIDANT ACTIVITY INDUCED BY METABOLIC PATHWAY ALTERATIONS DUE TO FRUCTOSECHANGES IN HYPERTENSION IN WISTAR RATS WITH *NIGHT-FLOWERING JASMINE*

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ABSTRACT

The present work aims to study the potential effect of antioxidant rich methanolic leaf extract of *Night Flowering Jasmine* (MLNFJ) on colonic motility and histopathological observations in fructose (10%) induced hypertensive rats. The study was conducted on 60 Male Albino Wistar rats (110-150g). Rats were randomly divided into a group of six, each group containing 10 animals. Group I was considered as normal control which received chow pellets and normal drinking water *ad libitum* for 6 weeks. Group II received fructose (10%) solution instead of normal drinking water for 6 weeks. Group III, IV, V received fructose (10%) solution instead of drinking water *ad libitum* and MLNFJ at different doses (100, 200, 400 mg/kg p.o) for 6 weeks. Group VI received fructose (10%) solution instead of drinking water *ad libitum* and Enalapril at a dose of 10mg/kg p.o for 6 weeks. The cumulative concentration-response curve (CCRC) of Ang II was shifted towards the right by MLNFJ treatment using an isolated strip of rat ascending colon. MLNFJ treatment increasedthe contractile characteristics of the rat ascending colon in the CCRC of ACh as compared to the fructose-treated group. Histopathological changes observed in fructose (10%) group showed cloudy swelling in renal tubules, sclerotic glomerulus and renal hypertrophy. The liver of the fructose (10%) group showed the presence of macro vesicular steatosis, fat accumulation and congestion of blood sinusoids around the central vein. The aorta of the fructose group showed increased thickness of tunica media. Heart of fructose (10%) group shows vacuolation of cardiomyocytes. MLNFJ treatment reduced fructose-induced tissue damage due to the consequence of metabolic syndrome. The total phenolic content of MLNFJ was found to be 42.91µg gallic acid equiv./mg of extract. MLNFJ is rich in flavonoids and therefore has powerful antioxidant properties. The findings show that by battling oxidative stress caused by fructose (10%) and reducing Ang II activity, MLNFJ may be able to prevent the development of high blood pressure caused by fructose.

KEYWORDS Fructose, *Night Flowering Jasmine* (NFJ), metabolic syndrome, hypertension, oxidative stress, kidney, liver, aorta, heart.

INTRODUCTION

Hypertension is currently one of the major risk factors for cardiovascular, neurological and renal events. Several studies demonstrated that excessive and chronic ingestion of ethanol causes cardiomyopathy, cardiac arrhythmias, heart failure and hypertension.[1-3] Similarly, numerous studies also indicate that diets high in carbohydrates, particularly sugars and even more particularly sucrose and fructose increase the risk of cardiovascular diseases including hypertension.[4] It has been reported that some metabolic abnormalities such as hyperinsulinemia, insulin resistance and hypertriglyceridemia as well as hyperactivity of the sympathetic nervous system and oxidative stress were frequently associated with the pathogenesis of both ethanol and sucrose induced-hypertension.[5] It is well known that hypertension can often lead to lethal complications if left untreated.[6] In spite of the large number of modern drugs, people largely use complementary and alternative medicine to prevent and cure illness[7] for curiosity and also the idea that combining it with conventional treatment would help.[8] A holistic or a spiritual health view and the belief that herbs are natural (and thus safe) also seem to be associate with the use of alternative medicine.[9] Traditionally, many of the folk remedies of plant origin

have long been used for the treatment of various ailments, usually as mixture of many plants in combination with honey, palm oil or limestone.[10] Therefore, there is an urgent need to develop new and effective drugs for the treatment of hypertension. *Night Flowering Jasmine* (NFJ) (Oleaceae) is widely distributed along subtropical, tropical to sub-Himalayan regions in the South East Asia. It has been extensively used as a therapeutic agent in the Ayurvedic healing traditions of South Asia. Traditionally used to treat sciatica, arthritis and malaria, however, reports from the literature have also indicated that the leaf oil from NFJ include hepatoprotective, anti-leishmanial, antiviral and antifungal activities. Local people of Andhra Pradesh, India use the whole tree for cancer, root for fever, sciatica, anorexia and bark as expectorant. Other research into the leaf extract of the NFJ have shown considerable immunological activity and water-soluble ethanol extracts from the leaves are reported to possess anti-inflammatory activity which, however, accompany development of ulcers in test rats. In addition, anti-oxidant studies on the acetone soluble ethyl acetate leaf extracts have shown significant activity against hydroxy and superoxide radicals, as wells as peroxide scavenging activity. Likewise, activity-guided isolation of compounds in NFJ flowers yielded iridoid glucosides that have exhibited antiplasmodial activity against Plasmodium falciparum. Besides these compounds, 4-hydroxyhexahydro benzofuran-7-one, nyctoside A, arborside C, arborside D, 6-hydroxyloganin, arbortristoside A, arbortristoside B and nyctanthoside have been reported.[11,12] So, the present investigations were undertaken to evaluate the protective activity of antioxidant rich MLNFJ on colonic motility and histopathology of kidney, liver, heart and aorta in fructose-induced hypertensive rats.

MATERIAL AND METHOD

Plant material

Fresh leaves of *Night Flowering Jasmine* were purchased locally and authenticated by the Department of Botany, Dr. APJ Abdul Kalam University, Indore (M.P.) and the specimen voucher assigned. After that herbarium file was submitted in Department.

Experimental animals

60 male Albino Wistar rats (110-150g) purchased from Mumbai Veterinary college (Mumbai, Maharashtra) were used in the current experimental study. The animals were kept under standard laboratory condition temperature 25 ± 1°C, Relative humidity 45-55 % and Photoperiod (12 h dark/ 12 h light). The protocol of the study was approved by Institutional Animal Ethical Committee (IAEC).

Drugs and chemicals

Fructose, petroleum ether (60-80°C), methanol and gallic acid were obtained from Modern Sciences Pharmaceuticals, Nashik. Acetylcholine (ACh), angiotensin II (Ang II), urethane, were obtained from Sigma, Mumbai. Fructose (10%), ACh, Ang II and urethane were prepared freshly using distilled water. ECA was dissolved in water and given orally according to the experimental protocol.

