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EVALUATION OF ANTIHYPERTENSIVE ACTIVITY OF NATURAL MIXTURE AND INVESTIGATION OF HERB-HERB INTERACTION

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ABSTRACT: Hypertension is a common clinical problem encountered in day-to-day practice. Although several synthetic pharmaceutical drugs already exist for the treatment of hypertension, these drugs can develop many complicated side effects. On the other hand in our Arabian countries, patient compliance forces the population to frequently recourse to traditional medicine which mainly uses plants. Because of most of the medicinal agents including herbs were reported to have potentially unexpected effects including toxicity and interactions, this study focused on the investigation of antihypertensive activity of four plants mixture along with studying their interaction. In this study ethanolic extract of a mixture composed of four plants *Hyphaene thebaica*, *Olea europaea*, *Origanum majorana*, and *Hibiscus sabdariffa* was investigated for its antihypertensive activity and interaction between it's including herbs. 50 g of each plant powder was mixed homogeneously and macerated in 70% ethanol for about 7 days. The effect of this extract was investigated at a dose level of 25 mg/kg. Blood pressure was measured by a non-invasive blood pressure recorder apparatus before and after treatment. The results of this comprehensive study revealed that aqueous extract of this natural mixture showed a significant decrease in SBP as compared to fludrocortisone-induced hypertensive control mice. It was revealed that aqueous extract showed a hypotensive effect nearly equal to slandered control (captopril). According to the histopathology study, aq. The extract showed a protective effect against alterations and pathological changes that were induced by hypertension in the kidney.

INTRODUCTION: Hypertension (HTN) is one of the most prevalent non-communicable disorders, affecting several millions of people Worldwide. Despite the availability of effective drug treatment and lifestyle adjustment programs, the frequency of HTN is increasing uncontrollably. Also, it is related with a rise in peripheral vascular resistance that can, in turn, lead to dangerous secondary health complications ranging from myocardial infarction, renal failure, strokes, and death, if not detected early in life and treated correctly [1]. A decrease in blood pressure (BP) is considered to be the primary determinant of a decrease in cardiovascular risk.

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Factors found to be linked with high BP are the result of a complex relationship between genetic and environmental elements, which can lead to activation or inhibition of one or more of the processes involved in the normal control of BP. Dietary factors and physical inactivity contribute to the genetic predisposition, while environmental factors like smoking, drinking, obesity and alcohol, thus making hypertension a preventable cause of morbidity and mortality [2].

The advantages of populations with hypertension leading a healthy lifestyle cannot be stressed enough, and this includes a controlled diet and regular exercise. The prime goal of treatment is to eliminate the risks factors associated with hypertension, without lessening the patient's quality of life [1, 3, 4, 5, 6, 7, 8, 9].

The prevalence of HTN in the Kingdom of Saudi Arabia was 26.1%. For males, it was identified to be 28.6%, while for females; it was at 23.9%. The urban population showed; the significantly higher prevalence of hypertension of 27.9% was noticed at urban settings. The predominance of chronic artery disease among hypertensive subjects was 8.2%, and 4.5% among normotensive subjects. Increasing weight revealed significant increment in the prevalence of HTN [10].

Medicinal plants have been used throughout human history for the treatment of various diseases. Kingdom of Saudi Arabia posses a variety of therapeutically active plants species. However, these plants are used by the public as an alternative to conventional medications. Medicinal plants, in particular, are widely used in Traditional Arabic Herbal Medicine for health maintenance and to treat various illnesses including chronic diseases [11].

The research studies have presented credible evidence that antihypertensive drug treatment is capable of preventing serious complications of hypertension in individuals with moderate and severe hypertension [12]. Hence in this regard, the current research has been designed to evaluate the potency of dried powder mixtures of four native species of Saudi Arabia namely *Hyphaene thebaica*, *Olea europaea*, *Origanum majorana*, and *Hibiscus sabdariffa* for their antihypertensive potency with the below-given goals;

- To discover a safe, natural pharmaceutical mixture with high efficacy in controlling hypertension.
- To design a pharmaceutical drug that depends on herbal medicine with limited side effects and follows patient compliance.
- The study seeks to develop information recommending the use of a particular herbal mixture and the underlying reasons for using herbal therapy.
- Pre-clinical study of herb-herb interaction (Synergistic or inhibitory interactions) and safety of herbal mixture.

