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Study on vascular mechanisms underlying the hypotensive effect of *Sorghum halepense* (L.) Pers.

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Abstract: *Sorghum halepense* L (Poaceae), ordinarily it is known as Johnson grass and locally as baru. This study was designed to find the vascular mechanisms underlying the hypotensive activity of *S. halepense*. In this study, effect of *S. halepense* seed extract/fractions on various blood pressure parameters were evaluated in normal and fructose induced hypertensive rats by invasive technique. Possible underlying hypotensive mechanism of active fraction was determined by using various pharmacological inhibitors. *S. halepense* extract/fractions vasorelaxant effect were also evaluated on rat aorta rings in organ bath and various intracellular signaling pathway inhibitors were used for determination of underlying mechanisms. *S. halepense* extract/fractions produced blood pressure lowering effect with most significant effect by its aqueous soluble fraction at dose of 10mg/kg. This effect was attenuated by pretreatment of atropine. Aqueous soluble fraction produced endothelium dependent vasorelaxation in rat aortic rings that was inhibited by pretreatment of atropine after phenylephrine induced contraction. The vasorelaxant effect of aqueous soluble fraction was attenuated by potassium channel blockers and also produced inhibitory effect on calcium entry through calcium channels. It also suppressed phenylephrine induced contraction like verapamil. By HPLC analysis found vanillic acid and naringinin in it. In conclusion, aqueous soluble fraction of *S.halepense* possess phytoconstituents which may be responsible for hypotensive and vasorelaxant effect of *Sorghum halepense.*

Keywords: *Sorghum halepense* (L.) Pers. hypotension, vasorelaxation, atropine, calcium channels, potassium channels, vanillic acid.

INTRODUCTION

Hypertension is a crucial threat for cardiovascular diseases with deaths of maximum nine million people worldwide each year (Kitt *et al.,* 2019). The existence of vascular damage is a common and an established medical cause of hypertension. Therefore, treatment of vascular diseases has become the most important intervention in the management of all forms of hypertension (Recognize, 2009). Presently, beta blockers, calcium-channel blockers, potassium channel openers and vasodilators are medicinal approaches used in reducing blood pressure (Musini *et al*., 2017). Current antihypertensive and vasodilators have high efficacy but produced non-compliance and side effects in patients (Siddique *et al.*, 2012). Therefore, there is a need to explore the novel or advanced antihypertensive and vasodilators which could raise the competency and decline the frequency of undesired effects of drugs (Jarari *et al.*, 2015). Medicinal plants are strong basis of medicine and possess therapeutics prospective or a source of curative supports (Oladeji, 2016). This is because of their enhanced traditional suitability and improved compliance with the human body and reduced side effects (Chanda, 2014). Pakistan has unique biodiversity and climate, containing pharmacologically effective medicinal plants with safety profile (Shinwari, 2010). Among the flora of Pakistan, *Sorghum halepense* L. is an imperious medicinal plant of family Poaceae. It is ordinarily known as Johnson grass, while in local language it is recognized as dadam and baru (Rambabu *et al*., 2016). It keeps incredible medicinal value comprising antihypertensive activity (Hussain *et al.*, 2008). It has also been traditionally used as depurative, demulcent, poison, diuretic and as a tonic. It is also used for ailments of urinary tract and blood. The *Sorghum halepense* has anti-inflammatory, catarrh, febrifuge, relieving headache, properties (Rambabu *et al*., 2016). Earlier studies has described its pharmacological effects against fungi as *Macrophomina phaseolina* (Javed *et al*., 2012) and central activity (Rambabu *et al.,* 2016). It also has been reported as cytotoxic, antioxidant and antidiabatic (Khan *et al.,* 2019). Similarly, its phytochemical analysis showed many biologically active

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compounds including aliphatic acids, polyphenols, flavonoids, flavones, sorgolenone, quinins and p-coumaric acid (Shah *et al*., 2019). Keeping in view, plant diverse phytochemical profile and expected pharmacological uses, the recent study was conducted to recognize the vascular mechanism underlying the hypotensive effect of *Sorghum halepense* as previously no study on this effect has been conducted.

MATERIALS AND METHODS

Chemicals and drugs

Methanol, hexane, dichloromethane, ethyl acetate and butanol were used for extraction and fraction of plants and fructose, L-NAME, indomethacin, atropine, hexamethonium bromide, captopril, propranolol, sodium nitroprusside were used for determination of underlying hypotensive mechanism. Phenylephrine, potassium chloride, TEA, 4-AP, barium chloride and glibenclamide were used for vasorelxation study protocol. All above mentioned chemicals and drugs were of standard analytical grade and purchased from Sigma Aldrich Pakistan. Sodium thiopental purchased from Abbott laboratories Ltd Karachi Pakistan for anesthetization of rats.

Animals

Male Sprague-Dawley rats (250-300g and 3-4 months) were taken from animal house of Faculty of Pharmacy, University of Sargodha-Sargodha, Pakistan. All animals were retained in familiarized conditions, for example persistent 12 h light/dark cycle and temperature of (22±2 ◦C). Standard food of pellets size and water ad libitum was delivered in accordance with NIH, Health Research Extension Act of 1985, 85-23 for housing and care of research animals. Written authorization for this study was taken from the local Institutional Ethics Committee of GCUF (No. IAEC/GCUF/2017/65).