Preparation of the extract

Leaves were washed and dried in sunlight. The powder obtained(1kg) was defatted using pet ether (60-80°C) and extracted with methanol by hot extraction method using soxhlet apparatus. The methanolic extract obtained was allowed for distillation to remove the excess quantity of methanol and to concentrate the product into a dry mass. The percent yield value was found to be 12.89 % w/w.[13]

Qualitative phytochemical analysis of plant extract

The MLNFJ extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate.[14,15] The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Total phenol determination

The total phenolic content of the plant extract was determined using spectrophotometric method (UV- 2600, Shimadzu). The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of NFJ, 2.5ml of 10 % Folin-Ciocalteu's reagent. Blank was concomitantly prepared, containing 0.5ml methanol, 2.5ml of 10% Folin-Ciocalteu's reagent. The samples were then incubated at room temperature for 45 min. The absorbance was recorded at 750 nm. Phenolic contents were measured using a standard curve obtained from various concentrations of gallic acid and expressed as microgram per milligram of gallic acid equivalents.[16]

Experimental protocol

A high fructose diet (fructose 10%, w/v) ad libitum for 6 weeks was used to induce hypertension in male Wistar rats (150–200 g). Every two days, fructose solution was made by dissolving fructose in distilled water.[17] 60 Albino Wistar rats were randomly divided into a group of six, each group containing 10 animals.

Group I was considered as normal control which received chow pellets and normal drinking water ad libitum for 6 weeks.
Group II received fructose (10%) solution instead of normal drinking water for 6 weeks.
Group III received fructose (10%) solution instead of drinking water ad libitum and MLNFJ at a dose of 100mg/kg p.o for 6 weeks.
Group IV received fructose (10%) solution instead of drinking water ad libitum and MLNFJ at a dose of 200mg/kg p.o for 6 weeks.
Group V received fructose (10%) solution instead of drinking water ad libitum and MLNFJ at a dose of 400mg/kg p.o for 6 weeks.

Group VI received fructose (10%) solution instead of drinking water ad libitum and Enalapril at a dose of 10mg/kg p.o for 6 weeks.

Relative organ weight (liver, kidney and heart)
Body weight of each animal was determined before treatment and before sacrifice. The liver, kidney and heart of each animal were dissected and weighed. Relative organ weight (ROW) was determined by using the formula.
ROW =Absolute organ weight (gm)/ Body weight of the rodents the day of sacrifice (gm) ×100

Measurement of blood pressure by the invasive (direct) method
After the treatment schedule was completed, a subset of five Rodents from each group was used for invasive blood pressure measurements described by Subramani Parasuramanetal (2012) Urethane (1200 mg/kg, i.p.) was used to anesthetize the rats. For blood pressure measurement left common carotid artery was cannulated using polyethylene tubing which was prefilled with heparinized saline (100 IU/ml) to prevent clotting. The cannula was connected to a pressure transducer by a direct method onto a chart data system (PowerLab4/35; AD Instruments, Australia).[18]

***Invitro* studies**
At the end of the treatment schedule, another subset of five Rodents was sacrificed. Rata sending colon was isolated and used for the cumulative concentration-response curve (CCRC) for Ang II[19] and Ach.[20] The physiological salt solution was made up of the following ingredients: (mM) NaCl, (118); KCl, (4.7); CaCl2, (2.5); MgSO4,(1.2); NaHCO3,(25); KH2PO4,(1.2) and glucose (11). The pH of the physiological salt solution was 7.4, maintained at 37ºC and aerated with carbogen (95 percent oxygen and 5 percent carbon dioxide). One end was attached to an aerator tube, while the other was attached to a lever. Each strip was given the optimum resting tension (1 g) and given 30 mins to equilibrate. The contractile response to each dosage of Ang II and Ach was measured for 60 seconds respectively.

Histopathological study
From individual groups kidneys, liver, aorta, and heart were isolated, weighed and fixed in 10% formalin.
Fixation: The process of autolysis virtually begins immediately after death. Therefore, rapid and adequate fixation after sampling is essential. This can be achieved by immersion of the tissue sample in an adequate volume off ixative solution. There are several methods off ixation including aldehydes, mercurials, alcohols, oxidizing agents, and picric acid derivatives. Tissue immersion in aldehyde (formaldehyde orglutar aldehyde) is the most frequently used fixation method in biomedical research.

Trimming: After fixation, tissue samples need to be properly trimmed to reach the adequate size and orientation of the tissue. This step is also important to reach a sample size that is compatible with subsequent histology procedures such as embedding and sectioning.

Pre-embedding: Pre-embedding is a sequential process that consists of dehydration of tissues in increased concentrations of alcohol solutions, then gradual replacement of alcohol by a paraffin solvent. Xylene has the advantage to be miscible in both alcohol and paraffin. As a result, the tissue sample is dehydrated and fully infiltrated by paraffin.

Embedding: After tissue has been dehydrated, cleared and infiltrated with embedding material like paraffin, agar, gelatin, which is then hardened. The mold is filled with melted paraffin and then immediately placed on a cooling surface. To trace each tissue specimen, the cassette with permanent tissue and study identification is placed on top of the metal base mold and incorporate dint he paraffin block before cooling. In this manner, the cassette will be used as a base of the paraffin block for microtome sectioning.

Sectioning: The objective of this step is to cut 4-5 mm-thick sections from paraffin blocks. The paraffin block is mounted on the microtome holder. Sections are cut as aribbon and are floated on a water bath maintained at45°C to stretch the paraffin section. A standard microscope glass slide is placed under the selected tissue section and removed from the water bath. Tissue sections are then allowed to dry, preferably in a thermostatic laboratory oven at 37°C.

Staining: There are many histochemistry staining techniques that can be applied to examine specific tissue or cells tructures. As most of the sedyes are water soluble, tissue sections should be rehydrated to remove paraffin (using xylene, alcohol solutions ending n water). Hematoxylin and Eosin (H&E) is the routine staining used to study histopathology changes in tissues and organs from animals in toxicity studies. Hematoxylin is a basic dye that has affinity for acid structures of the cell (mostly nucleic acids of the cell nucleus), and eosin is an acidic dye that binds to cytoplasm structures of the cell. As a result, H&E stains nuclei in blue and cytoplasm's in orange-red. After staining, a very thin glass should be placed over the tissue section to protect it and to enhance the optical evaluation of the tissue. This also allows tissue section storage for several years. Cover slipping process consists of gluing the cover slip glass over the tissue section on the microscope slide glass.[21]

Microscopic examination: Light microscopy was used to analyze the sections, and photos were captured at a resolution of 40x.

Statistics
For each group, the mean SEM values were determined. For statistical analysis, one-way ANOVA was performed, followed by Dunnetts multiple compare is on

tests. Statistical significance was de fined at a value of $p<0.05$.

