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## Effect of morin, a flavonoid against DOCA–salt hypertensive rats: a dose dependent study

Prahalthan P, Kumar S, Raja B\*

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar–608 002, Tamil Nadu, India

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## ABSTRACT

**Objective:** To determine the protective effect of morin, a flavonoid against deoxycorticosterone acetate (DOCA)–salt induced hypertension in male Wistar rats. **Methods:** Hypertension was induced in uninephrectomized rats by weekly twice subcutaneous injection of DOCA (25 mg/kg bw) and 1% NaCl in the drinking water for six consecutive weeks. Effect of morin against DOCA–salt induced hypertension was evaluated by measuring blood pressure and performing biochemical estimations and histopathological examination of renal tissues. **Results:** DOCA–salt hypertensive rats showed considerably increased systolic and diastolic blood pressure, serum hepatic marker enzyme activities such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma–glutamyl transpeptidase (GGT) and renal function markers (urea, uric acid and creatinine) in plasma. Oral administration of morin (25, 50 and 75 mg/kg bw) brought back all the above parameters to near normal level. Histopathology of kidney also confirmed the biochemical findings of this study. The effect at a dose of 50 mg/kg bw of morin was more pronounced than that of the other two doses (25 and 75 mg/kg bw). **Conclusions:** These findings indicate that morin exhibits strong antihypertensive effect against DOCA–salt induced hypertension.

### 1. Introduction

Cardiovascular disease (CVD) is a major health problem across the world, accounting for 30% of all deaths<sup>[1]</sup>. CVD has reached epidemic proportions in India<sup>[2]</sup> and is estimated to result in more than 3 million deaths each year<sup>[3]</sup>. Hypertension is a major risk factor for cardiovascular diseases and a large body of evidence suggests oxidative stress, an increase in the production of reactive oxygen species (ROS), as a strong underlying factor in hypertension<sup>[4,5]</sup>. Excessive ROS generation has been suggested to be involved in a variety of cardiovascular diseases including hypertension, inflammation in the cardiovascular system, cardiac hypertrophy, cardiomyopathies and ischemic heart disease<sup>[6–8]</sup>. Various genetic and environmental factors are known to be involved in the pathogenesis of primary hypertension, among which

excess sodium intake has long been regarded as the pivotal environmental factor for this disorder<sup>[9]</sup>. The long–term administration of deoxycorticosterone acetate (DOCA)–salt to rats induces sodium retention and high salt intake, producing volume–dependent hypertension<sup>[10]</sup>. Considerable evidence demonstrated that high blood pressure is accompanied by oxidative stress and impaired renal function in salt–sensitive hypertension<sup>[11]</sup>.

During the last few years, efforts have been made to increase the number of synthetic antihypertensive drugs, but their use is often limited because of their adverse effects. In recent years, dietary agents such as increased consumption of fruits, vegetables, whole grains, and fish have been shown to be important in the control of CVD including hypertension<sup>[12]</sup>. Flavonoids are polyphenolic compounds that can be found in dietary components such as food products, beverages and herbal medicines with different health benefits shown in a large number of studies<sup>[13]</sup>. Most flavonoids have an antioxidant activity<sup>[14,15]</sup>.

Morin (3,5,7,2',4'–pentahydroxyflavone; a light yellowish pigment) is a kind of flavonoid belonging to the group of flavonols (Figure 1), found in guava leaves<sup>[16]</sup>, onion, apple<sup>[17]</sup>

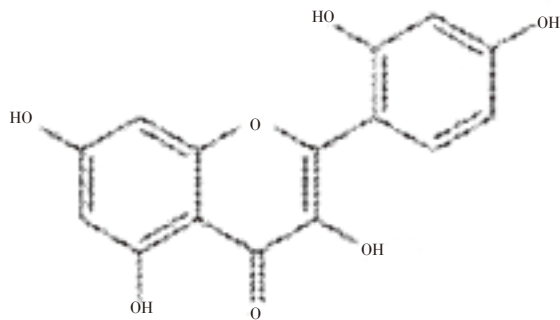
\*Corresponding author: Dr. Raja B, Assistant Professor, Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar–608 002, Tamil Nadu, India.