Plants material collection, extraction and fractionation

S. halepense seeds (8kg) were collected in September - 2016 from district of Sargodha-Pakistan which was identified and authenticated by plant taxonomist of Department of Botany Govt. College University Faisalabad (GCUF) Faisalabad-Pakistan. A voucher specimen (W-1065) of this specie was placed in the herbarium of Department of Pharmacy, Faculty of Pharmaceutical sciences, GCUF for further reference. *S. halepense* seeds (8kg) were shade dried and pulverized to a coarse constituency for extraction process. Concisely, the coarse seeds were soaked in 70% aqueous methanol solution and retained in solution for a total of 3 days for three times. After every 3 days, it was filtered above a porous cloth and at end of 9^{th} day, all the filtrate was combined, filtered through Whatman qualitative Grade 1 filter paper and then concentrated in a rotary evaporator at 40^{o}C to get a thick crude extract (Alamgeer *et al*, 2013).The % yield of crude extract of *S. halepense* was 31.25%. For preparation of fractions, 250g of crude extract was mixed in 250ml of distilled water and formerly solvent – Solvent extraction was done by using different solvents. Latterly, fractions were prepared concentrated by rotary evaporator (40^{o}C), that resulted into ethyl acetate soluble fraction (13.01g), butanol soluble fraction (24.21g), aqueous soluble fraction (61.50g). Very less quantity of hexane and dichloromethane soluble fractions were obtained and not used for investigation. Aqueous methanolic extract and fractions were stored in a refrigerator at -4°C, and used for further pharmacological analysis (Shih *et al*., 2008).

Phytochemical analysis

Total phenolic contents (TPC)

Total phenolic contents were determined by some modification of Folin–Ciocalteu reagent method. Sample of 1ml of each extract (in methanol) and gallic acid (in methanol) were mixed with 5ml of Folin–Ciocalteu reagent (dilute ten folds) and 4ml of sodium carbonate (20% w/v). Then after1 hr absorbance values were dignified at 765 nm. The calibration curve was designed via captivating absorbance by meaning of concentration. Total content of phenolic compounds in plant extracts were stated as mg gallic acid equivalent per gram (mg GAE/g) (Saleem *et al*., 2019).

Total flavonoid contents (TFC)

The total flavonoid contents of plant extracts were determined by aluminum chloride calorimetric technique with some modification. The diluted plant extract/catechin in a quantity of 0.5ml was mixed with 2ml of distilled water, 0.15ml of five percent $NaNo_2$ solution, 0.15ml of ten percent $AlCl_3$, four percent of NaOH solution and methanol to make volume 5 ml and mix properly. Absorbance of the reaction mixture was captured at 510nm after incubation of 15minutes. After that take catechin linear regression curve which is equivalent of the total flavonoid contents (TFC) of the extracts (Saleem *et al.,* 2019).

HPLC analysis of S. halepense

HPLC analysis of aqueous methanolic extract of *S.halepense* (AMSH) and aqueous soluble fraction of *S. halepense* (ASH) were done on a liquid chromatograph, prepared with a solvent pump (model 600), a 2996 photodiode array detector and attached with Empower v.2 Software (Waters Spa, Milford, MA, USA). A C18 reversed-phase packing column (Prodigy ODS (3), 4.6 × 150 mm, 5 μm; Phenomenex, Torrance, CA, USA) was used for parting. The UV/Vis acquisition wavelength was set in the range of 200–500 nm. The mobile phase was directly *on-line* degassed by using Biotech DEGASi, mod. Compact (Italy). Gradient elution was performed using the mobile phase water-acetonitrile (93:7, v/v, 3% acetic acid). All the sample solutions were centrifuged and the supernatant was injected into HPLC.The stock solutions of phenolic were made (1 mg/ml in a final

volume of 10 ml of methanol). Working solutions of mixed standards at the different concentrations (µg/ml) were made by dilution of stock solution with the mobile phase. Then the standards were injected into the HPLC-UV/Vis system. Each solid sample was weighted and solubilized in mobile phase in 1:1 (*w:v*) ratio. In this case, the obtained concentrations (µg/ml) correspond to the total amount (µg/mg). After solubilization, the sample was centrifuged at 12000 x *g*. All chromatograms obtained by each sample were recorded. The reported values were mean ± standard deviation of three independent measures (Zengin *et al.*, 2018).

Experimental protocols

Study of acute effect of S. halepense extract/fractions on blood pressure in normotensive and hypertensive rats

For this study, Male Sprague-Dawley rats (250-300g) were used and allocated into two major groups, Group I, in which rats receiving only vehicle (DW) and designated as normotensive rats (NTR) group with four subgroups (n=6) for evaluation of crude extract and each fractions respectively. Group II, designated as hypertensive rats (FHR) group, in which hypertension was induced with 6 weeks continuous feeding of 10% fructose with four subgroups (n=6) for evaluation of crude extract and each fractions individually. Briefly, rats of both groups were anesthetized with intra-peritoneal injection of sodium thiopental (70-90 mg/kg BW) for determination of effects of *S. halepense* extract and three fractions. Tracheostomy was performed to maintain respiration. For drug delivery, polyethylene catheter was inserted into juggler vein, whereas to determine arterial BP, cannula filled with heparinizes saline (100IU/ml) and connected to pressure transducer was inserted into carotid artery. After 30 minutes of stabilization, rats were administered with 0.9% sodium chloride (NaCl=vehicle) through juggler vein to ensure that the perceived effects were not due to the vehicle act (Siddiqi *et al.*, 2012). Then dose–response relationship of aqueous methanolic extract of *S. halepense* /fractions were measured on normotensive rats (NTR) at doses of 0.1, 0.5, 1, 5, 10 and 20 mg/kg and on fructose-induced hypertensive rats (FHR) at doses of 0.1, 0.5, 1, 5, 10 and 20 mg/kg. Blood pressure parameters (SBP, DBP and MAP) were recorded after administration of aqueous methanolic extract of *S. halepense* and fractions for 45 minutes from carotid artery by pressure transducer attached to Power Lab data acquisition system that connected to a computer equipped with Lab Chart 7 Software (Tom *et al*., 2011).