RESULTS

Dried and powdered leaves of plant of NFJ was subjected to soxhlation extraction process with methanol solvent and yielded 12.89 % w/w. The results of qualitative phytochemical analysis of the crude powder leaf of NFJ were shown in Table 1. methanolic extracts of NFJ leaves showed the presence of flavonoids, carbohydrate, proteins and amino acids, saponins, tannins, steroids and alkaloids. The total phenol content of MLNFJ was found to be 42.91µg gallic acid equiv./mg of MLNFJ (Figure 1). Chronic administration of MLNFJ (100,200&400mg/kg/day, p.o.) for 6 weeks in fructose (10%) fed rats significantly ($p<0.05$) shifted the CCRC of Ang-II to the right with suppression of maxima as compared to CCRC of fructose (10%) fed rats on isolated ascending colon Table 3. Chronic administration of MLNFJ (400mg/kg/day, p.o.) for 6 weeks in fructose (10%) fed rats significantly ($p<0.05$) increased percent response of ACh as compared to the CCRC of fructose (10%) fed rats, for the isolated ascending colon Table 4.

Table 1: The phytochemical investigation for various chemical constituents in MLNFJ.

Chemical constituents	Name of the test	Procedure	Observation	Result
Alkaloids	Mayer's test	MLNFJ +few drops of Mayer'sreagent	Orange ppt	+++
Tannins	Ferric chloridetest	MLNFJ was stirred with 10mL of hot distilled water, filtered +ferric chloride	blue-green orgreen precipitate.	+++
Steroids	Salkowski test	MLNFJ +chloroform + a few drops of conc. H2SO4,shaked well and allowed tostand for some time.	Red colorappeared at the lowerlayer	++
Saponins	Frothing test	1ml MLNFJ filterate +1mldistilled water.shake vigarously	Persistent foam whichlasted for at least 15 minutes.	++
Flavanoids	Shinoda test	MLNFJ+5ml 95% ethanol+drops of conc.Hcl+0.5g magnesium turnings	Pink colouration	+++
Carbohydrates	Benedict's test	MLNFJ+5ml benedict's reagent. Boil for 2min andcool.	Red precipitate	+
Protein and amino acids	Ninhydrin test	2-5 drops of Ninhydrin solution were added to MLNFJ and boiled in a water bath for 1-2 minutes.	Blue colour	++

[+++ = highly present, ++ = moderately present, + = slightly present]

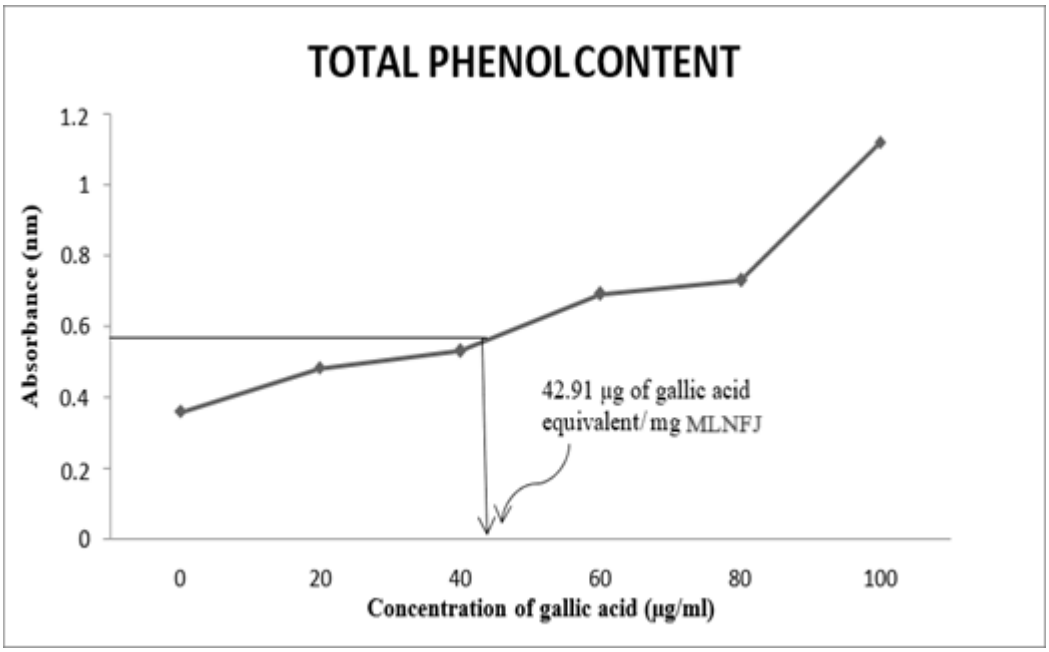


Fig. 1: Total phenolic content determination of MLNFJ by Folin-Ciocalteu's Method.

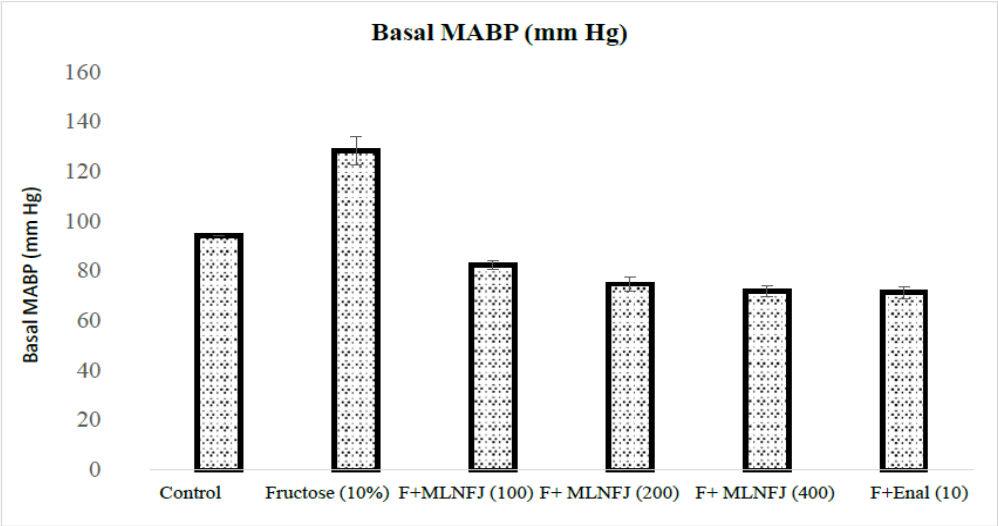


Fig. 2: Effect of MLNFJ (100, 200 and 400 mg/kg, p.o., for 6 weeks) on basal MABP (mm Hg)in Fructose (10%) treated hypertensive rats.

Table 2: Effect of MLNFJ (100, 200 and 400 mg/kg, p.o., for 6 weeks) on relative organweight in Fructose (10%) treated hypertensive rats.

