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PHYTOCHEMICAL EVALUATION AND ANTIHYPERTENSIVE ACTIVITY OF *MALUS DOMESTICA* PEEL IN EXPERIMENTAL ANIMALS

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ABSTRACT

Glucocorticoids are among the most frequently given medications. Excess glucocorticoid amounts, unfortunately, cause hypertension in 80-90 percent of individuals. *Malus domestica* Borkh (Family: Rosaceae), known as apple, has been utilized for different diseases since ancient times and is still popular today due to its high antioxidant, vitamin, and trace element content. The purpose of this study was to explore the antihypertensive effect of M. domestica peel extract (MDPE) in Dexamethasone (Dexa)-induced hypertension in Wistar rats. Animals were randomly grouped into 6 (n=5). Group I- vehicle, Group II & III- MDPE (200 & 400 mg kg-1day-1 p.o., respectively for 14 days), Group IV Dexa (1.8 mg kg-1 week-1 14 days, Group V and VI- Dexa (1.8 mg kg-1 week-1 with MDPE (200 and 400 mg kg-1 day1 p.o., 14 days). Dexa was given subcutaneously. Results obtained at the end of the dosing period revealed that MDPE treatment significantly reversed changes in mean arterial blood pressure (BP) measured by invasive method (IBP), Pressure rate index (PRI), heart rate (HR) and changes in vascular reactivity to various catecholamines. Heart weight and serum levels of Creatine kinase (CK-MB) and Lactate dehydrogenase (LDH) decreased significantly after MDPE treatment in Dexa-treated animals. This study confirmed that M. domestica peel extract treatment for 14 days partially reversed Dexa-induced hypertension in rats.

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Introduction

Hypertension increases the risk of various cardiovascular illnesses, including atherosclerosis and myocardial infarction. The production of reactive oxygen species (ROS) by vascular cells [1] and alterations of antioxidant enzymes have been implicated in the pathogenesis of vascular diseases. Most studies in hypertensive patients report increased levels of ROS such as superoxide anion, hydrogen peroxide and lipid peroxides in plasma, together with reduced levels of natural antioxidant vitamin C and decreased activities of antioxidant enzymes such as superoxide dismutase (SOD) or glutathione peroxidase (GPX) [2-4].

Glucocorticoids are often recommended medications for asthma, rheumatoid arthritis, eye disorders, skin disorders, organ transplant, glomerulopathies, malignancies, pain syndrome, and other illnesses [4, 5]. Glucocorticoids have anti-inflammatory and immunosuppressive properties. However, glucocorticoid medication in the long term results in hypertension and diabetes. Glucocorticoid therapy induces hypertension and impaired glucose metabolism in 80-90 percent of Cushing syndrome patients [5, 6].

In the current situation, we are seeking herbal medications to avoid such side effects. Herbal and nutritional supplements are widely used all over the world. A growing number of individuals are now embracing herbal medicines and nutritional supplements to treat cardiovascular disorders. Fruit-derived bioactive compounds are disease regulators because they have fewer negative effects than chemical substances.

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M. domestica is the most prominent and regularly consumed fruit. It is still used in popular medicine for many diseases and physiological problems due to its rich source of nutritional molecules and high amounts of bioactive elements. Polyphenols, polysaccharides (pectin), phytosterols, and pentacyclic triterpenes are the main ingredients of *M. domestica* [7].

It also has trace minerals and vitamins. According to Richardson *et al*. (2020), apples offer a variety of vitamins, including vitamin C, E, and -carotene, as well as vital minerals such as calcium, iron, potassium, manganese, zinc, magnesium, copper, and Sulphur [8].

Whole fruit has been used for jams and cakes from ancient times, except for the seeds. Apples, on the other hand, have at least partially inspired the proverb, "An apple a day keeps the doctor away." Asthma, acidity, arthritis, diarrhea, fever, obesity, headache, stomach pains, skin illnesses, and respiratory difficulties can all be treated with apples [9]. Apple vinegar can help cure anemia, since it includes iron in a very digestible form and vitamin B12 and folic acid. In addition, apple cider vinegar can help with asthma, kidney stones, arthritis, and skin disorders [10].

It also shows anti-oxidant [11], cholesterol lowering [12], antidiabetic [13] activities. Flavonoids isolated from apple peel extracts have been shown to be beneficial in treating hypertension and cardiovascular disease [14].