Determination of mechanism underlying the hypotensive effect of active fraction of S. halepense

For this purpose, active fraction at dose of (10mg/kg) was used. Briefly, male Sprague Dawely rats (n=3 for every drug) were pretreated with L-NAME (20 mg/kg), hexamethonium (30 mg/kg), sodium nitroprusside (SNP, 2.5mg/kg), methylatropine (1 mg/kg), propranolol (100 ug/kg) and captopril (2.5mg/kg), 10 min earlier to administration of single dose of aqueous fraction of *S. halepense* (ASH=10mg/kg) and after that changes in mean arterial blood pressure (MAP) was measured for 45 minutes from carotid artery by pressure transducer attached to Power Lab data acquisition system that than connected to a computer equipped with Lab Chart 7 Software (Shih *et al.*, 2008: Tom *et al.*, 2011).

Study of vasorelaxant activity of aqueous methanolic extract of S. halepense and fractions on isolated rat aorta

For this study, rats were anesthetized by use of sodium thiopental (70-90mg/kg BW; i.p) to obtained unconsciousness. After that sacrificed rats to cut the thoracic aorta and placed in Kreb Henslet solution. Then immediately removed the adhered connective tissues and fat. The aorta was cut into rings of about 3-4 mm in length. The aortic rings were dipped in a 20ml chamber bath having Krebs solution (composition, mM: NaCl, 122; KCl, 4.9; HEPES, 10; KH2PO4, 0.5; NaH2PO4, 0.5;MgCl2, 1.0; glucose, 11.0; CaCl2, 1.8, pH 7.3), retained at a 37°C, fixed with tungsten wire and constantly bubbled with oxygen. Every ring maintained at resting tension of 1g and allowed to equilibrate for at least 45 mints. Krebs solution was changed every 15 min during this period. For endothelium-denuded rings, the endothelial layer was removed by gently rubbing the internal surface of the vascular lumen with forceps (Pantan *et al.*, 2014).

After the rings were equilibrated, they were pre-contracted with PE (10 µM) until the stability of tension was established, followed by cumulative exposure to aqueous methanolic extract of *S. halepense* /fractions with (0.0001mg–1mg). Changes in tension were detected using isometric force transducers coupled with Power Lab data acquisition system and then connected to a computer equipped with Lab Chart 7 Software program (AD Intruments, Sydney, Australia).

The effect S. halepense extract/ fractions on K^+ (80mM) induced contraction

For this study, steady state contraction was achieved in aortic rings with PE (10 µM) and KCl (80 mM). After that, aqueous methanolic extract of *S. halepense* and fractions (0.0001 mg–1mg) were added cumulatively to obtained dose response relationship. Then the extent of relaxation was expressed as the percentage of relaxation over PE-induced or KCl induced contraction and compared (Pantan *et al.*, 2014). For extract and every fractions separate set of experiment were performed.

Determination of effect of membrane receptors and endothelial mediator's inhibitors on relaxation response of ASH

Endothelium-intact rat aortic rings were pre-incubated with atropine (1uM, a muscarinic receptor antagonist), L-NAME (100uM, a non-selective NO synthase inhibitor), or indomethacin (1uM, a cyclooxygenase inhibitor), to

investigate the role of muscarinic receptor, nitric oxide (NO) and prostacyclin for15 min before pre-contraction with PE (1uM) and left for stabilization. Then response was evaluated through addition of cumulative concentrations of ASH (0.0001 mg–1mg) in absence and presence of above mentioned inhibitors (de AF Da *et al.*, 2012).

Determination of effect of K^+ channels blockers presence on relaxation response of ASH

The contraction was obtained with PE both in presence and absence of potassium channel blockers in separate endothelium-intact aortic rings; specific K^+ channel blockers as nonselective potassium channel blocker TEA (5 mM), voltage operated potassium channel blocker 4-AP (1mM), K_{ATP} blocker glibenclamide (10 μM) and inward rectifying current blocker $BaCl_2$ (1mM) were used 30 min before PE (10 μM) induced contraction. Then, ASH (0.0001mg–1mg) was added cumulatively to obtained concentration response curve (Pantan *et al.*, 2014).

Effect of ASH on extracellular Ca^{2+}/ Ca Channels

To determine the effect of ASH on voltage operated calcium channel (VOCC), the aortic rings were stabilized under the Ca^{2+} free Krebs solution containing EGTA (1 mM) for 20 min, then rings were allowed in Ca^{2+} free solution K^+ rich Kreb solution to open the calcium channels. Then, $CaCl_2$ (0.3mg–5mg) was added cumulatively to construct a maximum response. After that, the rings were washed out with Ca^{2+} free Krebs solution for 20 min. Next, pre-incubated with ASH (0.3mg–5mg) before re-exposure to Ca^{2+} free K^+ Kreb solution. The percentages of contraction were compared, produced by $CaCl_2$ in the absence and presence of ASH. Same experiment performed for verapamil effect for comparison (Pantan *et al.*, 2014).

Table 1: Phytochemical analysis: Total phenolic contents and total flavonoid contents of aqueous methanolic extract of *S. halepense* and fractions

Content	AMSH	ESH	BSH	ASH
TPC (ug GAE/mg DE)	79+3.24	69+1.24	63+3.24	73+3.24
TFC (ug CE/mg DE)	124.34+1.24	114.34+1.24	101.34+1.24	124.34+1.24

Values expressed as mean ± SEM (n=3), TPC: Total phenolic contents; TFC: Total flavonoid contents; GAE: gallic acid equivalent; CE: catechin equivalent; DE: dry extract, AMSH: aqueous methanolic extract of *S. halepense*, ESH: ethyl acetate fraction of S. halepense, BSH: butanol soluble fraction of S. halepense and ASH: aqueous fraction of S. halepense.