Group (mg/kg) Parameters	**Control**	**Fructose (10%)**	**F+MLNFJ(100)**	**F+MLNFJ(200)**	**F+MLNFJ(400)**	**F+Enal (10)**
Liver weight (g/100 g BW)	3.474 ±0.14	4.464 ±0.20*	3.245 ±0.02#	3.306 ±0.09#	3.163 ±0.13#	2.850 ±0.11#
Heart weight (g/100 g BW)	0.389 ±0.02	0.449 ±0.02*	0.371 ±0.004#	0.362 ±0.008#	0.344 ±0.02#	0.304 ±0.01#
Left kidney weight (g/100 g BW)	0.391 ±0.02	0.434 ±0.02*	0.361 ±0.004#	0.360 ±0.008#	0.354 ±0.01#	0.308 ±0.009#
Right kidneyweight (g/100 g BW)	0.395 ±0.02	0.460 ±0.03*	0.384 ±0.01#	0.376 ±0.01#	0.337 ±0.02#	0.311 ±0.01#

All values are expressed as mean ±SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to the fructose- fed group. MLNFJ = Methanolic Extract of *Night Flowering Jasminear*. Enal = Enalapril, F =Fructose (10%). The liver, heart & left kidney weight significantly increased in rats fed with fructose (10%) compared to animals in the control group. Liver, heart & left kidney weight significantly decreased in MLNFJ (100,200&400 mg/kg) treated Fructose (10%) fed rats (Table 2).

Table 3: Effect of MLNFJ (100,200&400mg/kg/day, p.o.) for 6 weeks on CCRC of Ang-II on isolated ascending colon in fructose (10%) fed rats.

Treatmen tgroups (mg/kg)	**% Response and –Log M concentration of Ang-II**					
	9.01	8.71	8.41	8.24	8.11	7.81
Control	7.16 ±1.07	13.81 ±1.15	19.43 ±1.28	26.59 ±1.62	31.71 ±1.49	34.78 ±1.80
Fructose (10%)	44.24 ±1.66*	58.82 ±2.91*	75.19 ±3.72*	81.84 ±5.44*	93.35 ±5.17*	100 ±6.99*
MLNFJ (100)	9.20 ±1.24#	15.08 ±0.96#	21.48 ±0.86#	27.10 ±1.65#	34.27 ±2.45#	38.36 ±1.92#
MLNFJ (200)	6.13 ±0.20#	10.23 ±0.70#	17.39 ±0.67#	23.27 ±0.73#	27.87 ±1.06#	31.20 ±1.32#
MLNFJ (400)	6.13 ±0.80#	9.46 ±1.32#	15.08 ±1.68#	20.46 ±0.83#	24.04 ±0.96#	25.57 ±1.22#
Enalapril (10)	5.88 ±0.24#	10.23 ±0.54#	13.55 ±1.02#	16.87 ±1.11#	19.94 ±1.43#	22.76 ±1.15#

All values are expressed as mean ±SEM, n=5. All data are subjected to One Way ANOVA followed byDunnett's test. *

p<0.05 when compared to control and # p<0.05 when compared to fructose fed group.

Table 4: Effect of MLNFJ (100,200&400mg/kg/day, p.o.) for 6 weeks on CCRC of ACh onthe isolated ascending colon in fructose (10%) fed rats

Treatment groups (mg/kg)	% Response and –Log M concentration of ACh					
	4.6	3.86	3.56	3.38	3.26	2.96
Control	14.87 ±0.91	31.40 ±0.48	50.82 ±1.02	73.96 ±2.03	90.08 ±2.26	100 ±1.56
Fructose(10%)	14.04 ±1.2	20.24 ±1.65*	27.68 ±1.98*	34.71 ±2.49*	40.08 ±2.48*	43.80 ±2.39*
MLNFJ(100)	13.22 ±0.87	24.38 ±1.11	30.57 ±1.35	40.49 ±1.28	48.34 ±1.98	54.54 ±2.29
MLNFJ(200)	9.91 ±1.01	23.55 ±0.67	35.53 ±1.49	42.97 ±1.74	48.34 ±1.07	54.95 ±1.02
MLNFJ(400)	15.70 ±0.24	27.68 ±1.20	52.27 ±0.37#	68.18 ±0.83#	81.40 ±1.86#	92.97 ±1.73#
Enalapril(10)	14.46 ±1.04	26.44 ±1.46	40.08 ±1.93#	49.17 ±1.31#	63.22 ±1.46#	71.90 ±1.93#

All values are expressed as mean ±SEM, n=5. All data are subjected to One Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to fructose fed group.

