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EFFECTS OF *ECLIPTA ALBA* AND *BOERHAAVIA DIFFUSSA* ON NORMAL BLOOD PRESSURE AND HYPERTENSION IN RATS AND THEIR COMPARISON WITH AMLODIPINE

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ABSTRACT

Background: Hypertension is an immensely pervaded problem in today's population and is a unanimous major risk factor for heart diseases. Various natural herbs have been found to control the hypertension exquisitely without causing any significant adverse effect. *Eclipta alba* (EA) and *Boerhaavia diffusa* (BD) are two herbaceous plants. EA is used to treat hepatic-dysfunction, hair diseases and anemia since ancient time. Similarly, BD is a great treatment-option for renal and urinary disorders. They have also been found quite effective in the safe treatment of hypertension. But studies in this respect are very scanty and confirmatory role of these herbs in hypertension is yet to be established. In the present study, effects of EA and BD on normal blood pressure (NBP) and hypertension were studied. Their antihypertensive activities were also compared with those of Amlodipine (Amlo).

Methods: Adult Wistar rats of both sexes, weighing 140-150 g were randomized equally to make 9 groups (6 rats/ group). First 6 groups were given high fat diet for 49 days to produce hypertension. Then drugs were given for next 45 days without stopping high fat diet. 7^{th}, 8^{th} & 9^{th} groups were meant to see the effects of EA and BD on NBP; they were kept on normal diet and were given EA-200 mg/kg, BD-200 mg/kg, EA+BD (200 mg/kg of each) respectively for first 45 days. SBP (systolic blood pressure) was measured by 'Tail-cuff method' with the help of NIBP (non-invasive blood pressure measurement)-controller machine.

Results: All drugs showed significant antihypertensive activities. Rats on Amlo-10 mg/kg showed most significant reduction (39.54%), followed by rats on EA+BD (200mg/kg of each; 31.01% reduction), BD-200 mg/kg (28.91% reduction), EA-200 mg/kg (25.54% reduction), BD-100 mg/kg (20.68% reduction). EA, BD and EA+BD caused no significant reduction in NBP.

Conclusion: To conclude, EA and BD comprise significant antihypertensive activities with no harmful effects on (reduction in) NBP.

INTRODUCTION: Hypertension is a very serious and pervaded health problem in today's population. Both clinical and epidemiologic studies have revealed that hypertension is undoubtedly a major risk factor for atherosclerosis and its complications, such as stroke and myocardial infarction. Apart from conventional allopathic measures, there must be meticulous search for alternative treatment; therefore it is evident to

look for natural options & switch on to safer indigenous system of medicine like natural herbs. WHO (in 1980) has also recommended the evaluation of the effectiveness of plants in conditions where there are no safe modern drugs are available. *Eclipta alba* (or, *Eclipta prostrata*) is a perennial herbaceous plant (family Asteraceae). It grows usually in moist places as a weed throughout the world. *Eclipta alba* is reported to cause improvement in hair growth and color [1, 2].

This plant contains coumestans [3], i.e. wedelolactone (I) and demethylwedelolactone (II) etc. Coumestans have been found to comprise estrogenic activity. Wedelolactone has been found to express profound antihepatotoxic activity [4]; it also acts as an antibacterial and anti-hemorrhagic. *Eclipta alba* also has traditional external uses; *viz* athlete foot, eczema and dermatitis. Some of its constituents have neutralized the toxicity and lessened the bleeding caused by snake venom [5] and mushroom toxins.

Boerhaavia Diffusa (Punarnava) is widely distributed in tropic and subtropic zones [6]. "Punarnavine (an alkaloid)" is the active principle in its extract. It is exquisitely effective to cure renal problems [7, 8].

It is also useful in hepatitis, snake-bite, rheumatism, scabies, menorrhagia, angina, respiratory tract infections and as contraceptive. It is effective in the abdominal cancers [9]. It also has antiviral activities [10, 11, 12, 13]. These plants have been found to exert antihypertensive action also, but have got very scanty number of studies and references [14, 15].

Present study was done to observe the effects of these two herbs (separately and in combination) on normal and raised blood pressures; and to compare these effects with those of standard allopathic drug Amlodipine.

