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Antihypertensive activity of Gossypin against L-NAME induced hypertension in rats

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ABSTRACT

Gossypin (3,5,7,3,4-pentahydroxy-8-o-glucoside) a bioflavonoid was evaluated for protection against L-NAME(L-N^G-Nitroarginine methylester) induced hypertension in albino rats, based on its effects on body weight of animals and arterial blood pressure. Male adult albino rats (220-2600gm)were used in this study. The hypertensive activity was measured using AD 8 channel Lab chart solution instrument. The activity was carried out in acute and chronic models .In acute studies animals were divided into 5 equal groups of five rats each. group I was the normal group received normal saline and group II as control group received L-NAME (20mg/kg i.v after 30 minutes of arterial cannulation .Group III as standard received losartan (10 mg/kg b.w.i.v) after increase in B.P produced by L-NAME while group IV, V. received gossypin at a dose of 5,10, mg/kg b.w i.p after inducing hypertension. In chronic studies animals were divided into 6 equal groups of 6 animals each. Group I normal group received normal saline orally for 4wks and group II received L-NAME (40mg/kg p.o)for 4 wks Group III as STD group received losartan (20mg/kg p.o) while group IV.V.VI as treated groups received Gossypin(5,10,20,mg/kg b.w p.o)for 4 wks. After last dose the animals were examined for weight variation and B.P determination by invasive method using AD instrument. The left carotid artery was cannulated for B.P measurement and femoral vein for drug administration. The hypertension induced by L-NAME was indicated by elevated levels in systolic B.P and decrease in body weight of animals. In acute hypertension, L-NAME increases systolic B.P and mean B.P and decrease in heart rate which was administrated through carotid artery after 30 mins of stabilization of B.P. There was increase in systolic and mean B.P and decrease in heart rate. The drug was administered by I.P shows significant reduction in elevated b.p which was observed for 2hrs.In chronic hypertension L-NAME induced group shows increase in B.P and decrease in body weight. The treated groups has shown reduction in elevated B.P and managed weight of animals. We suggest that Gossypin had effect on hypertension in L-NAME induced hypertensive rat by acute and chronic administration.

Keywords: L-NAME, Gossypin, carotid artery, hypertension, BP, IP, PO.

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INTRODUCTION

Hypertension is a common disease that is defined simply as persistently elevated arterial blood pressure (BP). Although elevated BP was perceived to be necessary for adequate perfusion of essential organs during the early and middle 1900s, it is now identified as one of the most significant risk factors for cardiovascular disease in the United States. Increasing awareness and diagnosis of hypertension and improving control of BP with appropriate treatment are considered critical public health initiatives to reduce cardiovascular morbidity and mortality. Blood pressure is a result of many interconnected factors. It is the product of cardiac output and total peripheral resistance, while cardiac output is the product of stroke volume and heart rate . Nervous and endocrine systems as well as genetic and environmental factors work together in a complex system to determine an individual's blood pressure. A measure of systolic and diastolic normal blood pressure is considered to be equal or less than 120/80 millimeters of Mercury (mmHg); in humans hypertension is defined as blood pressure equal to or above 140/90 mmHg. Hypertension is considered to be a major risk factor for an array of cardiovascular and associated diseases, including heart failure, peripheral arterial disease and end stage renal disease. Hypertensive states have also been linked to increased risk for type 2- diabetes and cardio metabolic syndrome. In fact, numerous studies have found a continuous relationship between elevated systolic and diastolic blood pressure and cardiovascular morbidity and mortality. Early interventions and control of elevated blood pressure result in improved health outcomes. However, despite current research and the large number of available treatments, hypertension remains a problem in both developed and developing nations and it is now being diagnosed in adolescents and children. Corresponding with the aging population, Kearney reports that 26.4 % (972 million) of the world's population over the age of 20 had hypertension in 2000 and that will increase to 29.2% (1.56 billion) by 2025. Hypertension is a growing global problem and despite the decades of research its incidence continues to grow calling for more elucidation of its mechanisms and potential treatments. The Seventh Report of the Joint National Committee on the Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) is the national clinical guideline that was developed to aid clinicians in the management of hypertension. This chapter reviews relevant components of this evidence-based guideline with a focus on the pharmacotherapy of hypertension. Data from the National Health and Nutrition Examination Survey from 1999 to 2000 indicate that of the population of Americans with hypertension, 68.9% are aware that they have hypertension, and only 58.4% are on some form of antihypertensive treatment. Moreover, only 34% of all patients have controlled BP, which increases to only 53.1% when only those on