Kidney histopathology

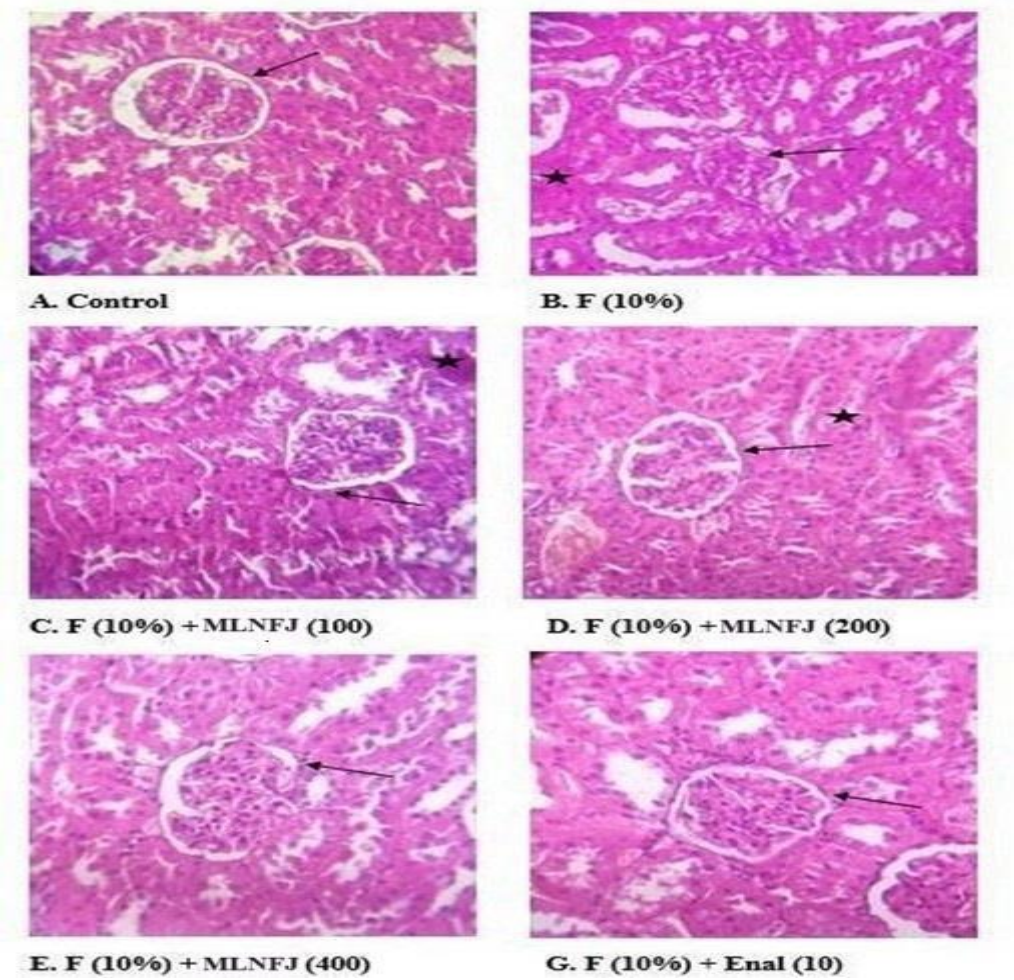
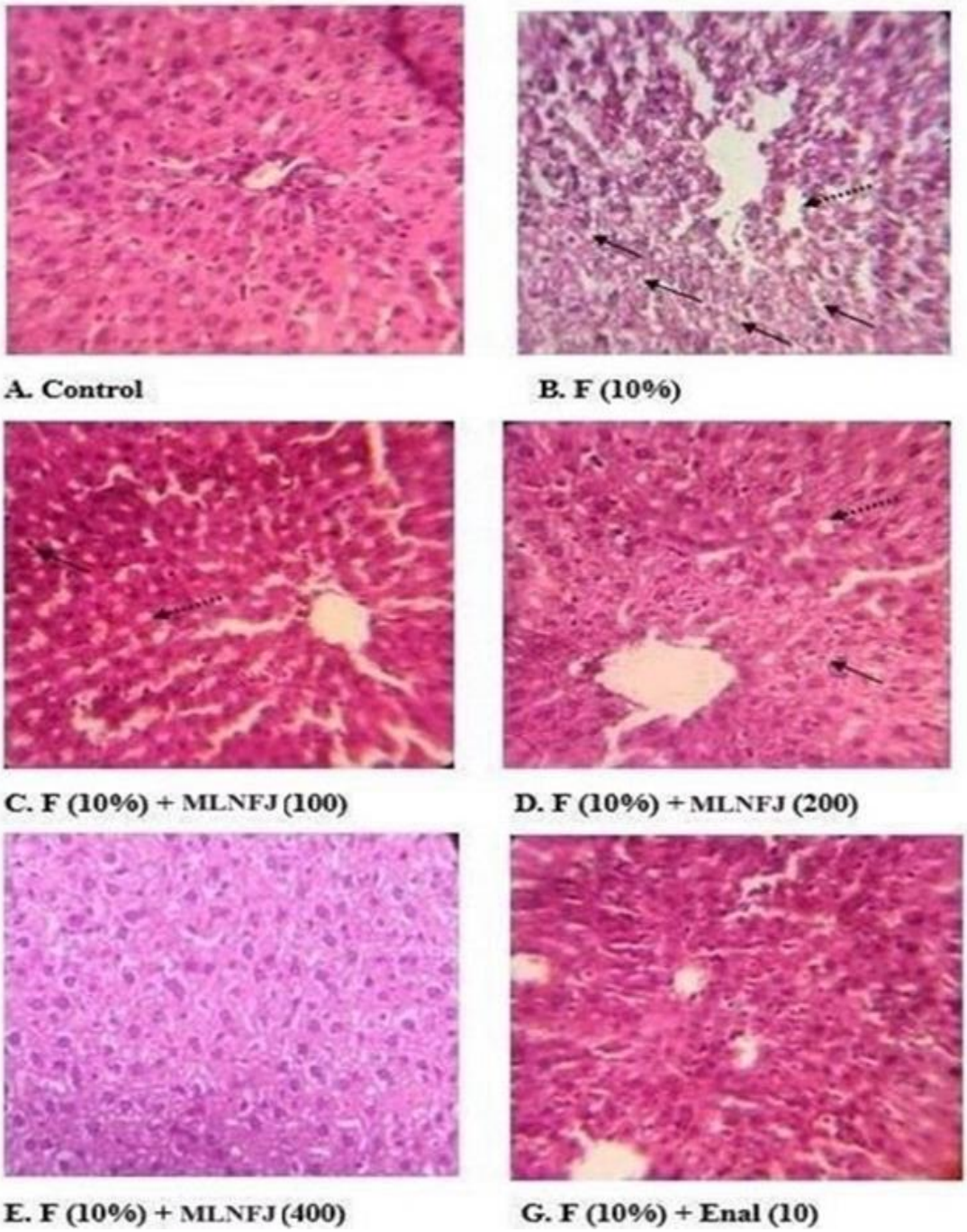


Fig . 3: A) Kidney of the control group showed the normal histological picture, normal glom 3: erulus (arrow). B) Kidney of Fructose (10%) group showed cloudy swelling in renal tubules(asterisks), clerotic glomerulus (arrow). C)&D) Kidney of MLNFJ (100&200 mg/kg) treated showed mild cloudy swelling in renal tubules(asterisks), normal glomerulus (arrow). E) &F) Kidney of MLNFJ (400 mg/kg) & Enal (10 mg/kg) group showed normal histological picture, normal glomerulus (arrow). (H&E, X 40). [MLNFJ-Methanolic extract of *Night Flowering Jasmine*, F- Fructose (10%),Enal-Enalapril.

Liver histopathology



Fig, 4: A) Liver of control rats showed normal hepatic lobules and hepatocytes with normalarchitecture. B) The liver of the Fructose (10%) group showed the presence of macrovesicular steatosis (Black arrows), fat accumulation (dotted arrow) and congestion of blood sinusoids around the central vein. C) Liver of MLNFJ (100 mg/kg) treated showed mild macrovesicular steatosis (Black arrows), fat accumulation (dotted arrow) and congestion of blood sinusoids around the central vein. D) Liver of MLNFJ (200 mg/kg) treated showed mild macrovesicular steatosis (Black arrows); fat accumulation (dotted arrow). E) &F) Liver of MLNFJ (400 mg/kg) & Enal (10 mg/kg) group showed normal hepatic lobules and hepatocytes with normal architecture. (H&E, X 40) [MLNFJ- Methanolic extract of *Night Flowering Jasmine*, F- Fructose (10%), Enal-Enalapril],