MATERIALS AND METHODS: Study was started in March 2011, at Department Of Pharmacology and Therapeutics, Chhatrapati Shahuji Maharaj Medical University, Lucknow, Uttar Pradesh, India.

Experimental animals: Adult healthy Wistar rats of either sex, weighing 140-150 g were used in study. Animals were obtained from CPCSEA-certified animal house [Indian Institute Of Toxicology Research, Lucknow, UP, India].

They were given food and water *ad libitum* and were kept in Institutional animal house under temperature, humidity and light & dark cycle-controlled environment [25 ± 2°C, 70%, 12 hrs' cycle).

High Fat Diet (HFD): It was prepared by Dayal Industries Pvt. Ltd., Barabanki Road, Lucknow, UttarPradesh, India (**table 1**).

TABLE 1: COMPOSITION OF HIGH FAT DIET

Ingredients	Approximate amount
Crude Fat (Prepared from Rice Bran)	15%
Crude Protein	16%
Acid Insoluble Ash	2.30%
Moisture	8%
Vitamins and Minerals	Appropriate quantity

Dosage forms, doses and sources of Drugs: Drugs were given orally with the help of feeding cannula.

Standard Drug- Amlodipine (Amlo): This was given having been dissolved in distilled water (after pulverization of tablets). It was purchased from market.

Tab. Amlodipine- Dose-10 mg/kgbw [16, 30]. (kgbw-kilogram of body weight)

Trade name- AMLOKIND (Mnufactured by Mankind Pharma Limited 236, Okhla, Phase -III, New Delhi).

Test Drugs: EA (*Eclipta alba*) & BD (*Boerhaavia diffusa*)– dried crude forms had been procured from IITC/Organic India Pvt. Ltd., Lucknow, India. Then their extracts were prepared. Extracts were given having been dissolved in distilled water just before administration. Extracts were otherwise kept at 4°C. {Dose of EA - 200 mg/kg [17]; Doses of BD- 200 mg/kg[18] and 100 mg/kg}. To see the combined effect; EA & BD, each in a dose of 200mg/kg were given together.

Preparation of extracts of test drugs: Dried plant material was ground to coarse powder. This powder was used for extraction. The exhaustive extraction of plant material was carried out using 80% ethanol in soxhlet apparatus.

The ethanolic extract was concentrated to dried residue by distillation (alcohol was allowed to evaporate). The thick paste thus obtained was stored at 4^{o}C till further use.

Measurement of SBP: Systolic blood pressure was measured by TAIL CUFF METHOD with the help of NIBP (Non-invasive blood pressure measurement) CONTROLLER MACHINE [ML125], AD Instruments (Australia) [22].

Statistical Analysis: Data were summarized as mean ± SD. Groups were compared by *'two factor (groups × days) analysis of variance (ANOVA)'* and the significance of mean difference within & among the groups was assessed by *Tukey post hoc test*. A two-sided (α=2) $p<0.05$ was considered statistically significant. Analyses were performed on *STATISTICA 6.0 (software)*.