So, in the light of the above literature, the present study was planned to study the effect of *Malus domestica* Borkh (Family: Rosaceae) peel extract in Dexamethasone-induced hypertension in rats.

Materials and Methods

Animals

Adult male Wistar rats of either sex (150-200 g) used in this study were procured from Bharat Serum and Vaccines Ltd., Thane, India.

Drugs and Chemicals

Adrenaline (Adr), noradrenaline (NA), phenylephrine (PE), serotonin (5-HT) and Urethane were obtained from Sigma-Aldrich, USA. Kits were used for the estimation of LDH and CKMB.

Plant Selection and Authentication

Malus domestica Borkh (Family: Rosaceae) Fruits were collected from the local market of Nasik. They were authenticated by Dr. A. Benniamin, Scientist and Head of Office, BSI, Pune (specimen number: LPS-1).

Preparation of Extract

Mature fruits were collected from the local market, and peels were carefully sliced using an apple peeler. Peels were collected, cut into small pieces, and extracted using a reflux condenser for about 6 hrs. The extract was filtered (twice), air-dried and concentrated under a boiling water bath (Yield: 15.24 % w/w). Chemical analyses were performed to identify phytoconstituents in the crude extract, such as phenolic acids, flavonoids, alkaloids, tannins, and saponins.

Acute Toxicity

An acute toxicity study was conducted on Male Albino mice weighing 25-30 gm (n = 3) per OECD 423 guideline down method. A single high oral dose, i.e., 2000 mg/kg of *M. domestica* peel extract, as recommended by the OECD guidelines 423 down method, was administered to the first animal (OECD Guideline for Testing of Chemicals, 2001) [15].

Induction of Experimental Hypertension by Dexamethasone

Animals were randomly grouped into 6 (n=5). Animals were randomly grouped into 6 (n=5). Group I- vehicle, Group II & III-MDPE (200 & 400 mg $kg^{-1}day^{-1}$, respectively for 14 days), Group IV Dexa (1.8 mg kg^{-1} $week^{-1}$ for 14 days, Group V and VI received Dexa (1.8 mg kg^{-1} $week^{-1}$ along with MDPE (200 and 400 mg kg^{-1} day^{1} 14 days). Dexa was given subcutaneously and MDPE through per oral route. After the dosing period, Blood pressure, Pressure-Rate Index, Heart Rate and changes in vascular reactivity to various catecholamines were recorded. The heart was isolated, and weight was recorded. Blood serum was used to estimate the quantity of Creatine kinase (CK-MB) and Lactate dehydrogenase (LDH).

Measurement of BP by Invasive Method (IBP)

After completion of the treatment schedule, rats from each group were anesthetized with urethane (120 mg/100 g). After cannulation of the carotid artery and stabilization for 15 min, mean arterial blood pressure (MABP), PRI and HR were recorded [16]. PRI is used as an index of myocardial oxygen demand [17].

Vascular reactivity to Adr (10 µg kg^{-1}), NA (10 µg kg^{-1}), PE (10 µg kg^{-1}) and 5-HT (10 µg kg^{-1}) was recorded.

Heart Weight

The animal heart was removed immediately after vascular reactivity was completed, weighed, and the heart weight in each group was recorded.

Estimation of Serum Parameters

Blood was collected by cardiac puncture after anesthetization using diethyl-ether. Serum was separated using an R-24 research centrifuge (Remi Instruments Ltd., Mumbai) at 3000 rpm for 15 min and used for estimation of Creatinine kinase [18] and Lactate dehydrogenase (LDH kit-Agappe Diagnostics Pvt. Ltd., India).

Histopathological Investigation

The heart was fixed in 10 % formalin and sent to the pathology laboratory for histological estimation.

Statistical Interpretation

Data was analyzed by one-way ANOVA followed by Dunnett's test. Values of *p<0.05 compared to control and #p<0.05 compared to the DEXA group were considered statistically significant.

Results and Discussion

Phytochemical analysis revealed the presence of carbohydrates, steroids, anthocyanidins, and flavonoids.

Acute Oral Toxicity Study

MDPE showed no signs of toxicity with a dose of 2000 mg/kg p.o. Hence 2000 mg/kg can be considered as the maximum tolerated dose, and 1/10th of this dose can be considered.