Aorta histopathology

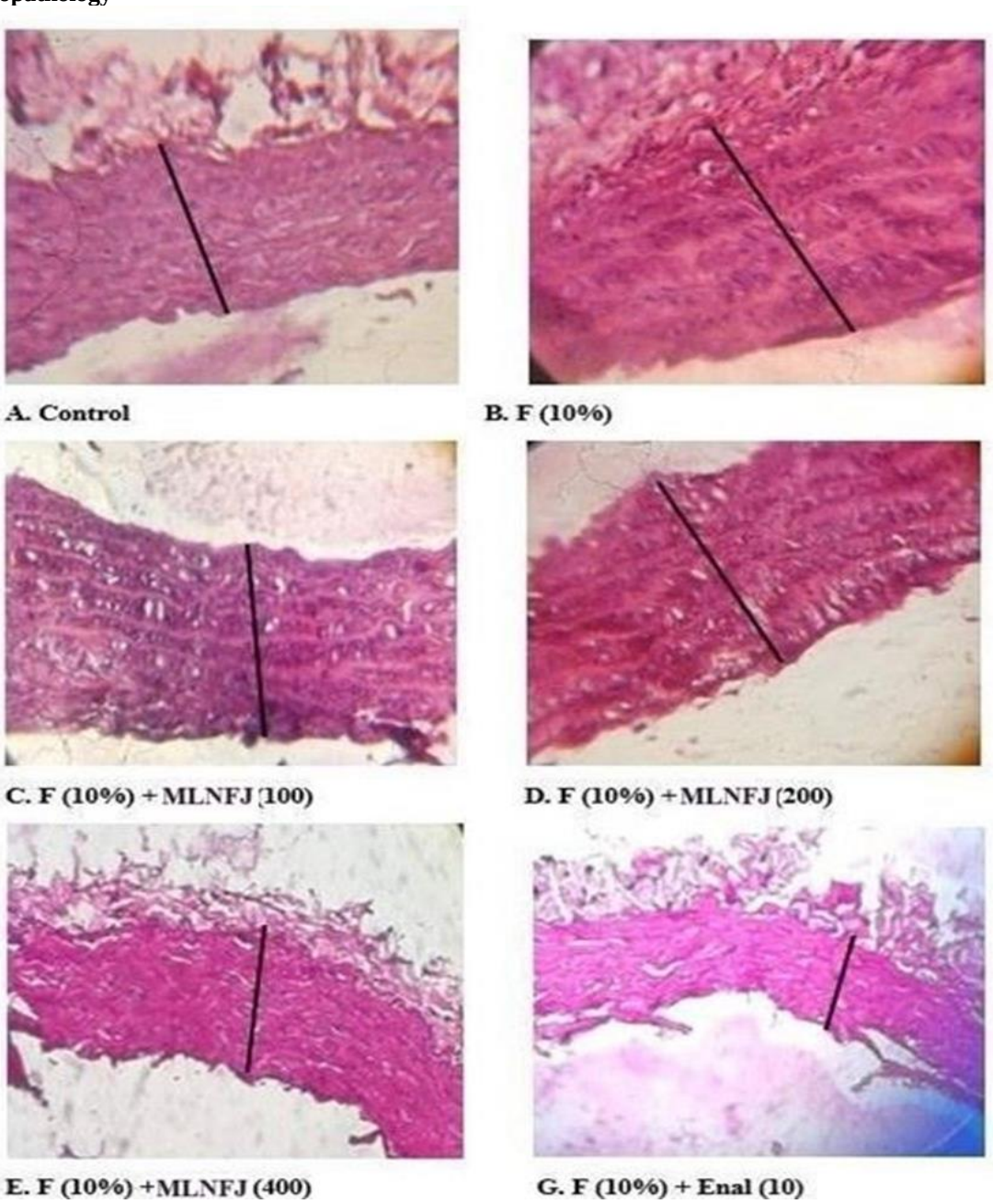


Fig. 5: A) Aorta of control rats B) Aorta of Fructose (10%) group showed increased thickness of tunica media (Blackline) C) & D) Aorta of MLNFJ (100 & 200 mg/kg) treated showed the mildly decreased thickness of tunica media (Blackline) E) Aorta of MLNFJ (400 mg/kg) treated showed a normal layer of tunica media (Blackline) D) Aorta of Enal (10 mg/kg) showed the decreased thickness of tunica media (Blackline), (figure: C, D, E, F were compared onlywith fructose 10% treated group). (H&E, X 40). [MLNFJ-Methanolic extract of *Night Flowering Jasminear* F- Fructose (10%), Enal-Enalapril]