treatment are evaluated[2]. Therefore, there are ample opportunities for clinicians to improve the care of hypertensive patients. Experimental models of hypertension are extremely important in the exploration of hypertension. One such established model is L-NAME hypertension, a pharmacologically induced form of experimental hypertension. Routes of effective administration include intravenous, intraperitoneal and oral. L-NAME produces a hypertensive state reflective of the dysfunction seen in essential hypertension via several mechanisms, including: inhibition of NO, SNS activity increasing total peripheral resistance, oxidative stress and arterial remodeling. Classically, L -NAME is known as an inhibitor of NOS leading to decreased NO, an important vasodilator. Acute and chronic inhibition of NO produces endothelial dysfunction, which has been clearly demonstrated by L-NAME. Moreover, the inhibition of NO by L-NAME in young SHR produces similar results to that of naturally aged SHR, supporting the role for NO inhibition in the study of essential hypertension. In addition to the reduction of NOS activity by L-NAME, the production of O_2^- by L-NAME may decrease NO further through its $ONOO^-$ -mediated uncoupling of NOS. While controversial, it has been reported that L-NAME may directly alter baroreceptor sensitivity in SNS leading to augmentation of blood pressure. It is hypothesized that NO is an inhibitory modulator of SNS outflow. Large volumes of evidence show that stimulation of SNS activity in L-NAME-induced hypertension can be modulated by reduced NO availability. Despite the method of induction, SNS activity is a contributor to acute and chronic L-NAME-induced hypertension. Gossypin was isolated from *Hibiscus vitifolius.* The yellow part of the petals of *Hibiscus vitifolius (*Malvaceae*)* (1.5g) extracted with methanol for 3 to 4 hrs concentrated to small volume under vacuum and was kept in refrigerator for 24 hrs. A large amount of yellow solid separated, which was filtered and washed with methanol. The yellow solid has a melting point 228-230^{O}C. Based on the spectral data (UV, IR, NMR and Mass) the compound was identified as gossypin. The flavonoid gossypin has been reported to exhibit anti-inflammatory action through inhibition of arachidonic acid metabolism .Gossypin significantly reduces the effect of paw edema and increased vascular permeability induced by histamine, 5-HT, bradykinin and Prostaglandin-E. Gossypin has also been shown to possess anti-hyaluronidase activity Antinoceptive activity of gossypin was also reported.

MATERIALS AND METHOD

Chemicals

L-NAME was purchased from Sigma chemical company. All other biochemical reagents and chemicals were of Analytical grade.

Animals

Healthy Wistar albino rats of either sex weighing between 220-260 g m were taken for the study. All the animals were procured from the Animal House of the H.S.K. College of Pharmacy, Bagalkot, and Karnataka. The animals were acclimatized by keeping them in the animal house facility of H.S.K .College of Pharmacy, Bagalkot for a week. They were housed in polypropylene (32x24x16 cm) cages containing husk as bedding material and maintained under controlled conditions of temperature (25±2 c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water *ad libitum*. Approval of the Institutional Animals Ethics Committee (IAEC) of H.S.K.College of Pharmacy, Bagalkot was taken for conducting antihypertensive activity. (Registration No. IAEC (HSKCP/IAEC, Clear / 2013-14 / 1-12).

Plant /Drug

Gossypin was obtained as pure drug from sigma chemical co. USA

Instrument used

AD instrument 8 channel, Lab chart solution.

Procedure

Healthy Albino rats (220-260gm) were used for the experiment. Animals were divided into 5 groups each group comprising of 6 animals each. The (blood pressure arterial) was determined by invasive method using AD instrument.

Group I	Normal
Group II	Control (L-NAME20mg/kg)
Group III	Standard (Losartan 10mg/kg)
Group IV	Gossypin (5mg/kg)
Group V	Gossypin (10mg/kg)

Blood pressure and heart rate were determined by the invasive method. Briefly, animals were anaesthetized by intraperitoneal administration of ketamine at the dose of 50mg/kg and a cannula was implanted in the femoral vein for drug administration. Another cannula was inserted in the left carotid artery for direct blood pressure measurement. Both cannula were filled with glucose-saline heparinized solution. The cannula inserted in the carotid artery was connected to a blood pressure transducer connected to AD instrument channel Lab chart solution for blood pressure recording. A stabilization period of 30 minutes was observed before any recording. Heart rate was also observed.