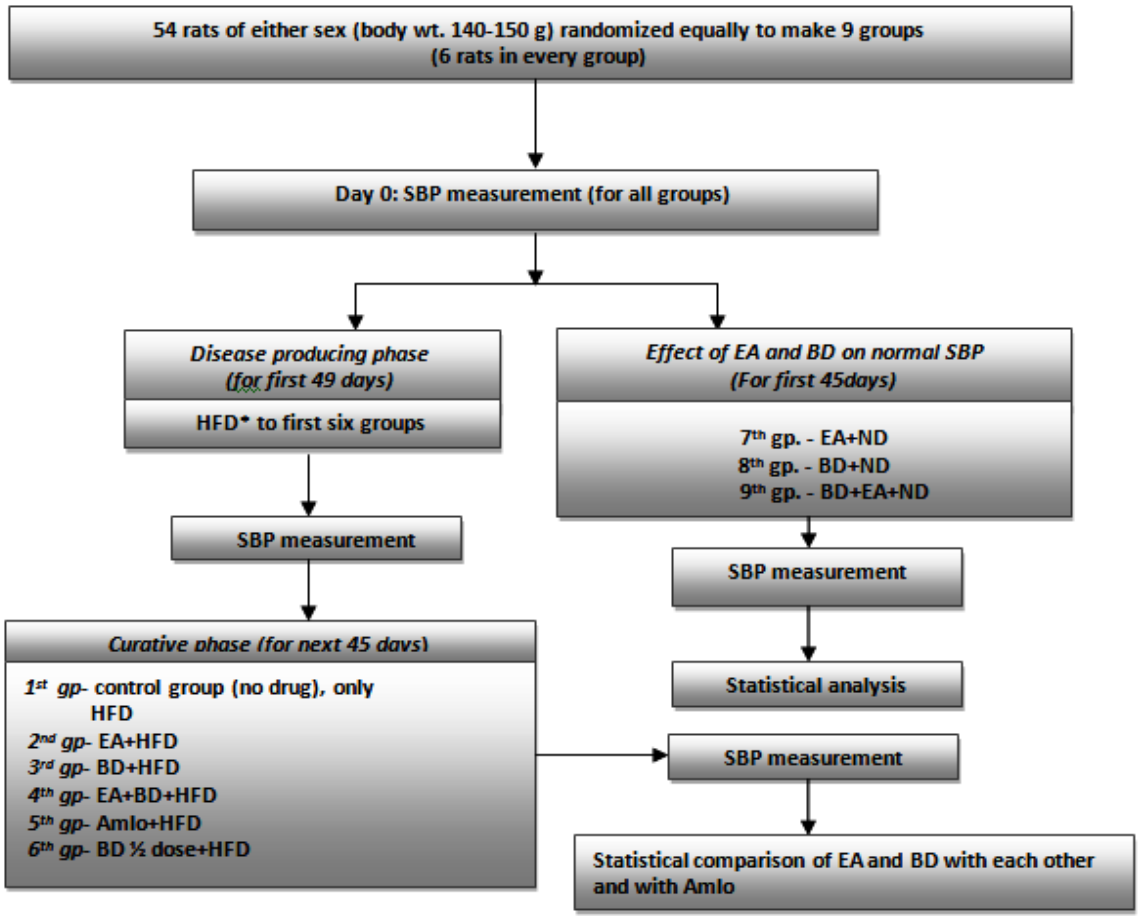

FIG. 1: LAYOUT OF EXPERIMENTAL PROTOCAL

EA- Eclipta alba, BD- Boerhaavia diffusa, Amlo- amlodipine, HFD- high fat diet, ND- normal diet, SBP- systolic blood pressure, gp- group. *HFD was given to produce hypertension [19, 20, 21].

RESULTS & DISCUSSION:

Effect of test drugs (EA, BD, EA+BD) on normal SBP: After drug-treatments (from day 0 to day 45) SBP decreased in all groups and evident highest in Group 9 (2.29% decrease) followed by Group 8 (1.47%) and Group 7 (1.04%).

ANOVA revealed insignificantly different SBPs among ($F=0.09$, $p>0.05$) and within (between days) the groups ($F=0.19$, $p>0.05$). SBPs in all groups decreased insignificantly ($p>0.05$) at day 45 as compared to those at day 0 (**Table 2a & Fig. 2a**).

TABLE 2a: PRE AND POST TREATMENT SBPs (mmHg) OF THE RATS ON NORMAL DIET (Mean ± SD, n=6)

Test drug groups	At day 0	At day 45	% change (day 0 to day 45)	F value (between days) (1, 30 DF)	F value (among groups) (2, 30 DF)
Group 7	109.33 ± 11.24	108.21 ± 12.17^{ns}	1.04%		
Group 8	107.50 ± 12.06	105.94 ± 11.69^{ns}	1.47%	0.19^{ns}	0.09^{ns}
Group 9	108.67 ± 12.11	106.23 ± 11.72^{ns}	2.29%		

ns – non significant; Non significant decrease in SBPs, since 'p' value >0.05.