Tel: +91–4144–239141

E–mail: drrajaau@gmail.com

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and other Moraceae which are used as dietary agents and also as herbal medicines<sup>[18]</sup>. Morin exhibits many biological activities, including antioxidant<sup>[19,20]</sup>, anti-inflammatory<sup>[21]</sup>, antitumour<sup>[22]</sup> activities and possibly even protective effects against chronic diseases<sup>[23]</sup>. Extensive literature survey has shown that no sufficient work has been done to study its antihypertensive effect. Therefore, the present study was designed to determine the dose-dependent effect of chronic administration of morin on blood pressure, activities of hepatic marker enzymes, renal function markers and histopathological findings against DOCA-salt induced hypertension in male albino Wistar rats.



**Figure 1.** Chemical structure of morin (3,5,7,2',4'-pentahydroxyflavone).

## 2. Materials and methods

### 2.1. Experimental animals

Male albino Wistar rats (*Rattus norvegicus*; 11–13 week old) were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamilnadu, India. They were housed (3 rats/cage) in polypropylene cages (47 cm × 34 cm × 20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22 °C and had free access to tap water and food. The rats were fed on a standard pellet diet (Kamadhenu Agencies, Bangalore, India). The whole experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Reg no: 160/1999/CPCSEA, Approval no: 622).

### 2.2. Drug and chemicals

Morin, DOCA and dimethyl formamide (DMF) were purchased from Sigma–Aldrich Chemical Company, St. Louis, Missouri, USA. All other chemicals used in this study were of the highest analytical grade and obtained from Sisco Research Laboratories (SRL) or Himedia, Mumbai, India.

### 2.3. Method of uninephrectomy

Left uninephrectomy was performed on all rats. Rats were anaesthetized with intraperitoneal injection of ketamine (75

mg/kg bw), kidney was visualized by a left lateral abdominal incision (1 cm long), and the left renal artery and ureter were ligated by silk thread, and then the left kidney was removed and weighed. The muscle and skin layer (incision site) were sutured with highly sterile suture needles.

### 2.4. DOCA-salt hypertensive rats

After uninephrectomy, UNX-rats were allowed to drink tap water *ad libitum*, with no further treatment, and DOCA-salt hypertensive rats were given 1% NaCl in the drinking water with weekly twice subcutaneous injection of DOCA [25 mg/kg bw in 0.4 mL of DMF (vehicle) with mild heating] for six consecutive weeks<sup>[24,25]</sup>.

### 2.5. Suspension of the test compound and mode of administration

Morin was freshly suspended in water<sup>[26,27]</sup> and was administered to rats orally using an intragastric tube daily for a period of six consecutive weeks. Rats were monitored daily for general health.

### 2.6. Experimental timeline

The rats were randomly divided into six groups each consisting of six rats. Group 1: UNX-rats; group 2: UNX-rats were treated with morin alone (75 mg/kg bw); group 3: DOCA-salt hypertensive rats; groups 4, 5 and 6 were hypertensive rats, which received different doses of morin 25, 50 and 75 mg/kg bw.

At the end of the sixth week, all the rats were anesthetized with intramuscular injection of ketamine and sacrificed by cervical dislocation. Blood was collected in two different tubes, *i.e.*, one with anticoagulant for the separation of plasma and another without anticoagulant for the serum. Plasma and serum were separated by centrifugation for the estimation of various biochemical parameters.

### 2.7. Measurement of blood pressure by non-invasive method

Systolic and diastolic blood pressures were recorded every week during the entire period of the study by tail-cuff method (IITC, model 31, Woodland Hills, CA, USA). The animals were placed in heated chamber at an ambient temperature of (30–34 °C) for 15 min and from each animal, 1–9 blood pressure values were recorded. The lowest three readings were averaged to obtain a mean blood pressure. All the recordings and data analyses were done using a computerized data acquisition system and software.