Heart histopathology

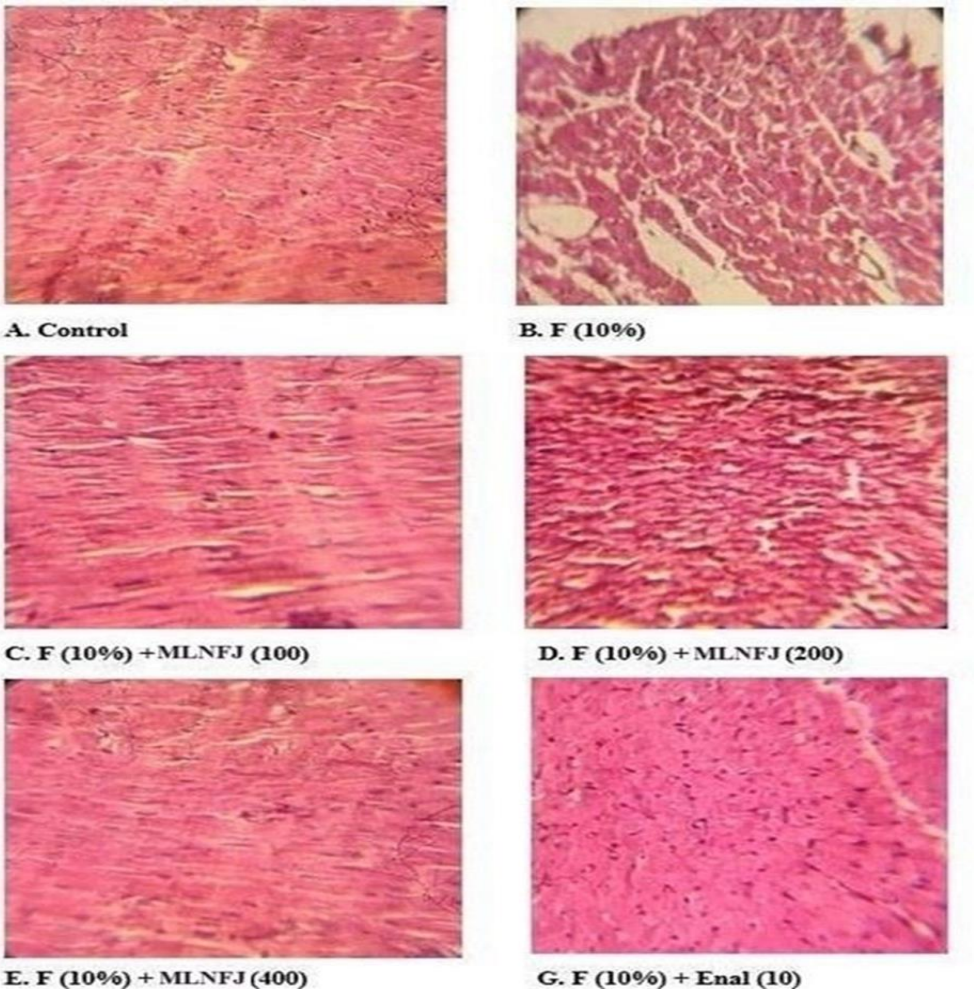


Fig. 6: A) Heart of the control group shows a normal histological picture. B) Heart of fructose 10% group shows vacuolation of cardiomyocytes (arrows)and mild hyaline degeneration (arrowhead). C), D), E) & F) Heart of MLNFJ (100,200&400 mg/kg) and Enal 10mg/kg treated showed mild hyaline degeneration (arrow head). (H&E, X 40). [MLNFJ-Methanolic extract of *Night Flowering Jasmine*, F- Fructose (10%), Enal-Enalapril.

Representative gross images of kidneys

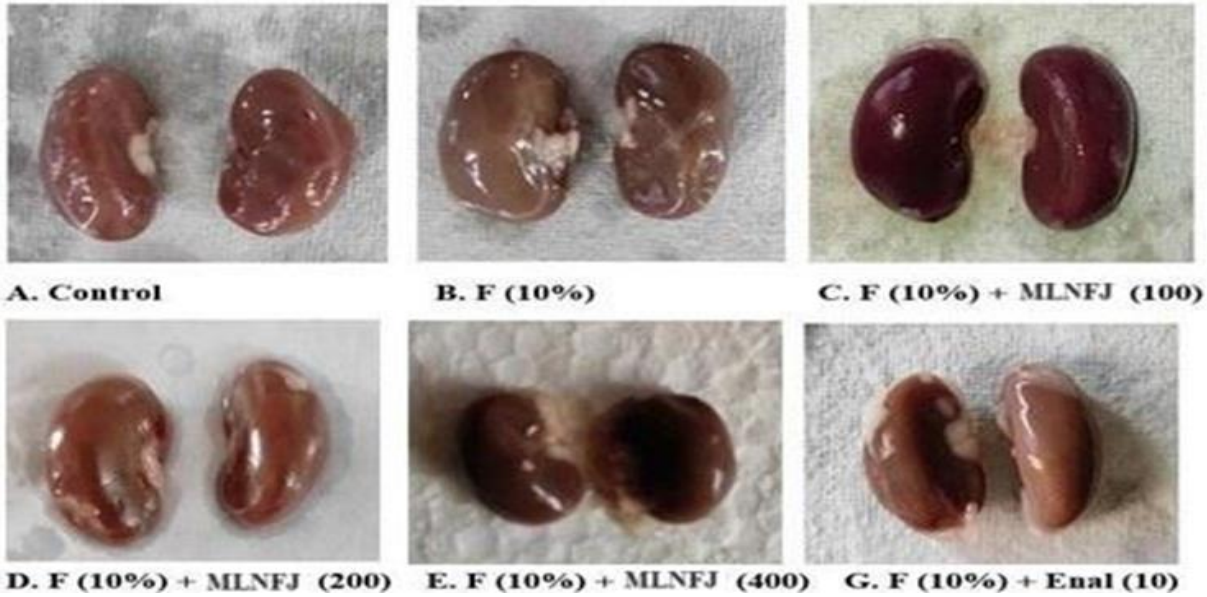


Fig. 7: A) Kidney of the control group. B) Kidney of Fructose (10%) treated group showing hypertrophy. C), D), E) and F) Kidney of MLNFJ (100, 200, &400 mg/kg) and Enal (10 mg/kg) treated groups. (MLNFJ-Methanolic

extract of *Night Flowering Jasmine*, F- Fructose (10%), Enal- Enalapril) Renal hypertrophy is observed in fructose-fed rats as compared to the control group. Renal hypertrophy is prevented by MLNFJ (100, 200, &400 mg/kg) and Enalapril (10 mg/kg) treatment as compared to fructose treated group (C, D, E&F).