Measurement of Mean Arterial Blood Pressure

The DEXA-treated group had significantly elevated blood pressure compared to the control animals. When comparing MDPE-treated animals to control animals, no significant changes in blood pressure were detected. When rats were given DEXA and MDPE (400 mg/kg), their blood pressure was much lower than in the DEXA-treated group **(Figure 1)**.

Pressure-rate Index (PRI)

Compared to control animals, the DEXA-treated group significantly improved the PRI. When DEXA was combined with MDPE (400), the PRI of the animals was significantly lower than in the DEXA-only group **(Figure 1)**.

Heart Rate

Compared to control animals, the DEXA-treated group had a substantial rise in heart rate. Animals given DEXA together with MDPE had a lower heart rate than those given DEXA alone **(Figure 1)**.

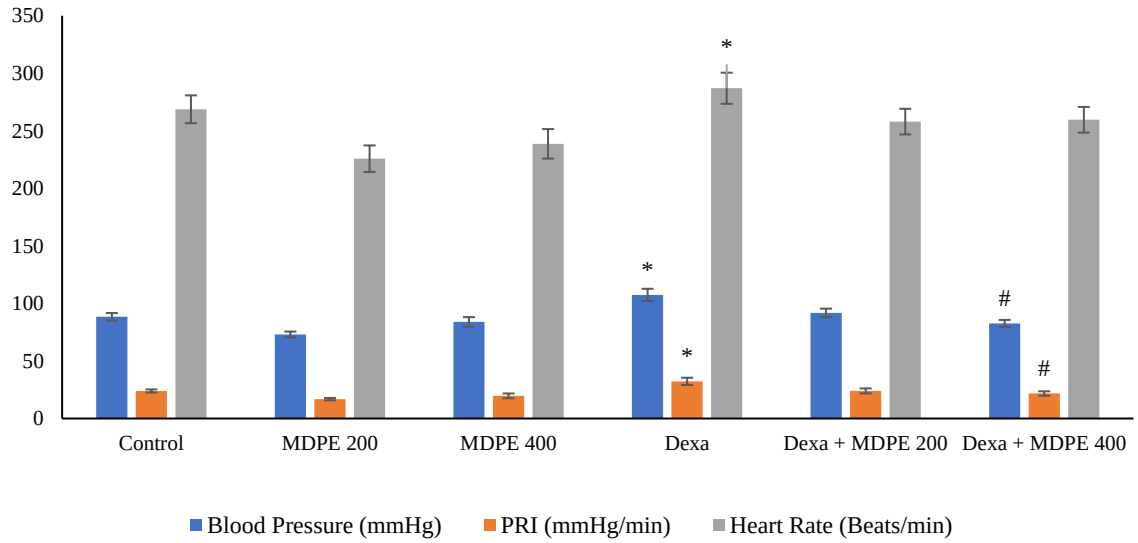

Figure 1. Effect of *Malus domestica* peel extract on mean arterial blood pressure, PRI and Heart Rate

Vascular Reactivity to Catecholamines

Phenylephrine

Phenylephrine (10 µg/kg, i.v.) exhibited increase in blood pressure in control animals. When compared to control animals, the DEXA-treated group had significantly higher blood pressure. Compared to the control group, the animals treated with MDPE exhibited no significant changes in blood pressure. When rats were given DEXA together with MDPE, their blood pressure was much lower than in the DEXA-only group **(Figure 2)**.

Noradrenaline

In control animals, the rise in blood pressure by NA (10 g/kg i.v.) was observed. Compared to control animals, the DEXA-treated group had significantly higher blood pressure. Whereas the MDPE exhibited no significant drop in blood pressure when contrasted with the control group. Compared to the DEXA group, animals treated with MDPE and DEXA had significantly lower blood pressure **(Figure 2)**.

Adrenaline

Adrenaline (10 g/kg i.v.) caused an increase in blood pressure in control animals. The DEXA-treated animals had considerably greater blood pressure than the control animals. There were no statistically significant changes between the MDPE-treated animals and the control group. The DEXA and MDPE-treated rats had lower blood pressure than the DEXA-only group, but the difference was not statistically significant **(Figure 2)**.

