

**ASSESSMENT OF ANTIHYPERTENSIVE ACTIVITY OF NOVEL SYNTHETIC HYBRID COMPOUND ON 2K1C INDUCED HYPERTENSIVE RATS**

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**Summary**

The present investigation was carried out to study the antihypertensive activity of the two derivatives (NS1 & NS2) of novel synthetic hybrid compound [N(phenyl)N' {1'6methyl-4-(2-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine- 5- carboxylic acid ethyl ester} 3' (4'' imino phenoxy) propane-2- ol thiourea] on 2K1C induced hypertensive rats. The left renal artery (LRA) was ligated by silk suture (no.4) in rats to produce two-kidney one-clip (2K1C) renovascular hypertension and kept for 6 weeks. After 6 weeks, respective treatments were started and continued for 7 days. On 7<sup>th</sup> day, 30 min after the last dose, the arterial blood pressure and heart rate (HR) of rats were measured by using data acquisition system. LRA ligated rats showed significant (P < 0.01) increment in mean arterial pressure (MAP) compared to non-ligated (normal) rats. NS1 decreased MAP and HR significantly (P<0.01) while NS2 significantly (P<0.01) decreased MAP without affecting heart rate when compared against LRA ligated rats. Standard drugs were significant (P<0.01) in reducing MAP as well as HR of LA ligated rats. In conclusion, both the derivatives (NS1 and NS2) of the novel synthetic hybrid compound showed significant reduction in the blood pressure of 2K1C induced hypertensive rats, while HR was only significantly reduced by NS1. The study needs further investigation to know the exact mechanism of antihypertensive activity of these hybrid compounds.

**Keywords:** Antihypertensive activity, heart rate, mean arterial pressure, novel synthetic hybrid compound, two-kidney one-clip.

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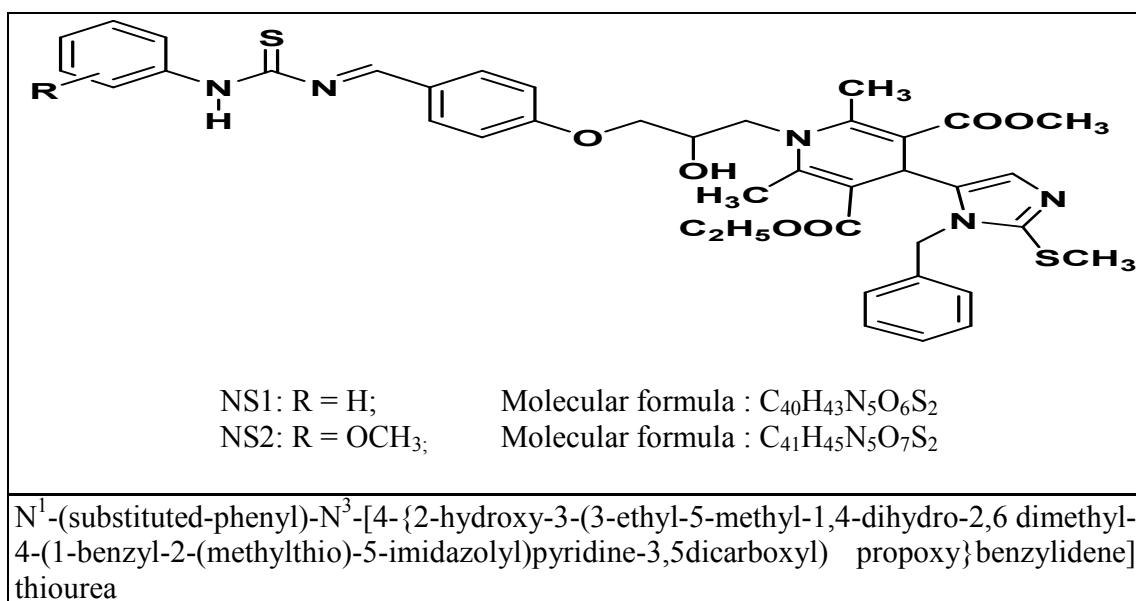
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### **Introduction**

Hypertension is a clinical condition of persistent raised arterial pressure, primarily due to increased vascular resistance in systemic circulation (1). It is one of the leading causes of disability, morbidity and mortality among the populace and most common chronic illness the world faces. It is one of the most important modifiable risk factor for stroke, renal vascular diseases and coronary heart disease (CHD) in Western and Asian population and CHD is estimated to be the most common cause of death globally by 2020 (2, 3).