MATERIALS AND METHODS:

Preparation of Ethanolic Extract of Plant Mixture: For the mixture, four medicinal plants named *Hyphaene thebaica*, *Olea europaea*, *Origanum majorana*, and *Hibiscus sabdariffa* were used. The plants were purchased from the local market in Obhur, Jeddah, KSA and authenticated by Dr. Samah Shabana, Pharmacognosy Department, Faculty of pharmacy (MUST). The plants were cleaned, shadow dried, and ground to powder. 50 g of each powder was weighed, and the powders were mixed homogeneously in equal ratio and macerated in 2 liters of 70% ethanol for about seven days. The macerated mixture was filtered, and then 50 ml of filtrate concentrated to dryness in a rotary evaporator under reduced pressure. After evaporation in a vacuum, the vacuum-dried ethanolic extract of the mixture was weighed and dissolved in a definite amount of ethanol in water to make a final concentration of 1mg/ml [13, 14, 15].

Fractionation for Preparing Aqueous and Organic Extracts: Vacuum-dried ethanolic extract of the mixture (80 g) was macerated in Hexane with the solvent: the solute ratio of 3: 1 for 24 h with frequent shaking using separating funnel to separate the aqueous extract from organic one. The organic fraction was dried under vacuum using rotary evaporator IKA- RV10, USA. The dried extract was weighed and dissolved in a definite amount of liquid paraffin (safer for mice), to make concentrations of 1 mg/ml. Also, the aqueous fraction was dried under vacuum and weighted, dissolved in a definite amount of distilled water to make the concentration of 1 mg/ml [13, 14, 15].

These two extracts were subjected to the investigation of their antihypertensive activity, their interaction and effect on different organs.

Animals, Drugs, and Chemicals: Male albino Swiss mice 15 - 30 gm were used. The study was conducted according to the National Institutes of Health guidelines for the care and use of laboratory animals. All animal care and experimental procedures were carried out with the ethics approval of the local regulatory authority. The animals were housed in standard cages at room ambient temperature (22° - 25 °C) and humidity (45 - 50%) along with 12/12 h light/dark cycle during experiment course. They were fed standard rat chow with water freely accessible. Captopril and fludrocortisone acetate are purchased from Sigma-Aldrich, USA. AST, Albumin kits, Creatinine kits, and urea kits were purchased from the local market. All other chemicals employed in the current study were of the analytical standard.

Mineralocorticoids Induced Hypertension: The hypertensive control group was kept on a diet high in sodium chloride, and drinking water was replaced by 2% sodium chloride solution. After they attain a weight of about 25 gm, they have been given fludrocortisone dissolved in sesame oil at a dose of 10 mg/kg was administered once daily for three weeks. The mice of systolic blood pressure of more than 170 mmHg were chosen to be hypertensive mice in the current study.

Blood Pressure Recording: Basal blood pressure and heart rate were measured using a non-invasive blood pressure recorder device (Ugo Basile instruments, Varese, Italy). Each rat was placed separately in a restrainer and appropriate cuff with the sensor around the tail and warmed to approximately 33 - 35 °C. The tail cuff was raised to a pressure above 200 mmHg. The systolic blood pressure and the diastolic blood pressure and the heart rate were measured immediately by the tail-cuff and pulse sensor [12, 13, 14].

Experimental Design: The mice were divided into 5 groups, each of 5 mice.

Assessment of Renal and Liver Functions in Mice: The blood sample was taken from each mouse from retro-orbital venous plexus. The serum was separated by centrifugation at 5000 rpm for 10 min. Kidney function was evaluated by estimation of serum creatinine, albumin, and urea by modified urease- Berthgot method. Serum samples were analyzed for total protein by the Biuret method, for creatinine (CRT) according to the Jeffe method, for blood urea nitrogen (BUN) by the modified urease-Berthelot method, and alanine transferase (ALT) activity by the enzymatic method, using a spectrophotometer and commercial colorimetric kits.

TABLE 1: EXPERIMENTAL GROUPS

Groups	Treatment
Normal Group	Administered once daily with normal saline 10 ml/kg body weight, IP for four weeks
Hypertensive group	Administered once daily with fludrocortisone dissolved in sesame oil at a dose of 10 mg/kg body weight for four weeks
Test group – I	Administered once daily with aqueous extract at a dose of 25 mg/kg body weight, IP for four weeks
Test Group – II	Administered once daily with organic extract at a dose of 3 mg/kg body weight, IP for four weeks
Standard Group	Administered once daily with captopril at a dose of 2 mg/kg body weight, IP for four weeks

Histopathological Assessment for Renal Injury: For optical microscopy, kidneys were quickly removed, fixed in 40% formalin for 48 h for fixation. The fixed organs were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax, then 4 - 5 µm thick sections were obtained by rotary microtome and stained with hematoxylin and eosin [12, 13, 14].

RESULTS: Results are expressed as Mean values. Statistical analysis is obtained by processing the results through SPSS software. All data were analyzed by one-way analysis of variance (ANOVA), and Students-test was used to determine the source of a significant effect. $P<0.05$ is taken as an acceptable level of significant difference from control.