5-HT

Compared to control animals, the DEXA-treated group had significantly higher blood pressure, while the animals treated with MDPE (400mg/kg) had significantly higher blood pressure. When rats were given DEXA together with MDPE, their blood pressure was much lower than in the DEXA-only group **(Figure 2)**.

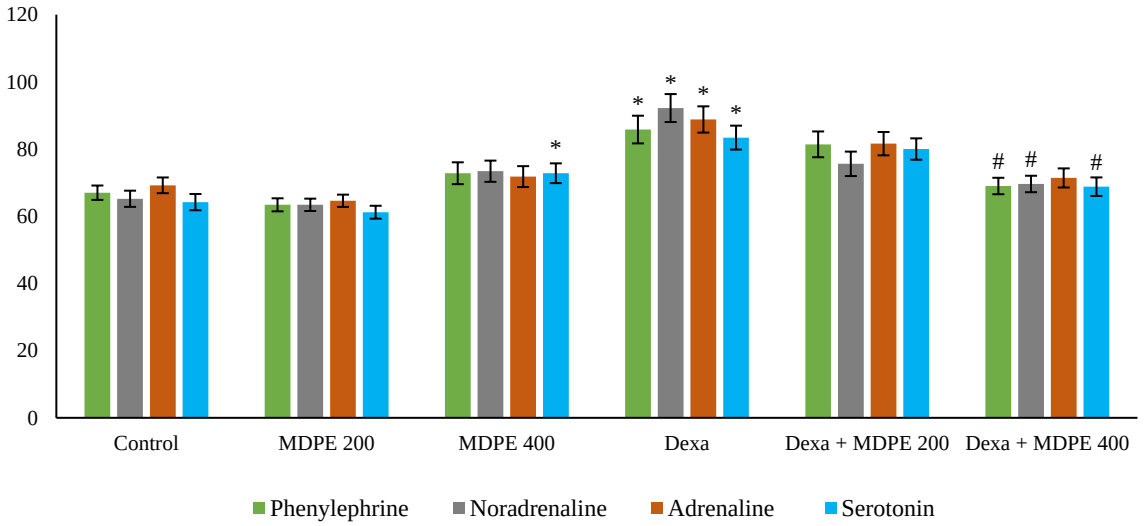

Figure 2. Effect of *Malus domestica* peel extract on vascular reactivity due to phenylephrine (10 µg/kg, i.v.), noradrenaline (10 µg/kg i.v.), adrenaline (10 µg/kg i.v.), serotonin (10 µg/kg i.v.) in Dexamethasone-induced hypertension in rats

Heart Weight

Compared to control animals, the DEXA-treated group had a substantial increase in heart weight, while the animals treated with MDPE substantially reduced heart weight. Compared to the DEXA group, animals treated with DEXA and MDPE considerably reduced heart weight **(Figure 3)**.

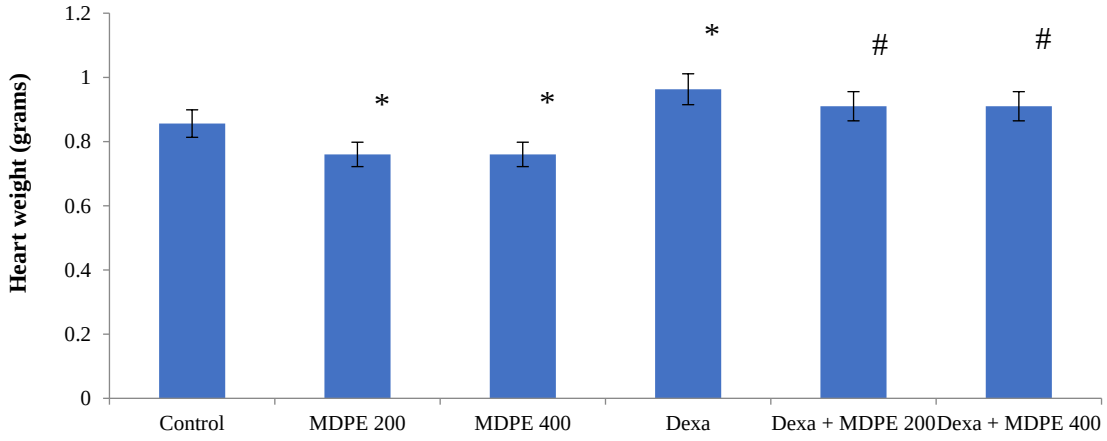

Figure 3. Effect of *Malus domestica* peel extract on heart weight

Serum Biomarker Analysis

Estimation of Creatinine Kinase (CK-MB)

Compared to control animals, the DEXA-treated group had considerably higher CK-MB values. Compared to the control group, MDPE (400 mg/kg) treated animals with significantly lower CK-MB levels. Animals with DEXA and MDPE (400 mg/kg) had significantly lower CK-MB values than the DEXA-treated group **(Table 1)**.