Table 2: HPLC analysis of aqueous methanolic extract of *S. halepense* and aqueous fraction

Contents	AMSH	ASH
	Quantity (ug/mg DE)	Quantity (ug/mg DE)
Gallic acid	nd	nd
Catechin	nd	nd
Chlorogenic acid	nd	nd
p-OH benzoic acid	nd	nd
Vanillic acid	0.20±0.02	0.12±0.07
Epicatechin	nd	nd
Syringic acid	nd	nd
3-OH benzoic acid	nd	nd
3-OH-4-MeO benzaldehyde	0.90±0.09	nd
p-coumaric acid	0.26±0.03	nd
Rutin	nd	nd
Sinapinic acid	nd	nd
t-ferulic acid	nd	nd
Naringin	nd	nd
2,3-diMeO benzoic acid	nd	nd
Benzoic acid	nd	nd
Naringenin	nd	2.68±0.27
Carvacrol	nd	nd

Values expressed as mean ± SEM (n=3), DE: dry extract, nd: not detected; AMSH: aqueous methanolic extract of *S. halepense:* ASH: aqueous fraction of *S.halepense*

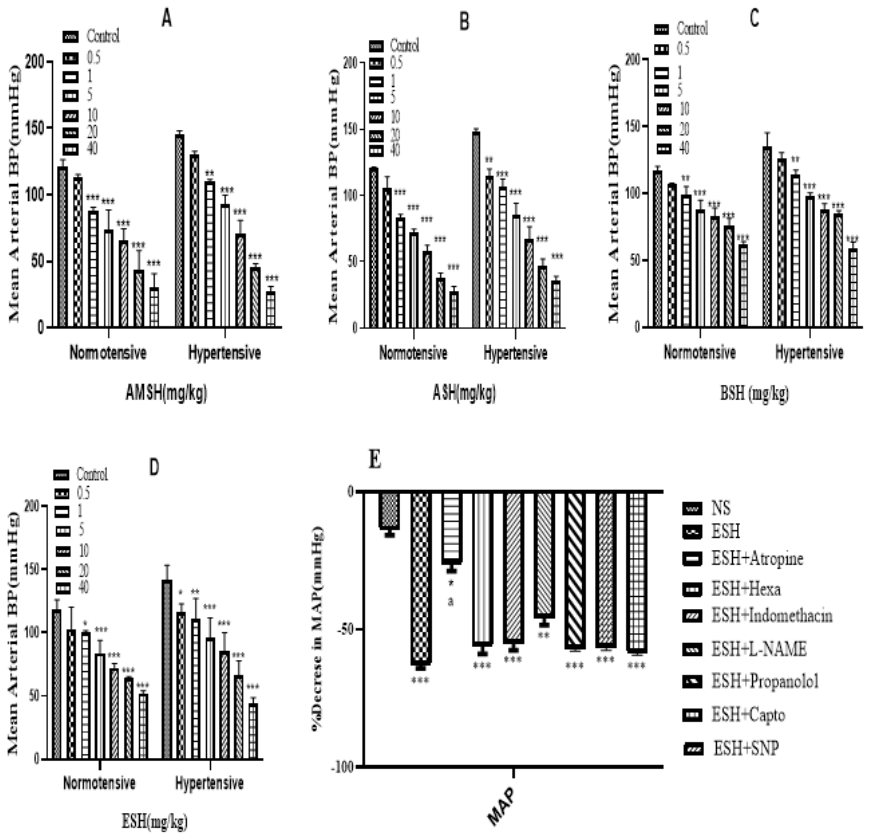

Fig. 1: Effect on mean arterial blood pressure produced by intravenous administration of different doses of (A) aqueous methanolic extract of *Sorghum halepense* (AMSH), (B) aqueous fraction of (ASH), (C) butanol fraction (BSH) and (D) ethyl acetate fraction (ESH), in normotensive and fructose induced hypertensive rats. Results were compared for reduction in MAP between normotensive and hypertensive rats for extract and each fractions different doses. Values expressed as mean ± SEM (n=6), ***p<0.001, **p<0.01, *p<0.05 as compared to control done by Two way ANOVA followed by Bonferroni post hoc. (E) Effect of intravenous administration of aqueous soluble fraction of *Sorghum halepense* (ASH 10mg/kg) on mean arterial blood pressure (MAP) of normotensive rats pre-treated with various antagonists. Values expressed as mean ± SEM (n=3), ***p<0.001, as compared to normal saline (NS) group while a=p<0.001, when compared to aqueous fraction of *S. halepense* (ASH) treated group analyzed by Two way ANOVA followed by Bonferroni post hoc test. Figure present effect on MAP with ASH in anesthesized rats pretreated with normal saline (NS), atropine (2mg/kg), hexamethonium (30mg/kg), indomethacin (5mg/kg), L-NAME (20mg/kg),propranolol (1mg/kg), captopril (2.5mg/kg) and sodium nitroprusside (SNP= 2.5mg/kg).

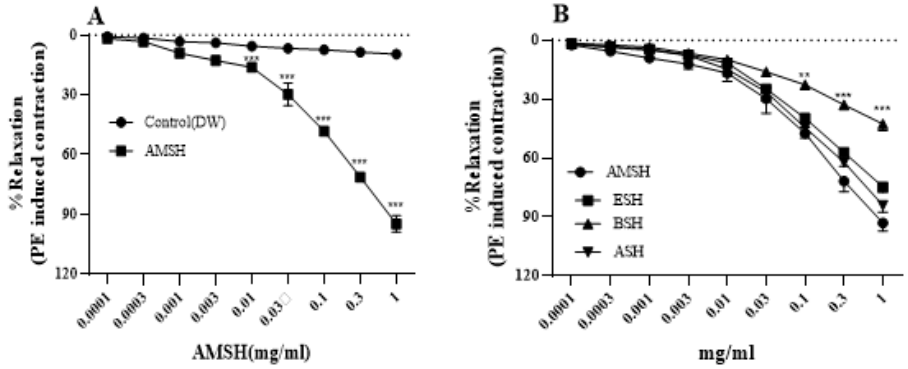

Fig. 2: Vasorelaxant effect in endothelium intact (E^+) rat aorta rings precontracted with PE of, (**A)** aqueous methanolic extract of *Sorghum halepense* (AMSH) compared with control (distilled water=DW), **(B)** ethyl acetate fraction (ESH), butanol fraction (BSH) and aqueous fraction (ASH) compared with AMSH as control . Data are expressed as a percentage relaxation from the PE-induced tone and are means ± SEM of (n=6), *** indicate p<0.001, **p<0.01, *p<0.05, 2 way ANOVA followed by a Boneferoni post-hoc test.