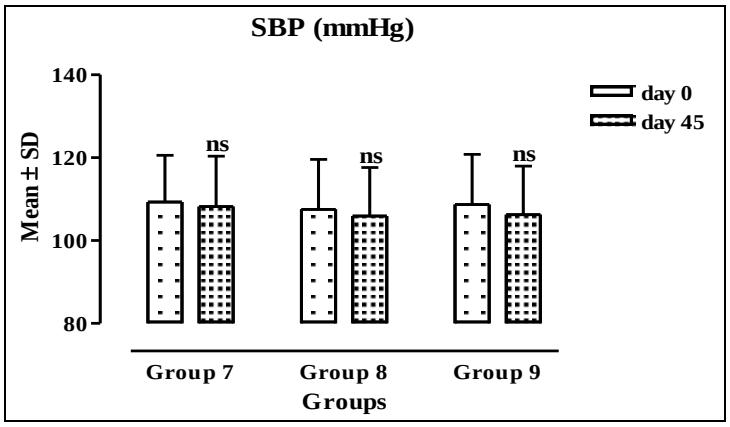

FIG. 2a: PRE AND POST TREATMENT MEAN (± SD) SBPs OF 7th, 8th & 9th GROUPS

ns p>0.05- day 0 vs. day 45.

Among the groups, SBPs in all groups were found similar (p>0.05) at day 0 and day 45 (**Table 2b and Fig. 2b**). It indicates that the test drugs have not shown significant effect on SBPs of normal (healthy) rats (on normal diet).

TABLE 2b: COMPARISON (p values) OF MEAN SBPs OF THREE (7th, 8th, 9th) GROUPS AT DAY 45 by *TUKEY TEST*

Comparisons	Mean difference (MD)	Tukey test (q value)	p value
Group 7 vs. Group 8	2.27	0.47	p>0.05
Group 7 vs. Group 9	1.98	0.41	p>0.05
Group 8 vs. Group 9	0.29	0.06	p>0.05

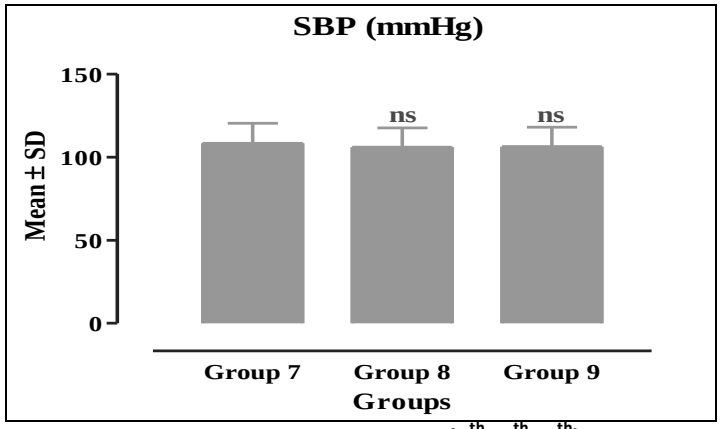

FIG. 2b. COMPARATIVE SBPs OF THREE (7th, 8th, 9th) GROUPS AT FINAL EVALUATION (day 45)

ns p>0.05- as compared to Group 7

Effect of test and standard drugs on SBPs of rats on HFD: After high fat diet (up to day 49), SBP increased in all groups; while after drug treatments (from day 49 to day 94) it decreased in all groups except in Group 1 (**Fig. 3a**). At final evaluation (i.e., at day 94), as compared to Group 1; SBP got maximum reduction in Group 5 (39.54%), followed by Group 4 (31.01%), Group 3 (28.91%), Group 2 (25.54%) and Group 6 (20.68%). ANOVA revealed significantly different SBPs among (F=6.77, p<0.001) and within (among days) the groups (F=67.39, p<0.0001) {**Table 3a**}.