Experimental procedure

For acute antihypertensive study, a solution of L-NAME, an inhibitor of nitric oxide synthase

was intravenously injection to normotensive Wistar rats (20 mg/kg) after the stabilization period at corresponding volume of 100µl/ 100 g bw. Losartan10mg/kg i.v and Gossypin was administered i.p at the doses of 5, 10mg/kg, twenty minutes after L-NAME administration, when the rise in blood pressure induced by L-NAME had reached the maximum. The BP and heart rate was observed for 2 hrs from 0min to 120 min.

Experimental procedure for L-NAME induced chronic hypertension.

Healthy Albino rats (220-260gm) were used for the experiment. Animals were divided into 6 groups each group comprising of 6 animals each. The (blood pressure arterial) was determined by invasive method using AD instrument. All the groups were administered L-NAME (40mg/kg b.w p.o/day for 4 wks) accept group I which receives normal saline for 4 wks. Group III were given STD drug (Losartan 20mg/kg b.w p.o /day for 4 wks) and group IV, V, VI received Gossypin at the dose of 5, 10,20mg/kg/day p.o for 4 wks)

Group I ----------Normal saline

Group II----------L-NAME (40mg/kg/day)

Group III----------STD (Losartan 20mg/kg/day)

Group IV----------Gossypin (5mg/kg/day)

Group V----------Gossypin (10mg/kg/day)

Group VI----------Gossypin (20mg/kg/day)

Blood pressure and heart rate determination

Blood pressure and heart rate were determined by the invasive method. Briefly, Animals were anaesthetized by intraperitoneal administration of ketamine at the dose of 50 mg/kg and a cannula was inserted in the left carotid artery for direct blood pressure measurement . cannula were filled with glucose-saline heparinized solution. The cannula inserted in the carotid artery was connected to a blood pressure transducer connected to AD instrument 8 channels, Lab chart solution for blood pressure recording. A stabilization period of 30 minutes was observed before any recording. Heart rate was also observed.

Statistical analysis

All values were expressed as mean ± SEM. The results were statistically evaluated using One-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison tests using Graph Pad software .Significance for difference between groups was evaluated for student's *t*-test to come to final conclusion.

RESULTS AND DISCUSSION

In acute hypertension the BP was observed for 120min in all groups. In group II, serves as control receives L-NAME there is a significant rise in BP after L-NAME administration (*p<0.05) after 30min. In L-NAME administration animals showed elevated BP and decreased in heart rate. In group III, standard group received Losartan showed reduction BP in L-NAME (**P<0.01) induced hypertension. In group IV and V, received gossypin (5 and 10 mg /kg) showed a significant (**p<0.01) decreased in L-NAME induced hypertension.

In chronic hypertension, Gossypin (5, 10, 20mg/kg) exhibited significant (***p<0.001) reduction in the hypertension as compared to control group. In this model, inhibition of NO-synthase permits the evaluation of the nitric oxide pathway, a physiologically important vasodilator, playing a major role in the regulation of systemic hemodynamic [20]. Acute intraperiotoneal administration of Gossypin in L- NAME-induced hypertensive rats showed a significant decrease in mean arterial blood pressure (MABP) that last more than an hour after administration. It is well known that L-NAME, administered either acutely by intravenous route or chronically by the oral induced sustained hypertension. But Chaswal et al [23] showed that the acute hypertension provoked by L-NAME administration may not involve the renin angiotensin system, which is at least partially responsible for the chronic response. The fact that Gossypin could reduce both the acute and chronic hypertension induced by L-NAME indicates that it may use another pathway. we postulate that the acute antihypertensive effect of gossypin might be mainly due to its ability to reduce the peripheral resistance via its vasodilating activities. It is also well known that in this model of experimental arterial hypertension, the sympathetic system tone and baroreflex to phenylephrine are significantly increased. Gossypin could also interfere with this system. Permanent increase in systemic blood pressure often leads to cardiovascular hypertrophy. Results with Losartan corroborate many other previous works and further confirm the involvement of the NO inhibition system in the development of this model of arterial hypertension. Phytochemical constituents like flavanoids and saponins in the plant. These bioactive phytomolecules are reported for their vasorelaxant and antihypertensive effects .With these may be gossypin is flavonoid showed vasorelaxant and anti-hypertensive activity by modifying NO-induced pathway.

Table 1: Normal hypertension level in normal rats.

Blood pressure	0min	30min	60min	120min
Systolic (mmHg)	158.8±5.96	151.7±3.39	148.1±3.09	138.2±0.89**
Diastolic(mmHg)	125.30±5.97	123.1±5.97	117.5±1.87	99.84±2.58**
Mean(mmHg)	142.1±3.88	137.5±2.94	132.8±2.26	119.0±1.56*
Heart rate (Beats /min)	329.1±18.41	330.2±27.46	319.7±17.03	323.8±4.93

All values are expressed as a Mean ± SEM, n=6, unpaired student *t*-test.