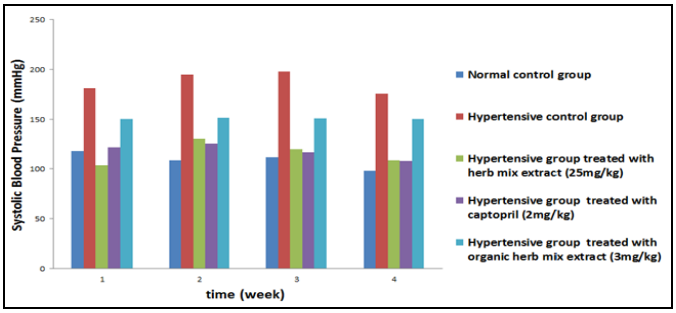

FIG. 1: EFFECT OF AQUEOUS (POLAR) EXTRACT, ORGANIC (NON-POLAR) EXTRACT, AND CAPTOPRIL ON BLOOD PRESSURE

Anti-Hypertensive Activity:

- Both aqueous and organic extracts of the herbal mixture showed a significant decrease in the mean blood pressure in hypertensive treated groups compared to the hypertensive control group.
- Polar (ethanolic and aqueous) extract of the herbal mixture showed a remarkable decrease in the mean blood pressure as the same as could happen by captopril.

TABLE 2: EFFECT OF HERBAL MIX POLAR EXTRACT, ORGANIC EXTRACT ON KIDNEY AND LIVER FUNCTIONS TESTS

GPs	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/l)	ALT (IU/ml)
Normal control	12.20	1.34	50.28	37.32
Hypertensive control	60.65	2.74	45.12	46.13
Hypertensive treated with aqueous herbal mix extract	47.34	2.13	53.13	41.43
Hypertensive treated with organic herbal mix extract	43.81	2.35	54.07	39.84
Hypertensive treated with captopril	46.64	1.95	63.10	49.51

Supportive Results:

a) Biochemical Study:

- The hypertensive control group showed a significant increase in serum level of urea, creatinine as compared to the normal control group.
- Hypertensive groups those were treated with both herbal mix polar, and organic extracts showed a significant decrease in serum level of urea, creatinine as compared to the hypertensive control group.
- Hypertensive groups, those were treated with both polar and organic extracts showed no change in serum level of Albumin and ALT.

b) Histopathology Study to Measure the Effect of Hypertension and Treatment with the Herbal Mixture on Kidney Tissues and Renal Tubules:

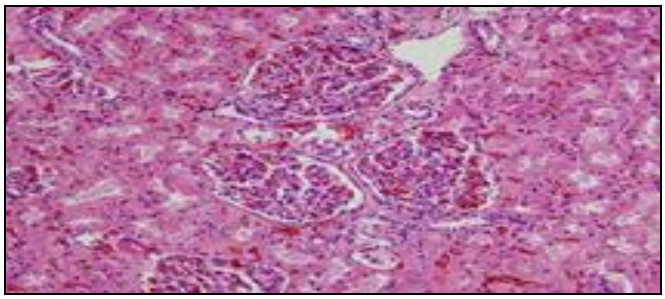

FIG. 2: NORMAL KIDNEY SECTION SHOWED THE VESSELS WITH NO NARROWING OR WALL THICKENING. THE BASEMENT MEMBRANE IS THIN AND WITHOUT INFLAMMATION OR THICKENING. BOWMAN'S CAPSULE THAT SURROUNDS THE GLOMERULAR IS THIN

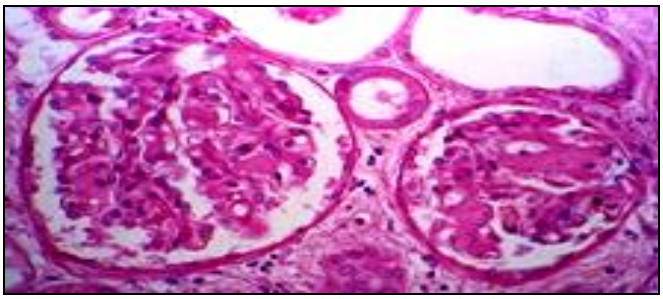

FIG. 3: HYPERTENSIVE CONTROL GROUP SHOWED CLOUDY SWELLING; SMALL BLOOD VESSELS SHOWED THICKENING OF THE WALL, PAREN-CHYMAL HEMORRHAGE AND INFLAMMATORY CELL INFILTRATE IN THE PARENCHYMA