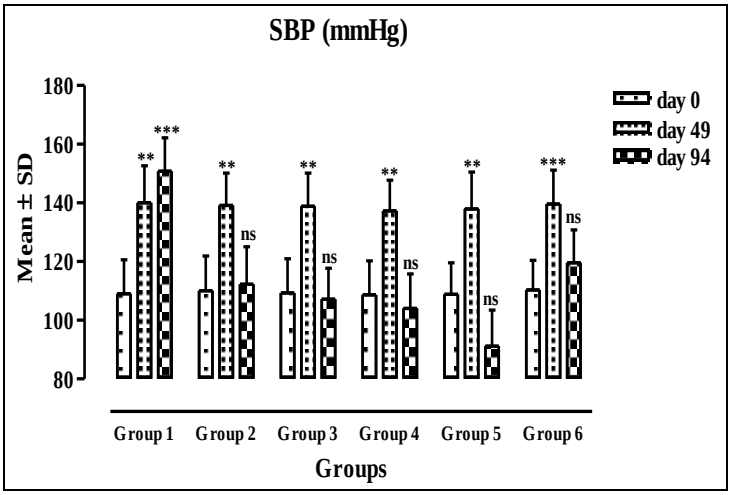

FIG. 3a: PRE AND POST TREATMENT MEAN (± SD) SBPs OF SIX GROUPS

ns p>0.05 or ** p<0.01 or *** p<0.001- as compared to day 0

SBPs in all groups increased significantly (p<0.01 or p<0.001) at day 49 as compared to day 0 (**Table 3a and Fig. 3a**). Mean SBP in Group 1 increased significantly (p<0.001) at day 94 also, as compared to that at day 49; while in other groups it lowered significantly (p<0.05 or p<0.01 or p<0.001) (Table 3a). Among the groups, SBPs in all groups were found similar (p>0.05) at day 0 and day 49. At day 94, mean SBPs differed significantly (p<0.05 or p<0.01 or p<0.001) in all groups except between Group 2 and Group 3, 2 and 4, 2 and 6, 3 and 4, 3 and 6, 4 and 6 (Table 3b).

TABLE 3a: PRE AND POST TREATMENT SBPs (Mean ± SD, n=6) OF SIX GROUPS

Drug groups	At day 0	At day 49	At day 94	% change w.r.t. Group 1	F value (among days) (2, 90 DF)	F value (among groups) (5, 90 DF)
Group 1	109.00 ± 11.64	140.07 ± 12.56[a]	150.75 ± 11.45[a]	-	67.39***	6.77***
Group 2	110.00 ± 11.88	139.16 ± 10.94[a]	112.25 ± 12.84[b]	25.54%		
Group 3	109.33 ± 11.62	138.85 ± 11.31[a]	107.17 ± 10.59[b]	28.91%		
Group 4	108.67 ± 11.58	137.23 ± 10.47[a]	104.00 ± 11.76[b]	31.01%		
Group 5	108.83 ± 10.78	137.93 ± 12.59[a]	91.14 ± 12.34[b]	39.54%		
Group 6	110.33 ± 10.13	139.54 ± 11.64[a]	119.57 ± 11.26[b]	20.68%		

***- p<0.001; w.r.t.- with respect to; [a]p<0.01 or [a]p<0.001- as compared to day 0; [b]p<0.05 or [b]p<0.01 or [b]p<0.001- as compared to day 49

Further, at final evaluation (day 94), mean SBPs in Group 1, 2, 3, 4 and 6 remained significantly ($p<0.05$ or $p<0.01$ or $p<0.001$) higher (respectively 39.54%, 18.80%, 14.95%, 12.36%, 23.78% higher) as compared to that of Group 5 (**Table 3b and Fig. 3b**).

TABLE 3b: COMPARISON (p VALUE) OF MEAN SBPs AMONG SIX GROUPS (CURATIVE PHASE) AT DAY 94 BY *TUKEY TEST*

Comparisons	Mean difference (MD)	Tukey test (q value)	p value
Group 1 vs. Group 2	38.50	8.04	p<0.001
,, vs. Group 3	43.58	9.10	p<0.001
,, vs. Group 4	46.75	9.76	p<0.001
,, vs. Group 5	59.61	12.45	p<0.001
,, vs. Group 6	31.18	6.51	p<0.001
Group 2 vs. Group 3	5.08	1.06	p>0.05
,, vs. Group 4	8.25	1.72	p>0.05
,, vs. Group 5	21.11	4.41	p<0.05
,, vs. Group 6	7.32	1.53	p>0.05
Group 3 vs. Group 4	3.17	0.66	p>0.05
,, vs. Group 5	16.03	3.35	p<0.05
,, vs. Group 6	12.40	2.59	p>0.05
Group 4 vs. Group 5	12.86	2.69	p<0.05
,, vs. Group 6	15.57	3.25	p>0.05
Group 5 vs. Group 6	28.43	5.94	p<0.01