### 2.8. Biochemical estimations

The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) were assayed by the method of Reitman and Frankel<sup>[28]</sup>, Kind and King<sup>[29]</sup>, Rosalki and Raul<sup>[30]</sup>, respectively. The levels of urea, uric

acid and creatinine in plasma were estimated by diagnostics kit (Qualigens Diagnostics, Mumbai, India) based on the method of Fawcett and Scott[31], Caraway[32] and Tietz[33].

2.9. Histopathological examination of renal tissues

The kidney tissues obtained from all experimental groups were washed immediately with 0.9% saline and then fixed in 10% buffered neutral formalin. After fixation, the kidney tissues were processed by embedding in paraffin wax. Then, the tissues were sectioned (5-6 μm) and stained with haematoxylin and eosin (H&E) dye and examined under a high power microscope (Nikon ELWD 0.3/OD75; Japan) and photomicrographs were taken.

2.10. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) using Statistical Package for the Social Science (SPSS) software version 12.00. Results were expressed as mean±SD for six rats in each group. A value of P<0.05 was considered to be statistically significant.

3. Results

3.1. Effect of morin on blood pressure

Figures 2 and 3 showed the dose dependent effect of morin in UNX and DOCA-salt hypertensive rats. The systolic and diastolic blood pressure was considerably (P<0.05) increased in DOCA-salt hypertensive rats (group 3) compared to UNX-rats (group 1). Oral administration of morin (25, 50 and 75 mg/kg bw) for a period of six consecutive weeks considerably (P<0.05) decreased systolic and diastolic blood pressure in DOCA-salt treated rats (groups 4, 5 and 6). The effect exerted by 50 mg/kg bw of morin was better than the other two doses (25 and 75 mg/kg bw). Oral administration of morin alone (75 mg/kg bw) to UNX-rats (group 2) did not show any effect.

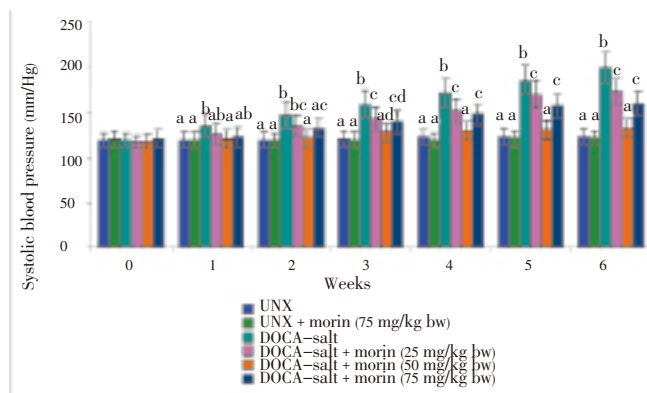


Figure 2. Effect of morin on systolic blood pressure of UNX and DOCA-salt hypertensive rats. Values are expressed as mean±SD for six rats in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

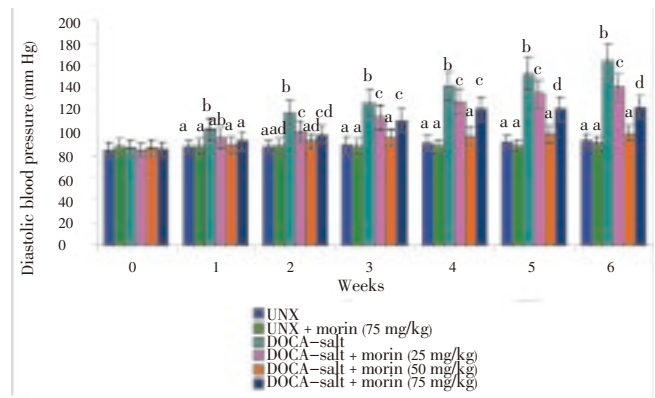


Figure 3. Effect of morin on diastolic blood pressure of UNX and DOCA-salt hypertensive rats. Values are expressed as mean±SD for six rats in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