Antihypertensive agents with several mechanism of action such as vasodilation,  $\beta$ -blockage,  $\text{Ca}^{++}$  ion channel blockage, ACE inhibition etc are currently available. A problem often faced in the treatment of hypertension is its complex and heterogenous pathogenesis. In many instance, treatment with a single drug cannot adequately control the illness. Sometimes the use of therapeutic agent alone in the treatment of disease may be limited by the side effects caused by its own action. The combinations of drug with different pharmacotherapeutic effects are feasible and it can be applied either to overcome the side effect of single drug or to add beneficial effects. The principle of combination drug therapy can be achieved by either concomitant administration of two or more single active drugs or by drugs in which the single active agents are combined in one molecule, so called hybrid molecule. This hybrid molecule often consists of different pharmacophoric groups which are linked to each other via spacers. Although it is tempting to designate every molecule which produces more than one biological effect as a hybrid molecule, there are certain restrictions. First, the hybrid molecule must contain two or more pharmacophoric groups which exert biological action via different receptors. Secondly, to be of clinical value, the biological properties must be present in the same concentration range and third, the hybrid molecule must be resistant to metabolic processes which after administration would result in regeneration of the original drugs (4).

Today's trend in antihypertensive drug design is to club together two or three molecules having different sites/mechanism of action. Being impressed by such recent trends, a superior vasodilator drug with beta-blocking activity was synthesized in our chemistry lab. Though vasodilation reduces the blood pressure (BP) by directly relaxing peripheral vascular smooth muscles, the reduction in BP leads to a reflex increase in sympathetic tone followed by increase in heart rate, cardiac output and plasma renin activity which attenuates antihypertensive effect. It was reported that these undesirable effect of vasodilator could be eliminated or minimized by simultaneous treatment with beta-blocker and the possible increase in peripheral vascular resistance induced by beta blocker was eliminated or minimized by concomitant use of vasodilators. We have chosen two vasodilator having different mechanism of action, one is thiourea skeleton, which is reported to posses vasodilator activity upon structural modification & the other is  $\text{Ca}^{++}$  ion channel blocker [ 3-methyl-5-ethyl-1,4-dihydro-2,6-dimethyl-4-(1-phenylmethylthi-5-imidazolyl)pyridine -3,5-dicarboxilate] attached through beta-blocking moiety (propranolamine). The above synthesized compounds and their derivatives were subjected to preliminary pharmacological investigation.



**Figure.1. Basic chemical structure of the synthesized hybrid NS series compounds**

2K1C is very commonly used model for renal hypertension. In renovascular hypertension, Renin Angiotensin Aldosterone System (RAAS) plays an important role in control of cardiovascular homeostasis affecting both blood pressure and fluid volume and is one of the most important etiological candidates in hypertension (3, 5). Experimentally the renal hypertension is produced by renal artery constriction, which activates RAAS and sympathetic nervous system. A number of factors like decreased blood volume may lead to sympathetic stimulation in this model. Renin is secreted by kidney when sympathetic activity is increased. Renin converts angiotensinogen to angiotensin-I. Angiotensin-I is converted to angiotensin-II by angiotensin converting enzyme (ACE). Angiotensin-II is a potent vasoconstrictor and increase BP. Angitensin-II also causes release of aldosterone leading to salt and water retention resulting in increased blood volume and hypertension (6).

In Two Kidney One Clip hypertension the ligature is applied to artery of one kidney and contralateral kidney is left untouched (7). This results in a sustained increase on BP due to increased plasma renin activity (PRA), which in turn increases the circulating angiotensin-II, a potent vasoconstrictor. However there is no salt and water retention because of the other normal kidney being intact. Thus the resultant hypertension at this stage is renin angiotensin dependent. After about 6 weeks, the increased angiotensin-II releases aldosterone from adrenal cortex leading to a gradual retention of salt and water which decreases the renin production. From this stage onwards, hypertension is volume dependent. Hence salt and water balance is critically involved in pathogenesis of renovascular hypertension (7, 8).