Lactate Dehydrogenase (LDH or LD)

LDH levels in the DEXA-treated group were considerably higher than in the control group. Compared to the control group, animals treated with MDPE (200 and 400 mg/kg) demonstrated substantially reduced LDH levels. Animals treated with DEXA plus MDPE (200 and 400 mg/kg) had significantly lower LDH levels than the DEXA-only group **(Table 1)**.

Table 1. Effect of *Malus domestica* peel extract on Creatine kinase values.

Treatment	Ck-MB	LDH
Control	62.45±4.56	331.3±11.21
MDPE 200	60.7±3.58	235.2±9.85*
MDPE 400	38.26±2.41*	179±6.14*
Dexa	64.14±3.98*	494.1±12.21*
Dexa + MDPE 200	64.10±4.12	307±8.25#
Dexa + MDPE 400	50.64±3.96#	267.2±7.67#

Histopathological Study

Section of heart from Control group I (A) showing normal cardiac architecture. Group II (B) and Group III (C) showed normal cardiac architecture as compared to Group I. Significant myocardial damage and infiltration of inflammatory cells as compared to the Control group were observed in Dexamethasone treated group (D). Sections of the heart from Group V (E) and VI (F) showed near-normal cardiac architecture compared to group IV. It significantly prevented myonecrosis, as indicated by a significant reduction in the infiltration of inflammatory cells and vacuolar changes compared to the Dexamethasone group **(Figure 4)**.

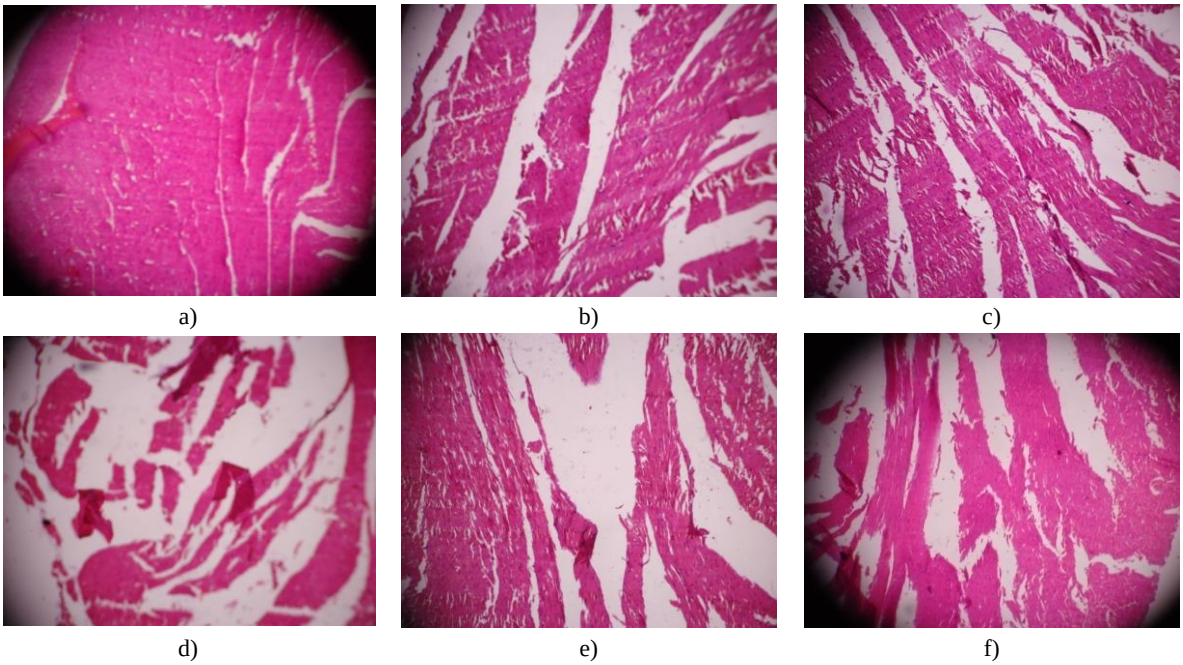

Figure 4. Effect of *Malus domestica* peel extract on cardiac morphology

According to one study, just 25% of those undergoing current hypertension therapy can keep their arterial blood pressure within normal limits. There are several reasons for this, including pharmacological side effects and the relatively high cost of therapy. The Ayurvedic system focuses on individualizing therapy and ensures that treatment for hypertension and many other cardiovascular illnesses is reasonable [19].