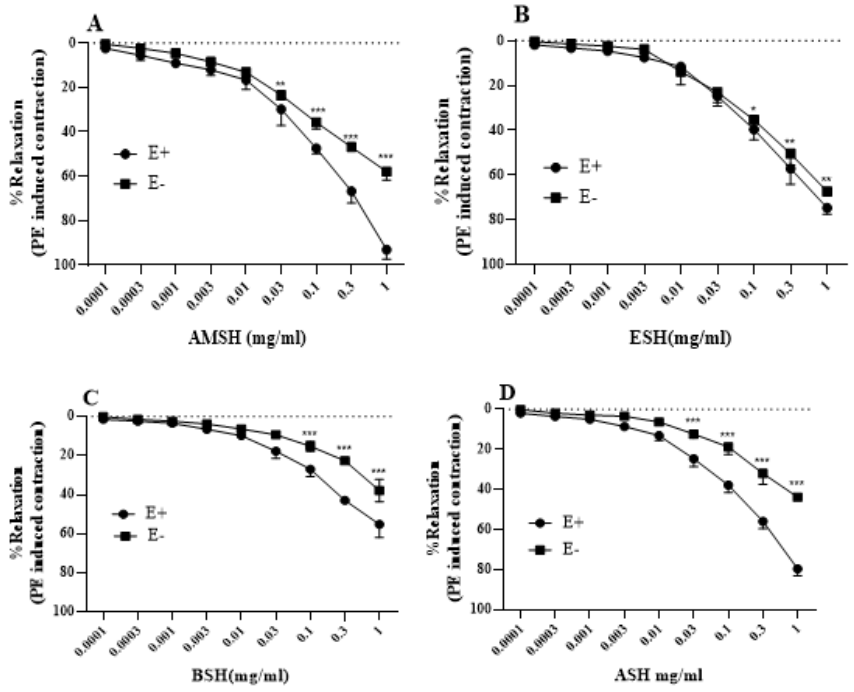

Fig. 3: Vasorelaxant effect in endothelium intact (E^+) and endothelium denuded(E^-) rat aorta rings pre-contracted with PE, (A) aqueous methanolic extract of *Sorghum halepense* (AMSH), (B) ethyl acetate fraction (ESH), (C) butanol fraction (BSH) and (D) aqueous fraction (ASH). Data are expressed as a percentage relaxation from the PE-induced tone and are means ± SEM of (n=6), *** indicate p<0.001, ***p*<0.01, **p*<0.05, 2 way ANOVA followed by a Boneferoni post-hoc test.

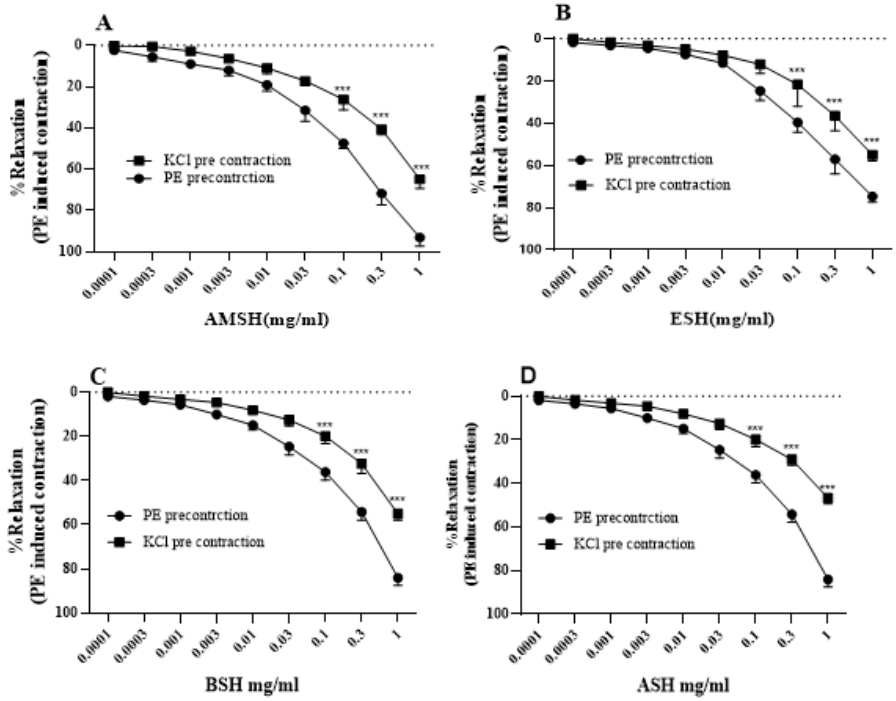

Fig. 4: Vasorelaxant effect of (A) aqueous methanolic extract of *Sorghum halepense* (AMSH), (B) ethyl-acetate fraction (ESH) (C) butanol fraction (BSH), (D) aqueous fraction (ASH) in rat aorta rings pre-contracted with PE and KCl. Comparison done between relaxation against PE and KCl induced contraction. Data are expressed as a percentage relaxation from the PE-induced tone and are means ± SEM of (n=6), *** indicate *p*<0.001, ***p*<0.01, **p*<0.05, 2 way ANOVA followed by a Boneferoni post-hoc test.