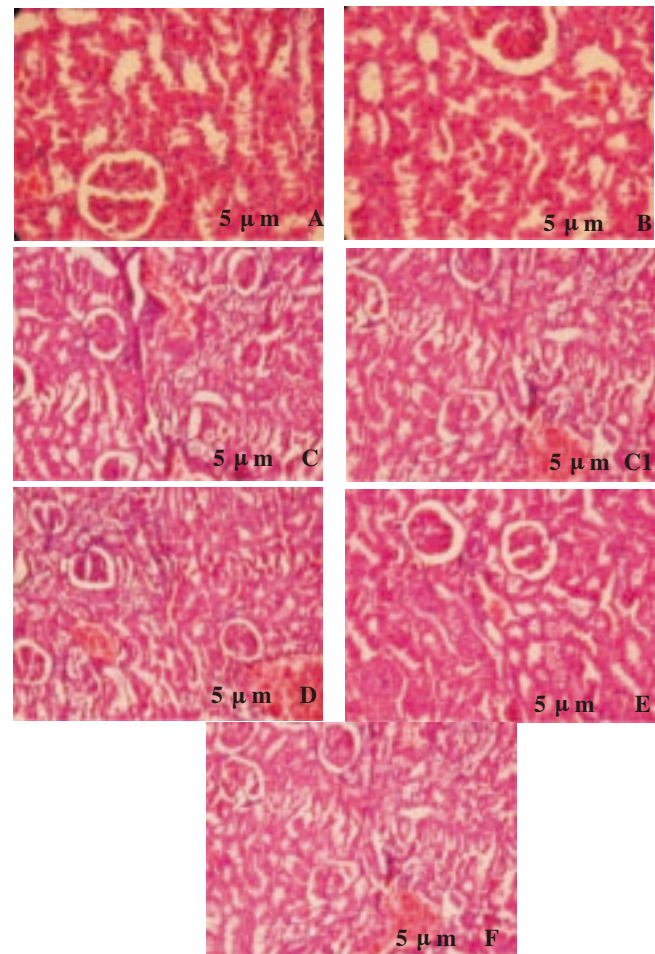


Figure 4. Histopathological changes in the kidney (H&E 40×). A: UNX-rat kidney (group 1) showing normal glomeruli and tubules; B: UNX + morin alone (75 mg/kg bw) treated kidney (group 2) showing normal architecture without pathological changes; C & C1: DOCA-salt hypertensive kidney (group 3) showing segmented glomerulo nephritics in glomeruli, tubules contain protein precipitate and cloudy swelling, small blood vessels showing thickening of the wall, parenchymal hemorrhage and inflammatory cell infiltrate in the parenchyma; D: DOCA-salt + morin (25 mg/kg bw) treated kidney (group 4) showing mild swelling in tubules and few inflammatory cells; E: DOCA-salt + morin (50 mg/kg bw) treated kidney (group 5) showing fewer inflammatory cells and near normal architecture; F: DOCA-salt + morin (75 mg/kg bw) treated kidney (group 6) showing mild glomerular hypertrophy and less congestion in interstitial vessels.

**Table 1**

Effect of morin on the activities of serum AST, ALT, ALP and GGT of UNX and DOCA-salt hypertensive rats (mean±SD) (n=6).