### **Materials and methods**

#### **Animals:**

Wistar albino rats of either sex (150-200 g) were housed in clean environment at a temperature of  $25 \pm 1^\circ\text{C}$  and relative humidity of 45 to 55%, under 12/12h light/dark cycle. The animals had free access to food pellets and water *ad libitum*. The research protocol was approved by Institutional Animal Ethics Committee (IAEC) of AISSMS College of Pharmacy, Pune, India.

#### **Chemicals:**

Atenolol was received as a gift sample from Acme Formulation Pvt. Ltd., Himachal Pradesh. Ketamine (Ketmin50, Themis Medicare Ltd), urethane, propranolol (Ciplar 40mg, Cipla), ethanol and heparin (Thromboparin injection, Charls Pharma Inc) were procured from local market.

#### **Surgical procedure for induction of hypertension in rats:**

Hypertension in rats was induced by clipping the left renal artery (LRA), as previously described (9). Male Wistar rats weighing 150 to 200 g were anesthetized with ketamine (80 mg/kg, i.p.). Fur on the back was shaved and skin was disinfected with alcohol (70 % v/v). An incision was made in the left lumbar area parallel to long axis of the rat. Renal pedicel was exposed with the kidney retracted to abdomen. LRA was dissected, cleaned and tied with the help of surgical silk suture (4.0). The left kidney was placed in position and skin incisions were closed by surgical absorbable suture. After recovery the rats were placed in clean cages and maintained under standard food pellets and 1% sodium chloride solution instead of water (10).

#### **Determination of antihypertensive activity of NS1 and NS2: (7, 10)**

Two kidney one clip model of kidney ligation was used to produce hypertension in rats. The rats were divided in two groups, Group 1 consists of six rats and were not undergone LRA ligation. Group 2 consist of 30 rats and were undergone for the LRA ligation. They were anaesthetized with ketamine (80 mg/kg, i.p.) and the LRA was ligated. After LRA ligation, the rats were housed and provided with 1% sodium chloride salt solution instead of water. After 6 weeks of LRA ligation the rats were divided into 5 Groups consisting of 6 rats in each group. Group 2(a) received vehicle [saline (0.9% sodium chloride salt solution), 1 ml/kg, i.p.], which served as LRA ligated control. Group 2(b) and 2(c) received a daily dose of standard drugs propranolol (30 mg/kg, i.p.) and atenolol (10 mg/kg, i.p.) respectively. Group 2(d) and 2(e) received a daily dose of test drugs NS1 (10 mg/kg, i.p.) and NS2 (10 mg/kg, i.p.) respectively. The dosing of rats was continued for seven days. On 7<sup>th</sup> day, 30 min after the respective treatments, the rats were anaesthetized with urethane (1.25 g/kg, i.p.) and blood pressure and heart rate were recorded. Doses of test compounds were selected on the basis of their LD<sub>50</sub>.

**Measurement of Heart Rate (HR):**

ECG was recorded with the application of subcutaneous needle electrodes to the flexor aspect of limbs of rat and connected with the SS2L electrode transducer which was connected with the Four Channel Data Acquisition System (BIOPAC System, Inc, MP 35) with the respective ECG transducer. The ECG of rats was recorded, and the heart rate was measured by the ECG graph that was displayed in the screen of the respective computer. Measurement of heart rate was done from R-R interval of ECG recording. After measurement of heart rate (HR), the mean arterial pressure (MAP) was measured.