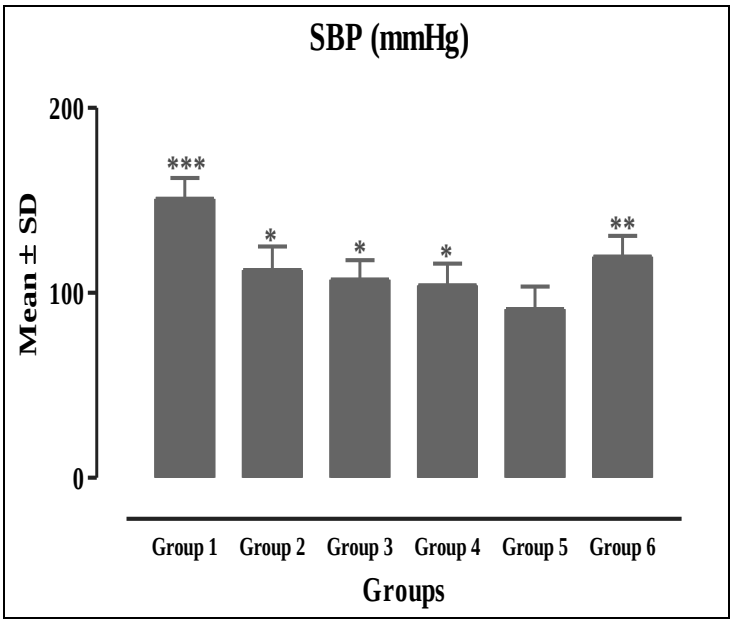

FIG. 3b: COMPARATIVE SBPs OF SIX GROUPS (CURATIVE PHASE) AT FINAL EVALUATION (DAY 94)