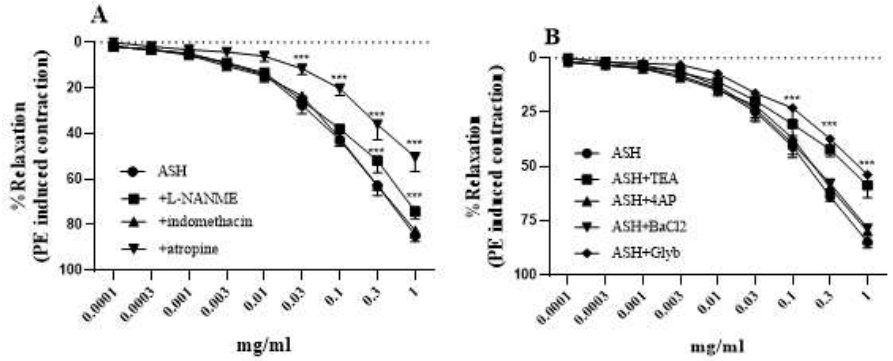

Fig. 5: Vasorelaxant effect of aqueous fraction of *Sorghum halepense* (ASH) in rat aorta rings pre-contracted with PE in absence and presence of, (A) L-NAME (100uM), indomethacin (10uM) and atropine (1uM), (B) tetraethylammonium (TEA=5mM), 4 aminopyridine (4AP=1mM), barium chloride ($BaCl_2$=1mM) and Glybenclamide (Glyb).Comparison done between relaxation response of ASH in presence of blockers with control response of ASH. Data are expressed as a percentage relaxation from the PE-induced tone and are means ± SEM of (n=6), *** indicate p<0.001, **p<0.01, *p<0.05, 2 way ANOVA followed by a Boneferoni post-hoc test.

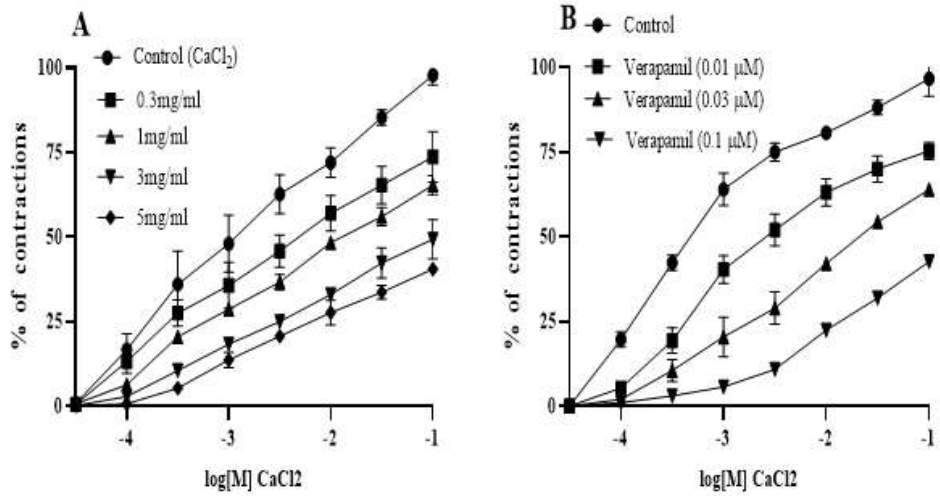

Fig. 6: Vasorelaxant effect of aqueous fraction of *Sorghum halepense* (ASH) (0.3mg, 1mg, 3mg, and 5mg/ml) (A) and verapamil (0.01uM, 0.03uM, 0.1uM) (B) on cumulative concentration response curve produced by addition of $CaCl_2$ in rat aorta rings. Data are expressed as a percentage relaxation from the $CaCl_2$-induced tone and are means ± SEM of (n=6), *** indicate p<0.001, 2 way ANOVA followed by a Boneferoni post-hoc test.

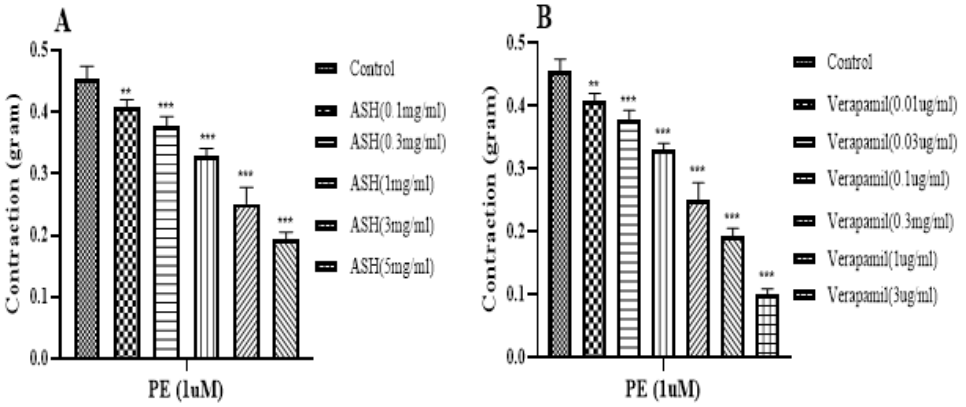

Fig. 7: Inhibitory effect of pre-incubation with various doses of aqueous soluble fraction of *Sorghum halepense* (ASH) (A), and various doses of verapamil (B) on contraction produced with PE in rat aorta rings in calcium free Kreb-Henselet buffer compared with response of PE not pre-incubated with ASH and verapamil, taken as control. Data are expressed as an inhibitory effect from the PE-induced tone and are means ± SEM of (n=6), *** indicate p<0.001, **p<0.01, , 1 way ANOVA followed by a Dennett's test.