**Measurement of Mean Arterial Pressure (MAP):**

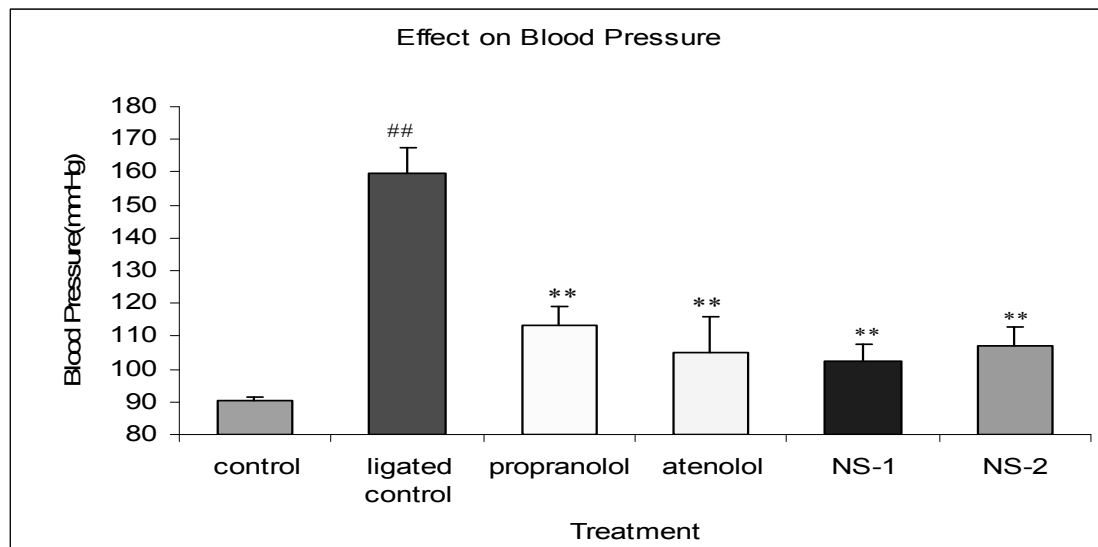
Anaesthetized rats were injected with heparin (100 IU/ml) to prevent the coagulation of blood in the catheter. The left carotid artery was exposed and cannulated with the catheter PE-50 (polyethylene-50) prefilled with heparinized 0.85% NaCl solution, for the measurement of the MAP. The catheter (PE-50) was connected to the blood pressure transducer which was connected with the Four Channel Data Acquisition System (BIOPAC System, Inc, MP35). After half hour of cannulation, when the arterial blood pressure reaches to stabilized condition (equilibrium), blood pressure was noted down.

**Statistical analysis:**

The results are expressed as mean  $\pm$  S.E.M. The statistical significance of difference between groups was determined using one-way analysis of variance (ANOVA) followed by Dunnett's test. Mean values were considered significantly different if  $P < 0.05$ . The software used for the statistical analysis was INSTAT and Graph Pad Prism.