*p<0.05 or **p<0.01 or ***p<0.001- as compared to Group 5

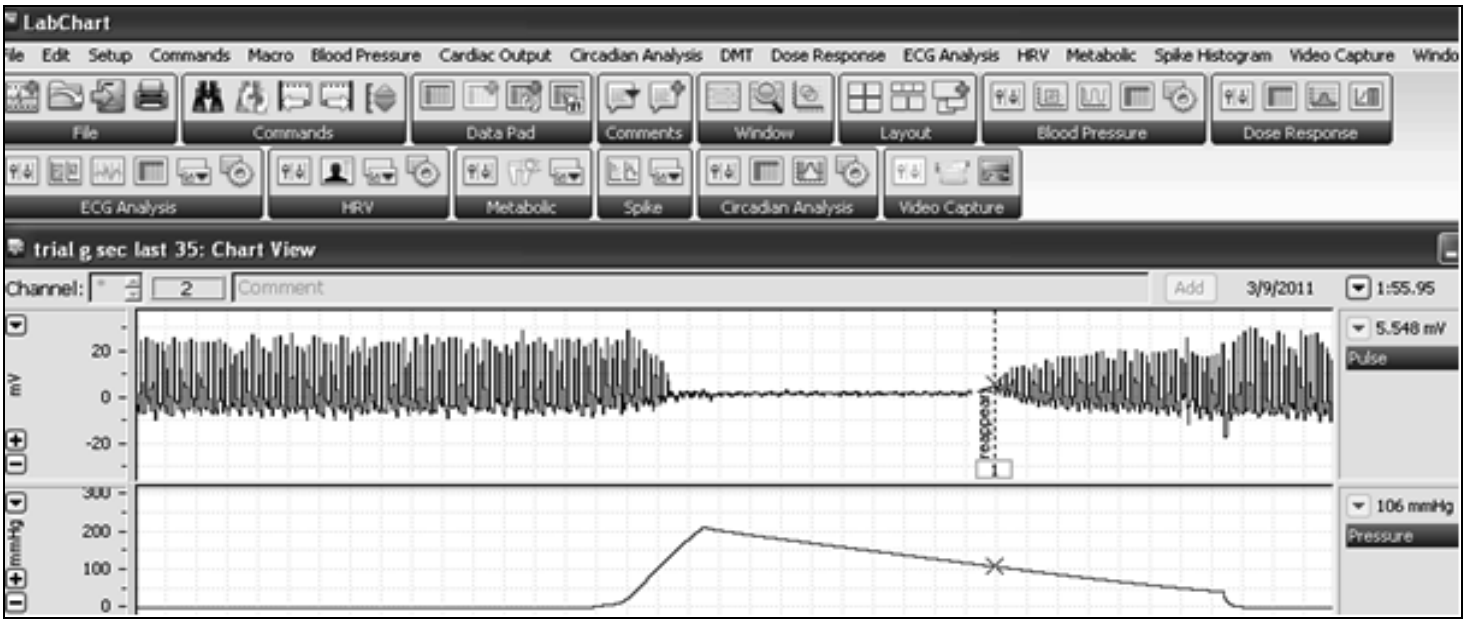

FIG. 4: GRAPHICAL RECORDING OF SBP OF A RAT AT DAY 0

Thus, Amlo was found to be most effective; followed by EA+BD, BD, EA and BD ½ dose; however, all drug-options produced significant improvement. EA and BD were found to be safe in a state of having normal BP. We chose two types of dose for BD because it seldom has previous studies on this topic. Amlo provides a therapeutic BP-steady state after a treatment for 6 weeks i.e., 42 days [23]. *Boerhaavia* provides its appreciable effect in 30 days or so [18, 24]. But, *Eclipta alba* has been found to establish its appropriate effect in 45-60 days [14, 17]. Hence, we chose a period of 45 days for curative phase in our study. EA and its active constituent- culumbin, exhibit remarkable anti-hypertensive activity on anesthetized rats [25]. EA is known to increase endothelium-dependent vaso-dilation in hypertensives [26]. Diuretic effect, which contributes to its anti-hypertensive action, can be due to the potassium content of the leaves, as potassium is known to reduce BP through diuresis or increased sodium excretion [27].

In BD, Liridodendrin & Hypoxanthine are active antihypertensive agents and the former is Ca^{+2} channel antagonist; BD is diuretic by increasing renal blood flow, which contributes to its antihypertensive actions [15]. Both the herbs are antihyperlipidaemic also, i.e., a property which helps in improvement of hypertension. Many of their other components may also play role separately or in combination against hypertension. Future works are undoubtedly needed to elucidate the concerned mechanisms.

Preclinical safety profile of EA and BD: In an acute toxicity study; it was found that alcoholic extract of EA was safe up to 5000 mg/kg *p.o. (per oral)* given to mice and no signs for behavioral as well as physical changes were found [28]. On the other hand, decoction (which has activities almost equivalent to those of extract) of BD-leaves caused no signs of toxicity up to a dose of 5000 mg/kg (p.o.) in mice in another study [29].

In the present study, Amlo was found to be more efficacious than herbal drugs. This difference could be due to use of less-potent doses of EA and BD (i.e. 200 mg/kg of ethanolic extract), secondly due to short duration of treatment. Though, we started our test drugs with lower doses, but it is not so difficult to take these herbs in some greater amount, because these drugs are quite free from adverse or toxic effects even at much higher doses. Longer duration is also not a major problem, because if the patient wants to compare, allopathic drugs are also taken for long period, sometimes lifelong; i.e. more studies with higher doses & longer durations are needed to be performed to evaluate our conclusions.

CONCLUSION: Study reveals that *E. alba* and *B. diffusa* are significantly effective remedies against hypertension. Also, if these herbs are taken together, greater benefit than any of the individual herbs was obtained in the study. These herbal drugs didn't express any detrimental effects on experimental animals. Therefore, *Eclipta alba* and *Boerhaavia diffusa*, as a low-cost herbal therapy, offer an inexpensive, safe & effective measure to combat a major public health problem of hypertension.

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