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
UNX	68.31±4.57 <sup>a</sup>	28.75±1.78 <sup>a</sup>	76.27±5.03 <sup>a</sup>	14.69±0.92 <sup>a</sup>
UNX + morin (75 mg/kg bw)	67.06±3.95 <sup>a</sup>	26.16±1.33 <sup>a</sup>	75.16±4.43 <sup>a</sup>	13.43±0.79 <sup>a</sup>
DOCA-salt	122.11±11.84 <sup>b</sup>	65.23±5.60 <sup>b</sup>	137.13±12.75 <sup>b</sup>	32.96±3.06 <sup>b</sup>
DOCA-salt + morin (25 mg/kg bw)	103.06±9.37 <sup>c</sup>	57.81±4.68 <sup>c</sup>	109.06±9.92 <sup>c</sup>	25.09±2.28 <sup>c</sup>
DOCA-salt + morin (50 mg/kg bw)	73.08±5.18 <sup>a</sup>	32.91±2.23 <sup>d</sup>	81.06±5.75 <sup>a</sup>	17.66±1.43 <sup>d</sup>
DOCA-salt + morin (75 mg/kg bw)	92.47±7.95 <sup>d</sup>	49.65±3.77 <sup>c</sup>	96.76±7.83 <sup>d</sup>	22.96±1.97 <sup>c</sup>

Values not sharing a common superscript differ significantly at  $P<0.05$  (DMRT).**Table 2**

Effect of morin on the levels of urea, uric acid and creatinine in plasma of UNX and DOCA-salt hypertensive rats (mean±SD) (n=6).

Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
UNX	19.36±1.33 <sup>a</sup>	1.36±0.08 <sup>a</sup>	0.88±0.06 <sup>a</sup>
UNX + morin (75 mg/kg bw)	18.03±1.26 <sup>a</sup>	1.24±0.06 <sup>a</sup>	0.83±0.04 <sup>a</sup>
DOCA-salt	44.06±3.92 <sup>b</sup>	3.73±0.32 <sup>b</sup>	2.87±0.27 <sup>b</sup>
DOCA-salt + morin (25 mg/kg bw)	35.06±2.98 <sup>c</sup>	3.02±0.24 <sup>c</sup>	2.19±0.20 <sup>c</sup>
DOCA-salt + morin (50 mg/kg bw)	22.67±1.63 <sup>d</sup>	1.89±0.13 <sup>d</sup>	1.06±0.07 <sup>d</sup>
DOCA-salt + morin (75 mg/kg bw)	30.16±2.44 <sup>c</sup>	2.59±0.19 <sup>c</sup>	1.76±0.15 <sup>c</sup>

Values not sharing a common superscript differ significantly at  $P<0.05$  (DMRT).

### 3.2. Effect of morin on the activities of hepatic marker enzymes

The activities of AST, ALT, ALP and GGT were depicted in Table 1. DOCA-salt hypertensive rats (group 3), exhibited a considerable ( $P<0.05$ ) increase in the activities of these marker enzymes in serum when compared to UNX-rats (group 1). Oral administration of morin (25, 50 and 75 mg/kg bw) considerably ( $P<0.05$ ) decreased the activities of these enzymes in serum of DOCA-salt treated rats (groups 4, 5 and 6). The morin at a dose of 50 mg/kg bw was more effective for reducing these enzyme activities compared with other two doses (25 and 75 mg/kg bw).

### 3.3. Effect of morin on renal function markers

In DOCA-salt hypertensive rats (group 3), the levels of urea, uric acid and creatinine in plasma were considerably ( $P<0.05$ ) increased when compared to UNX-rats (group 1). Oral treatment with morin (25, 50 and 75 mg/kg bw) throughout the experimental period to DOCA-salt treated rats (groups 4, 5 and 6) considerably ( $P<0.05$ ) decreased the levels of these markers in plasma. Among the three different doses tested, 50 mg/kg bw of morin elicited the highest significant effect (Table 2).

### 3.4. Effect of morin on the histopathology of kidney

Histopathological changes in the kidney tissue of all groups were evaluated microscopically (Figure 4A-F). Figure 4A showed the histological section of UNX-rats (group 1), which exhibited normal glomeruli and tubules. UNX-rats treated with morin alone (75 mg/kg bw) showed

normal kidney architecture without pathological changes (group 2; Figure 4B). However, DOCA-salt hypertensive rats kidney showed segmented glomerulo nephritics in glomeruli, tubules contain protein precipitate and cloudy swelling, small blood vessels show thickening of the wall, parenchymal hemorrhage and inflammatory cell infiltrate in the parenchyma (group 3; Figures 4C and C1). The above changes were reduced in kidney of rats treated with morin (25, 50 and 75 mg/kg bw) (groups 4, 5 and 6; Figures 4D-F). From the histopathological findings, oral administration of morin at a dose of 50 mg/kg bw remarkably minimized the structural changes in kidney than other two doses (25 and 75 mg/kg bw).