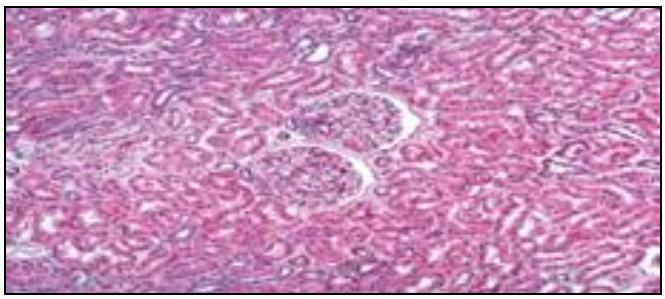

FIG. 4: THE HYPERTENSIVE GROUP TREATED WITH THE ORGANIC EXTRACT OF THE HERBAL MIXTURE SHOWED A MILD PROTECTIVE EFFECT ON RENAL TUBULES IN RENAL CORTEX

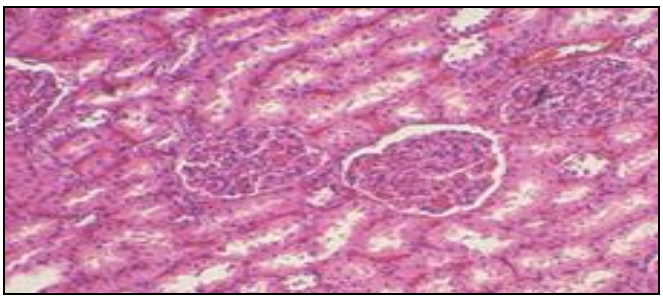

FIG. 5: THE HYPERTENSIVE GROUP TREATED WITH THE AQUEOUS EXTRACT OF THE HERBAL MIXTURE SHOWED A PROTECTIVE EFFECT ON RENAL TUBULES IN THE CORTEX REGION OF KIDNEY

DISCUSSION: *Hyphaene thebaica, Olea europaea, Origanum majorana,* and *Hibiscus sabdariffa* are rich in phytochemicals, the effects of which have been widely investigated. They have been used in the human diet as an extract, herbal tea, and a powder. Their bioactive compounds have been demonstrated to have antioxidant, anti-hypertensive and hypocholesterolemic properties. In the present study, we have focused the attention on the potential application of the herbal mix of *Hyphaene thebaica*, *Olea europaea*, *Origanum majorana*, and *Hibiscus sabdariffa* in the prevention or counteraction of hypertension.

The polar and non-polar extracts of herbal mixture induced a potent anti-hypertensive activity. However, the polar (aqueous) extract showed a protective effect on kidney functions by decreasing the serum level of urea & creatinine, and these results were supported by the histopathological study.

The current herbal mix revealed cytoprotective and antioxidant properties. In this study, it has been demonstrated that the mixture of the extracts exerts higher cytoprotective and antioxidant activities than every single extract separately. Both of these functions are critically involved in cardiovascular protection and hypertension control as reported in the literature. It is a well-proven fact that the oxidative stress is indeed a cause of hypertension, and then antioxidants should have beneficial effects on hypertension control with reduced oxidative damage to the tissues.

Nahida T, in one of their review article has given the note of about 49 natural drugs which bears the antihypertensive potency not only that these drugs are still being used in the management of hypertension as the conventional medications have shown to be having much accountable adverse drug reactions [16]. It does not mean that the natural substances are exempted from having adverse drug reactions but are managed with ease without altering either the quality of life or the treatment cost [17].

If antihypertensive medication is to give it must be given on an individual basis. Whereas, every individual would have consumed one or the other kind of the natural antihypertensive product in their lifetime, which further justifies the usage of natural products application in the management of hypertension in a better way than the conventional medications.

In the current research, the herbal mix showed no toxic effect on liver and kidney functions according to biochemical results that support our suggestion **Table 1.** These four herbs synergized each other to get a strong antihypertensive action. Which also proves that the combination of the herbs not only synergistically helpful in the management of hypertension also shows better in the protection of vital organs supported by the histopathological data, which are essential for the maintaining of good quality of life in every individual.

Further concrete evidence of the usage of the four plants as a crude drug in the management of hypertension shall be done by evaluating each plant separately for its efficacy against hypertension and comparing the results for the better outcome. Or on the other hand, one can try for separating the individual active constituent responsible for antihypertensive action. By till then, the usage of the mixture of the four drugs can be expected to be beneficial in the management of hypertension as it is cost effective and devoid of any untoward adverse drug reactions and proves beneficial for the usage in humans for the management of hypertension.

CONCLUSION: Our results revealed that polar and non-polar extracts of the natural mixture that was used in this study showed significant antihypertensive effects that indicate the synergistic action of its herbal contents. It was revealed that aqueous extract showed an anti-hypertensive effect nearly equal to slandered control (captopril).

According to the histopathology study, the aqueous extract showed a protective effect against alterations and pathological changes that were induced by hypertension in the kidney that indicated there was no hazardous herb-herb interaction.

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CONFLICT OF INTEREST: Nil

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