DISCUSSION

The present study indicates the effect of antioxidant-rich methanolic extract of *Night Flowering Jasminear* (MLNFJ) on colonic motility and histopathological observations in the fructose (10%) induced hypertensive rats. Flavonoids are a diverse class of bioactive polyphenolic compounds found in a wide variety of dietary plants and herbs. Flavonoids have cardio-vasculo protective properties and may help prevent the development or progression of several cardiovascular disorders, especially hypertension.[22] Consumption of fructose may result in the buildup of advanced glycation end products (AGE) in smooth muscle cells, leading to a change in the contractile activity of the intestinal smooth muscles.[23] It has been found that fructose diet promotes sympathetic activity while decreasing parasympathetic activity.[24] Endothelin-1 (ET-1) and angiotensin II (Ang II), the most powerful vasoconstrictors, are elevated in fructose hypertensive rats, according to L. T. Tran et al. (2009). The presence of a link between the endothelin and renin-angiotensin systems, that could influence the development of fructose-induced hypertension is well established.[25] MLNFJ, which is rich in flavanoids, reduces fructose-induced hypertension in rats by inhibiting the production of ET-1 and Ang II. MLNFJ (100,200,400 mg/kg) shifts the CCRC of Ang II to the right, indicating an inhibitory action on Ang II receptors. In the CCRC of AChthe contractile properties of rat ascending colon are increased by MLNFJ (100,200,400 mg/kg) treatment as compared to fructose treated group. This indicates an improvement in parasympathetic activity which is decreased in fructose hypertensive rats. The higher MLNFJ dose (400mg/kg) appears to have a greater effect. The kidneys are responsible for long-term blood pressure management, any injury to this organ has the potential to exacerbate or prolong systemic hypertension. Fructose has been shown to have negative effects on renal tissue in various studies. In rats, high fructose consumption causes functional and structural renal impairments that are dose- and time-dependent. Two conditions are likely to have a negative impact on the kidneys: 1) the kidney receives an increased load of fructose by the augmented urinary excretion of this sugar when exposed to higher doses.[26]; and 2) The first and limiting enzyme in fructose metabolism, fructokinase (also known as ketohexokinase or KHK), is highly expressed in renal tissue, particularly in the portion of the proximal tubule.[27] Fructose administration in the food (60%) or drinking water (10%) caused hypertension, hyperuricemia, and hypertriglyceridemia, there is a progressive increase in these parameters as fructose intake increases. Furthermore, fructose causes kidney hypertrophy, glomerular hypertension, cortical vasoconstriction, and preglomerular arteriolopathy. The elevation in blood pressure was most likely caused by preglomerular vascular disease and fructose-induced cerebral vasoconstriction.[28] Furthermore, fructose caused proximal tubular hyperplasia, tubular cell proliferation, and focal tubular damage in normal rats, as evidenced by type III collagen deposition in the interstitium, an increase in -smooth muscle actin–positive myofibroblasts, and a rise in macrophage infiltration.[29] A well- known stimulus that causes vasoconstriction and so promotes systemic hypertension is minor damage to renal tissue.[30] In the present study, kidney of the control group showed the normal histological picture, normal glomerulus. Kidney of Fructose (10%) group showed cloudy swelling in renal tubules, sclerotic glomerulus. Kidney of MLNFJ (100&200 mg/kg) treated showed mild cloudy swelling in renal tubules and normal glomerulus. Kidney of MLNFJ (400 mg/kg) & Enal (10 mg/kg) group showed normal histological picture and normal glomerulus. Fructose is linked to biochemical changes that can lead to metabolic syndrome (MetS), nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes. It is processed by the liver, which induces lipogenesis. Hepatic insulin resistance and dyslipidemia are caused by the triglycerides. The Maillard reaction may be involved in the formation of fructose-derived. advanced glycation end products (AGEs). Fructose is ten times more reactive than glucose, but its plasma concentration is just 1% of glucose. Fructose is elevated in several tissues of diabetic patients where the polyol pathway is active, reaching the same order of magnitude as glucose. It's possible that fructose's high reactivity, either directly or through its metabolites, contributes to intracellular AGE development and vascular complications.[31] Increased fructose consumption has been associated to the development of obesity, dyslipidemia, and impaired glucose tolerance in clinical investigations, and a role in the development of hepatosteatosisis hypothesized. Advanced glycation end products (AGEs) are formed when fructose undergoes a nonenzymatic reaction.[32] The liver is the primary organ in which fructose metabolism occurs fast, resulting in increased hepatic synthesis of glycogen and free fatty acids.[33] Nonalcoholic fatty liver disease(NAFLD) is the most frequent condition in industrialized countries, affecting 15–20% of the general population.[34] Epidemiological studies have linked NAFLD to excessive fructose consumption.[35, 36] MLNFJ has strong antioxidant characteristics that may help to minimize oxidative stress and prevent the generation of free radicals. In fructose hypertensive rats, administration of MLNFJ (100,200,400 mg/kg) reduced fat deposition in the liver. Liver of control rats showed normal hepatic lobules and hepatocytes with normal architecture. The liver of the Fructose (10%) group showed the presence of macrovesicularsteatosis, fat accumulation and congestion of blood sinusoids around the central vein. Liver of MLNFJ (100 mg/kg) treated showed mild

macrovesicular steatosis, fat accumulation and congestion of blood sinusoids around the central vein. Liver of MLNFJ (200 mg/kg) treated showed mild macrovesicular steatosis; fat accumulation. Liver of MLNFJ (400 mg/kg) & Enal (10 mg/kg) group showed normal hepatic lobules and hepatocytes with normal architecture. Chronic consumption of high-fructose corn syrup (HFCS) raises uric acid production in the liver. Hypertension is caused by an increase in uric acid levels, which causes oxidative stress and endothelial dysfunction.[37-41] HFCS raises free oxygen radicals and lowers nitric oxide (NO) production. NO synthase activity is also reduced in the aorta of fructose-fed rats.[42-45] The damage of vascular endothelial cells caused by oxygen free radicals lowers NO production and NOsynthase activity.[46, 47] Endothelial damage occurs as a result of low NO levels. Hypertension can arise when NO levels are reduced, resulting in decreased vascular relaxation.[48,49] Kho et al. evaluated histological alterations in the thoracic aorta of high fructose-fed rats by staining with H&E. When compared to the control group, the endothelial layers of the thoracic aorta were roughened, layers of the tunica intima media were increased, and adipocytes were hypertrophic.[50] In this study, aorta of Fructose (10%) group showed increased thickness of tunica media. Aorta of MLNFJ (100 & 200 mg/kg) treated showed the mildly decreased thickness of tunica media. Aorta of MLNFJ (400 mg/kg) treated showed a normal layer of tunica media. Aorta of Enal (10 mg/kg) showed the decreased thickness of tunica media. Insulin resistance is linked to increased oxidative stress, endothelial dysfunction, and cardiovascular disease.[51] Thus, fructose-rich diets are major cause of heart disease, as indicated by ventricular dilatation and hypertrophy, impaired ventricular contractile performance, and inflammation.[52] The administration of high fructose disrupted the normal histology of rats' kidneys, aorta, and hearts. The fructose group's heart showed mild hyaline degeneration, necrosis, and multifocal regions of mononuclear cell aggregation and cardiomyocyte vacuolation.[53] In this study, heart of the control group showed a normal histological picture. Heart of fructose (10%) group shows vacuolation of cardiomyocytes and mild hyaline degeneration. Heart of MLNFJ (100,200&400 mg/kg) and Enal 10mg/kg treated showed mild hyaline degeneration. MLNFJ has potent antioxidant properties, which may reduce oxidative stress, and suppress free radical formation. In fructose hypertensive rats, administration of MLNFJ (100,200,400 mg/kg) reduced fat deposition in the liver, reduced aortic wall thickening, and prevented glomerulosclerosis and cardiomyocyte vacuolation. MLNFJ (100,200,400 mg/kg) has shown a protective effect on histology of kidney, liver, aorta and heart as compared to fructose (10%) treated rats. The metabolic syndrome caused by fructose is linked to glomerular hypertension and renal microvascular abnormalities in rats. In fructose (10%) fed rats, glomerular hypertension, renal hypertrophy and cortical vasoconstriction are all indicators of renal dysfunction.[54,55] Renal hypertrophy was seen in fructose-fed rats compared to control rats, and it was reversed by MLNFJ(100,200,400 mg/kg) and enalapril (10 mg/kg) administration. Thus, MLNFJ has antioxidant activity and reverses metabolic syndrome in fructose induced hypertensive rat model.

CONCLUSION

Methanolic leaf extract of *Night Flowering Jasmine* (MLNFJ) could prevent the development of hypertension in rats caused by fructose (10%), probably by battling oxidative stress caused by fructose and Ang II, as well as decreasing Ang II activity. The histopathological studies also revealed the protective effect of MLNFJ on kidney, liver, aorta and heart. The effect appeared to be more pronounced at a dose of MLNFJ 400 mg/kg.

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