## 4. Discussion

Increased concentrations of aldosterone lead to increased reabsorption of sodium ions and water from epithelial cells in the distal nephron of the kidney, thereby influencing blood pressure levels[34]. In addition, increased aldosterone concentrations may activate oxidative stress through an unregulated NADPH oxidase in the DOCA-salt model[35]. In agreement with previous report[25] we also observed that systolic and diastolic blood pressure was considerably increased in DOCA-salt hypertensive rats (group 3), which might be due to increased oxidative stress and decreased bioavailability of nitric oxide. Daily oral administration of morin, resulted in a remarkable reduction in systolic and diastolic blood pressure which might be due to the antioxidant ability of this compound. Flavonoids and triterpene were recently shown to reduce hypertension in experimental animal models[36]. Several cohort studies have

suggested that high intake of flavonoids may decrease the risk of coronary heart diseases<sup>[37]</sup>.

Oxidative stress and alteration of cellular redox state are linked to many types of acute and chronic liver and kidney injury. Disruption of liver tissue architecture and vacuolation under hypertension and nitric oxide-deficiency are an indication of hepatic fatty infiltration and hepatocellular injury<sup>[38]</sup>. AST, ALT, ALP and GGT are the relatively liver specific enzymes. Their estimation in the serum is useful as a quantitative marker of the extent and type of liver damage. In our results, AST, ALT, ALP and GGT activities were increased considerably in the serum of DOCA-salt hypertensive rats (group 3), which is a clear evidence for liver damage. The reason behind this elevation may be due to the necrotic and oxidative action of liver tissues which causes leakage of these enzymes from hepatocytes as a result of membrane damage<sup>[39]</sup>. Treatment with morin considerably reduced the activities of these enzymes. It is suggested that morin aids in parenchymal cell regeneration in liver, thus protecting membrane integrity, thereby decreasing enzyme leakage.

The kidney plays a central role in the regulation of the balance of the body salt and water, and then disordered regulation of renal functions is responsible for the altered balance of salt and water in pathophysiological states including some experimental models of hypertension<sup>[40]</sup>. Our results revealed that a considerable increase in plasma urea, uric acid and creatinine levels might indicate a hypertension in DOCA-salt rats (group 3) and may be due to kidney damage caused by the oxidative stress by increasing the formation of superoxide. These findings are correlated well with the kidney histology examination. Oral administration of morin considerably reduced the raised plasma urea, uric acid and creatinine levels, bringing about remarkable recovery in kidneys as evidenced microscopically. Considerable reduction in these levels might be due to the antioxidant property of morin. These findings suggest that renal damage is remarkably prevented by treatment of morin in DOCA-salt hypertensive rats, which also might be beneficial in the control of blood pressure. Interestingly, it has been suggested that kidney and cardiac injury can be avoided or minimized by reducing oxidative stress through increased intake of antioxidants<sup>[41]</sup>.

UNX-rats treated with morin alone (75 mg/kg bw) showed normal appearance of the kidney without any pathological changes. This indicates that morin does not possess any adverse effect under normal conditions. Thus, histopathological findings of the present study confirmed the biochemical observations of this study.

In conclusion, our results demonstrate that morin, possesses strong antihypertensive effect against DOCA-salt hypertensive rats, which is evidenced by a considerable

decrease in blood pressures, hepatic and renal function markers. Our results also revealed that the renal damage was ameliorated by morin supplementation. Thus, it is a safe antioxidant. Among the three dosages tested, 50 mg/kg bw exhibited the highest considerable antihypertensive effect. However, further studies are needed to find out the exact mechanism of action of morin.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgments

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