Effect of ASH on intracellular Ca^{2+} release

Vascular reactivity of ASH was evaluated on Ca^{2+} influx either through, receptor-operated Ca^{2+} channels (ROCs) and Ca^{2+} release from internal store(s).The rings were pre-contracted with KCl (80 mM) to provide Ca^{2+} loading into SR. Then, the aortic rings were washed and exposed to Ca^{2+} free Krebs solution containing EGTA (1 mM), followed by activating transient contraction by PE (10 μM) in Ca^{2+} free solution before and after pre-incubation with ASH different doses (0.01mg–5mg/ml). The percentages of contraction activated by PE in the absence of ASH was considered as control and compared with PE induced contraction in presence of different doses of ASH. Same experiment performed with verapamil (0.01ug/ml – 3ug/ml) for comparison (Pantan *et al.*, 2014).

STATISTICAL ANALYSIS

The results are expressed as mean ± standard error mean (SEM).Statistical analyses were completed with one way ANOVA followed by a Dennett's test and two way analysis of variance followed by Bonferroni tests using Graph Pad Prism version 8.0. By this done illustration of graphs. Differences were considered significant at *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$.

RESULTS

TPC and TFC of AMSH and fractions

Total phenolic contents and total flavonoid contents were evaluated against gallic acid and catechin as standards equivalents, presented in table 1.

HPLC analysis of AMSH AND ASH

HPLC analysis of AMSH and ASH was performed and obtained constituents were specified in table 2. Phytochemical analysis exposed the presence of vanillic acid, 3-OH-4-MeO benzaldehyde and p-coumaric acid in aqueous methanolic extract of *S. halepense* (AMSH) while vanillic acid and naringinin in aqueous soluble fraction (ASH).

Acute hypotensive and antihypertensive effect of AMSH and fractions

Effect on blood pressure parameters after administration of vehicle is negligible. AMSH and fractions caused a significant reduction of mean arterial blood pressure (MAP) at different doses in normotensive and in fructose induced hypertensive rats as given in fig 1. The aqueous methanolic extract of *S. halepense* (AMSH) reduced the MAP in normotensive rats by 6.73%, 27.45%, 39.23%, 45.87%, 64.14% and 75.23% and in fructose induced hypertensive rats by 54.66%, 69.97% and 79.25 % at the doses of 0.5,1, 5, 10, 20 and 40 mg/kg, respectively. However, aqueous soluble fraction (ASH) among other fractions reduced MAP in normotensive rats by 51.93%, 68.66% and 77.01 % and in fructose induced hypertensive rats by 54.89%, 71.75% and 79.28 % at the doses of 10, 20 and 40 mg/kg, respectively. Effect of ASH was observed significant in both normotensive and hypertensive rats at 10mg/kg where safest reduction of blood pressure. While butanol and ethyl-acetate soluble fraction produced less percentage of reduction as compared to aqueous soluble fraction.

S. halepense induced hypotension by activation of muscarinic receptors

S. halepense aqueous soluble fraction (ASH) hypotensive response was inhibited by pre-treatment of atropine. Treatment with ASH (10 mg/kg) decreased (MAP) upto 62.79±1.28 mmHg in normal rats whereas, pre-treatment with atropine reduced this decrease to 26.23±2.42 mmHg. However, pre-treatment with indomethacin, L-NAME, hexamethonium, captopril, propranolol and sodium nitropurruside produced non-significant effect on MAP (fig. 1E).

Effect of aqueous methanolic extract and fraction on rat aorta

The aqueous methanolic extract of *S.halepense* (AMSH) produced vasorelaxation at (0.0001 to 1mg/kg) in endothelium intact ring pre-contracted with PE (10uM) as compared to control (D.W). The relaxation at dose of 1mg/kg was 94.73±1.61% (fig. 2A). Moreover, all fractions of this extract produced vasorelaxation but maximum effect was produced with aqueous soluble fraction (ASH), as 84.69±1.16 % relaxation at dose of 1 mg/kg (fig. 2B). Endothelium denudation has effect on the vasorelaxation of ASH (fig. 3).

Effect of aqueous methanolic extract and fraction on K^+ (80mM) induced contraction

AMSH and ASH produced vasorelaxation in rings pre-contracted with KCl. Although at 1mg/kg produced less effect on vasorelaxation as compared with PE (fig. 4).

S. halepense active fraction has effect on membrane receptors

Pre-treatment with atropine (1uM, a muscarinic receptor antagonist), significantly inhibited the vasorelaxation response of ASH in intact rat aortic ring. It produces 67.62% relaxation as compared to control ring. L-NAME (100uM, a non-selective NO synthase inhibitor) and indomethacin (1uM, a cyclooxygenase inhibitor) did not affect the vasorelaxation of ASH (fig. 5A).

S. halepense active fraction vasorelaxation attenuated by K channels blockers

Pre-treatment with K_{ATP} (Glibenclamide) produced highly significant attenuation of vasorelaxation of ASH from 88.69±1.16 % to 53.79±1.53% as compared to control. K_{IR} blocker ($BaCl_2$=1mM) significantly attenuate ASH induced relaxation 66.79±0.62% and with non-selective K

blocker (TEA) produced 67.05±0.62% relaxation as compared to control. However, pretreatment with K_V did not produced any decrease in vasorelaxation as compared to control (fig. 5B).