The minimum value of *p<0.05, **p<0.01 as compared to 0 min.

Table .2: Effect of L-NAME (20mg/kg) induced acute hypertension in rats

Blood pressure	0min	30min	60min	120min
Systolic (mmHg)	151.3±3.74	176.8±10.37	183.80±5.74**	169.5±5.13*
Diastolic(mmHg)	121.3±1.77	147.4±11.91	147.6±5.70**	132.6±4.72*
Mean(mmHg)	134.2±1.05	161.9±11.20	167.7±5.04**	148.3±6.10**
Heart rate(BPM)	378.6±13.07	275.0±7.18	281.9±9.10	281.9±5.76***

All values are expressed as a Mean ± SEM, n=6, unpaired student *t*-test .The minimum value of *p<0.05, **p<0.01, ***p<0.001 as compared to 0 min.

Table 3: Effect of (Losartan 10mg/kg) in L-NAME induced acute hypertension in rats

Blood pressure	0min	30min	60min	120min
Systolic (mmHg)	161.9±5.62	172.3±11.53	215.5±10.08**	153.1±3.33*
Diastolic(mmHg)	129.3±8.83	133.7±5.95	170.5±9.75*	118.7±3.44*
Mean(mmHg)	145.7±6.95	151.2±6.87	192.9±7.37**	136.0±2.98*
Heart rate(BPM)	342.3±25.22	337.5±14.25	313.5±12.38*	397.4±2.25*

All values are expressed as a Mean ± SEM, n=6, unpaired student *t*-test .The minimum value of *p<0.05, **p<0.01, compared to 0min.

Table 4: Effect of Gossypin (5 mg/kg) in L-NAME induced acute hypertension in rats

Blood pressure	0min	30min	60min	120min
Systolic (mmHg)	145.7±2.12	155.4±1.51	164.5±4.11**	167.9±3.46***
Diastolic(mmHg)	114.4±3.80	122.3±1.56	125.0±3.78	124.6±3.18
Mean(mmHg)	130.6±2.85	138.8±1.50	144.8±3.92*	146.2±3.03**
Heart rate(BPM)	350.8±20.7	343.9±18.9	351.00±15.17	351.00±19.75

All values are expressed as a Mean ± SEM, n=6, unpaired student *t*-test .The minimum value of *p<0.05, **p<0.01, ***p<0.001 as compared to 0 min.

Table 5: Effect of Gossypin (10mg/kg) in L-NAME induced acute hypertension in rats

Blood pressure	0min	30min	60min	120min
Systolic (mmHg)	164.4±4.36	162.6±8.81	205.3±17.80*	141.7±3.68*
Diastolic(mmHg)	137.2±8.78	129.4±8.53	440.5±280.90*	111.3±3.85*
Mean(mmHg)	151.1±6.30	145.00±8.16	181.2±15.61*	126.5±2.54*
Heart rate(BPM)	321.6±18.33	317.9±19.21	314.5±22.29**	387.9±16.33**

All values are expressed as a Mean ± SEM, n=6, unpaired student *t*-test .The minimum value of *p<0.05, **p<0.01, as compared to 0 min.

Table 6: Effect of Gossypin against L-NAME induced chronic hypertension

Treatment	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean BP (mmHg)	Heart rate (BPM)
Normal	139.30±3.193	103.3±2.745	121.20±2.985	402.20±4.73
L-NAME(40mg/kg)	245.5±9.223[a]	173.80±4.111[a]	198.50±6.543[a]	287.8±3.70[a]
Losartan(20mg/kg)	153.30±6.724***	121.60±4.713***	141.00±5.067***	328.3±3.91***
Gossypin(5mg/kg)	173.4±3.948***	117.80±1.361***	141.00±1.756***	292.0±2.61***
Gossypin (10mg/kg)	149.60±6.688***	112.9±5.023***	131.30±5.742***	302.11±3.31***
Gossypin (20mg/kg)	157.60±5.994***	115.5±4.823***	134.50±4.702***	294.10±3.31***

All values are expressed as a Mean ± SEM, n=6, One Way Analysis of Variance (ANOVA), followed by multiple comparisons Dunnet's test. The minimum value of [a]$p<0.05$, as compared to normal group.***$p<0.001$ as compared to control group.

CONCLUSION

In this we conclude that gossypin possess a significant cardio protective activity against Isoproterenol induced myocardial damage and anti-hypertensive agent against L-NAME induced hypertension in rats.

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