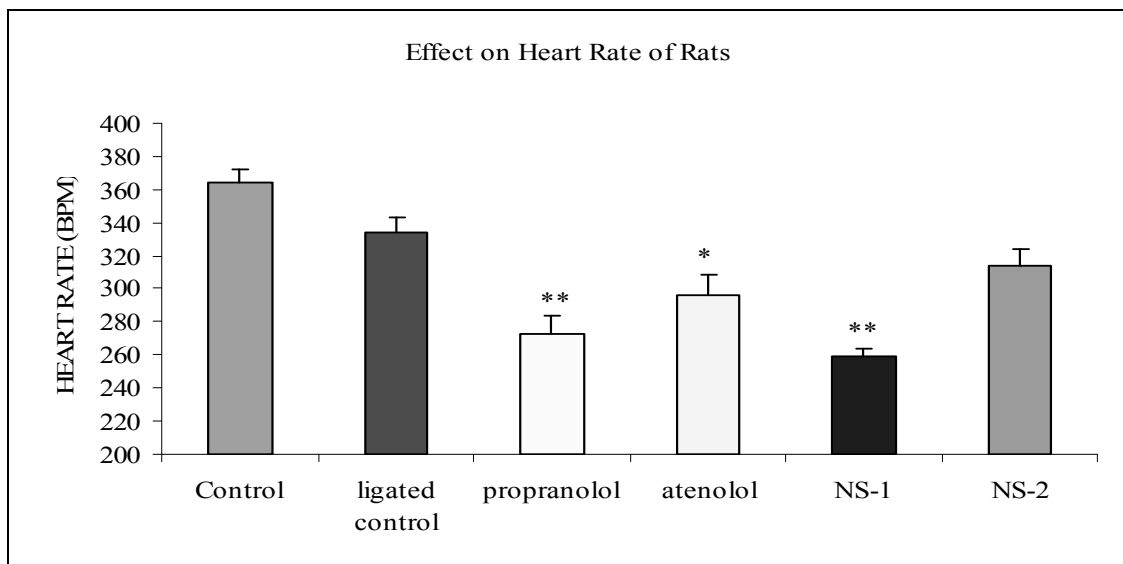
**Results**

LRA ligation had significantly ( $P < 0.01$ ) increased MAP compared to non-ligated rats. However, the heart rate of LRA ligated rats has not shown significant changes as compared to non-ligated rats. Treatment with NS1 (10 mg/kg, i.p.) and NS2 (10 mg/kg, i.p.) produced a significant ( $P < 0.01$ ) reduction in MAP. NS1 produced a significant decrease in heart rate while NS2 has not shown any significant decrease in the heart rate. Standard drugs atenolol (10 mg/kg, i.p.) and propranolol (30 mg/kg, i.p.) significantly ( $P < 0.01$ ) reduced MAP and heart rate of hypertensive rats. The fall in MAP produced by NS1 was greater than atenolol and propranolol while the effect of NS2 on MAP was less than atenolol but was greater than those produced by propranolol. NS1 showed greater reduction in HR compared to atenolol and propranolol but NS2 has not shown any significant reduction in HR.



**Figure. 2. Effect of test and standard drugs on mean arterial pressure of hypertensive rats.**

Results are expressed as mean  $\pm$  SEM. n=6, Data was analyzed by one way analysis of variance (ANOVA) followed by Dennett's' test. ##P < 0.01 as compared to non- ligated rats, \*P < 0.05 and \*\*P < 0.01 as compared to LRA ligated control group.



**Figure. 3. Effect of test and standard drugs on heart rate of hypertensive rats.**

Results are expressed as mean  $\pm$  SEM. n=6, Data was analyzed by one way analysis of variance (ANOVA) followed by Dennett's' test. ##P < 0.01 as compared to non- ligated rats, \*P < 0.05 and \*\*P < 0.01 as compared to LRA ligated control group.

**Discussion & conclusion**

The aforementioned study reveals that the two derivative of novel synthetic hybrid molecule consisting of three different pharmacophore with different site/mechanism of action possess significant antihypertensive activity. The first active pharmacophore of drug is thiourea, which was reported to have vasodilator effect on blood vessels and thereby produces antihypertensive effect. This antihypertensive effect was mediated by decrease in the total peripheral resistance due to dilation of blood vessels. The second active pharmacophore is dihydropyridine, a reported calcium channel blocker which shows antihypertensive effect by restricting the calcium entry into the smooth muscle (blood vessels), and there by produces vasodilatory effect. Dihydropyridine moiety also decreases the cardiac output and heart rate. The third active pharmacophore is propanolamine, which was reported to have  $\beta$ -blocking activity (nonselectively).  $\beta_1$ -adrenoceptor blocker produces the reduction in the heart rate and  $\beta_2$ - adrenoceptor blocker produces vasodilaory activity in the peripheral blood vessels.

All the pharmacophoric group of this hybrid molecule is reported antihypertensive, so the overall activity of both derivative of hybrid molecule was expected to have antihypertensive activity. This experimental study shows that both the derivative NS1 and NS2 have antihypertensive activity on the 2K1C induced hypertensive rats.

Since the pioneering working of Goldblatt and colleagues, 1K1C and 2K1C methods have been used to induce hypertension in rats for different purposes (11, 12). The animal models of hypertension share many features which are common to human hypertension. Most of the models have been developed by utilizing the etiological factors that are presumed to be responsible for human hypertension such as excessive salt intake, hyperactivity of RAAS and genetic factors. Since regulation of blood pressure is multifactorial, the effectiveness of an antihypertensive agent in one model dose not necessarily mean that the mechanism of action of a given agent in given model is related to the pathogenesis of elevated blood pressure. (7).

The two kidney one clip model of renovascular hypertension increases the blood pressure by stimulating the RAAS system and intake of salt increases the blood volume.  $\beta$  - adrenoceptor antagonism is expected to inhibit renin release. (13), so the reduction in the renovascular hypertension by the NS1 and NS2 shows that it may act by inhibition of  $\beta$ -adrenoceptor.

Bradycardia effects of compounds may result from calcium entry and beta-adrenoceptor blocking (14). But the NS2 shows insignificance in producing bradycardia, so it may be due to less/non  $\beta_1$ -blocking activity or calcium channel blocking activity.

The mentioned hybrid compounds showed significant antihypertensive activity which might be beneficial for use in hypertension when single drug significantly fails to reduce blood pressure of hypertensives. However, study needs further investigation to understand the exact mechanisms antihypertensive activity of novel synthetic hybrid compounds.

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