Regardless of the vast literature of drugs for the treatment of hypertension, many novel antihypertensive drugs are being introduced. Natural products contribute majorly to the pharmacotherapy of various chronic diseases [20]. *Malus domestica*

peel is claimed to possess potential antioxidants, flavonoids, anthocyanidins and inorganic nitrite, which are claimed to have good antihypertensive activity.

In the current investigation, Dexamethasone was used to induce hypertension in rats. Several mechanisms have been postulated for Dexamethasone to cause hypertension. As a probable mechanism for increased vascular tone and organ destruction, hypertension may be coupled with an increase in oxidative stress.

Mammalian cells can produce oxygen metabolites, reactive oxygen species (ROS), through the activity of numerous enzymes. ROS are primarily generated in vascular cells by NADPH oxidases, uncoupled nitric oxide synthase, xanthine oxidase, and mitochondrial sources. ROS generation by these sources is elevated in hypertension, which not only contributes to hypertension but also causes vascular disease and dysfunction. ROS generation in other organs, including the kidney and brain areas, is believed to have a role in BP control [21, 22].

Various plant extracts have been studied for their hypotensive properties. Traditional herbal extracts include [23] blueberry, [24, 25] chocolate, [24] purple maize, purple sweet potato, and red radish [26]. After two weeks of therapy, the Brazilian folk medicine *Cecropia glaziovii Sneth* lowered mean systolic blood pressure by 20 mmHg, whereas single dosage treatments of the extracts had no effect [25]. After 3 weeks of oral treatment, radish leaf extracts high in polyphenols showed a similar tendency to lower blood pressure [27]. In all of this research, the presence of phytochemicals was found to be linked with blood-pressure-lowering abilities.

Polyphenols, particularly flavonoids, have increased endothelial function and antioxidant activity, resulting in cardioprotective benefits [28, 29].

The DEXA-administered group had significantly higher BP and PRI than the control animals. No significant changes in BP or PRI were observed when comparing M. domestica peel extract (200 and 400 mg/kg p.o. for 14 days) to control animals. When rats were given DEXA coupled with *M. domestica* peel extract (400 mg/kg p.o. for 14 days), their BP and PRI levels were significantly lower than in the DEXA-only group.

Regarding vascular reactivity to catecholamines, the DEXA-treated mice had significantly higher blood pressure than the control animals. The *M. domestica* peel extract (400 and 200 mg/kg) treated animals had normal blood pressure. Rats given DEXA combined with *M. domestica* peel extract had significantly lower blood pressure than the DEXA-only group.

In the case of CK-MB and LDH biochemical analyses, the DEXA-treated group significantly rose in CK-MB value compared to control animals. Animals given M. domestica peel extract (400 mg/kg, p.o. for 14 days) had significantly lower levels than the control group. Animals given DEXA coupled with M. domestica peel extract (400 mg/kg p.o. for 14 days) had significantly lower LDH levels than the DEXA-only group. The results of this study are in accordance with Olasehinde *et al.* (2022) [30], Doseděl *et al*. (2021) [31] Zhang *et al*. (2020) [32].

Conclusion

The present study highlights the effect of *Malus domestica* peel extract on hypertension induced by Dexamethasone. Thus, this study concludes that *Malus domestica* peel extract has a protective effect on dexamethasone-induced hypertension in Wistar rats. This study reinforces claims that eating more fruits and fruit-based products reduces disease risk factors and give insight into the creation of apple-based natural health products.

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Conflict of interest: None

Financial support: None

Ethics statement: Experimental procedures and the study protocol was approved by IAEC of MGV's Pharmacy College, Nashik (Protocol number: MGV/PC/XXVIII/01/12-13).

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