S. halepense active fraction does has effect on extracellular calcium induced contraction

S.halepense active fraction (ASH) at (0.3mg to 5 mg/ml) alter the contraction produced due the addition of $CaCl_2$ (1uM to 3mM) in rat aortic rings in absence of extracellular calcium similarly as verapamil. The maximum contraction produced by 3mM $CaCl_2$ was 103.50±3.57% of contraction, which reduced by 5mg/ml of ASH up to 55.66±1.78 % contraction (fig. 6)

S. halepense active fraction vasorelxation effected by intracellular calcium release

Pre incubation of rat aortic rings with *S. halepense* active fraction (ASH) different doses (0.3mg to 5 mg/ml) lessen the contraction produced due the PE (1uM) as control. PE (1uM) produced maximum contraction as 0.455±0.008 g while 5 mg/ml of ASH reduced it to 0.19±0.005 g similar to verapamil effect after PE induced contraction (fig. 7).

DISCUSSION

The investigation on the natural products to get inventive drugs, has open substantial changes in field of drug. Natural products get importance in last decade for treatment of different diseases (Junior *et al.,* 2013).The work on medicinal plants extract and fractions raises each year and subsequently numerous natural products become available as new antihypertensive and vasorelaxant drugs (Kazama *et al.,* 2012). This study showed the use of aqueous methanolic extract of *S. halepense* and fractions in hypertension and vasorelaxation. Moreover, this study identified the vascular mechanisms underlying the hypotensive effect of *S. halepense* most active fraction. This study also identified active constituents in plant extract and active fraction that are accountable for these effects. The results presented in this study showed us that the active components existing in the aqueous soluble fraction are proficient to induce hypotensive and vasorelaxation actions with direct participation of the muscarinic receptor, calcium channels and potassium channels.

Evaluation of hypotensive and antihypertensive effect of aqueous methanolic extract of *S. halepense* and fractions in normotensive and fructose induced hypertensive rats at different doses showed the dose dependent hypotensive/antihypertensive effect with maximum effect at dose of 10mg/kg of aqueous soluble fraction of *S. halepense* (ASH). Therefore this fraction at this dose was selected for further studies to estimate underlying mechanism of action. Pre-administration of atropine, attenuated the degree of hypotension of ASH treatment. In previous studies it has been reported that activation of M2 muscarinic cholinergic receptors subsequent the release of neurotransmitter acetylcholine, that in turn regulated by parasympathetic control of heart rate by direct activation of G protein dependent maintenance of ion channel activity or by modulation of cAMP dependent responses (Harvey and Belevych, 2003). Moreover, this regulation occurred at level of autorhythmic (pacemaker) cells in the sinoatrial node (SAN),where sympathetic and vagal parasympathetic activity via G-coupled leads to decreased depolarizing currents carried by hyperpolarization-activated cyclic nucleotide-gated cation (HCN) and L-type Ca^{2+} channels (Mighiu and Heximer, 2012). Furthermore, binding of acetylcholine to muscarinic receptor (M2), causes activation and dissociation of G- protein heterodimers that ultimately activates the G protein coupled inward rectifying potassium channels and decreased sinus rate. Also, it has been reported that M_2 receptors lessen the contractile forces of the atrial cardiac muscle and diminish the conduction velocity of the atrioventricular node (AV node) and resulted in decrease of blood pressure (Ang *et al.,* 2012; Moss *et al.,* 2018). So we assume that ASH may be acting through muscarinic receptors for decrease of blood pressure and need to be evaluated further.

Mediators such as phenylephrine and high K^+ were used to differentiate the effect of *S. halepense* on endothelial and smooth muscle cells of isolated rat aorta. AMSH and fractions induced vasorelaxation with maximum effect with ASH after contraction with PE. Moreover, ASH produced endothelium dependent mechanisms. Further experiment were performed with ASH on endothelium intact ring for evaluation of mechanisms.

Pretreatment of aortic rings with atropine, a muscarinic receptor blocker (Guedes *et al.,* 2004) before PE contraction, inhibited the vasorelaxation effect of ASH. It has been reported, in vasculature, muscarinic receptors activation is involved in vasorelaxation and direct activation of muscarinic M3 linked NO release played important role (Knox *et al.,* 2019). Pretreatment of intact rat aortic ring with indomethacin, a prostaglandin inhibitor and L-NAME (NO synthesis inhibitor) (de AF Da *et al.,* 2012) did not modify vasorelaxant effect of ASH. These findings showed that the endothelium-dependent vasodilatory effect of ASH may be mediated through muscarinic receptor that led to the formation of NO (Ahmed *et al.,* 2018).

S. halepense vascular effects was also studied on smooth muscle cells. It has been reported that PE is known to cause aortic contraction by increasing the intracellular Ca^{2+} concentration through the release of Ca^{2+} from sarcoplasmic reticulum (SR) and influx through opening of voltage dependent calcium channels (Le *et al.,* 2001). To evaluate this possibility, rat aortic rings were

pretreated with different concentrations of ASH in Ca^{2+} free/EGTA medium (Ahmed *et al.,* 2020). Interestingly, this pretreatment suppressed PE individual contractions compared to control response of PE, indicating the inhibitory effect of ASH on Ca^{2+} release from the internal Ca^{2+} store. Thus, it seems likely that the vascular effect of ASH may involve a reduction of IP3- dependent Ca^{2+} release from sarcoplasmic reticulum (SR) sensitive to phenylephrine as reported in many previous studies (Lee *et al*., 2001).Membrane associated channels are also involved in smooth muscle relaxation specially while contracted with PE and KCl. This depend on the opening of cell surface voltage-gated Ca^{2+} channels (VGCCs), which are activated by cell membrane depolarization and cause an increase in $[Ca^{2+}]i$ (Kirschstein *et al.,* 2009). It is apparent that an agent inhibits high K^+ induced contractions could be a possible Ca^{2+} entry blocker (Karaki *et al,* 1984). ASH inhibited K^+ (80 mM)-induced vascular smooth muscle contraction concentration-dependently and significantly reduced the Ca^{2+}-induced contraction in aortic rings, similar to verapamil. These findings showed that ASH may also has an inhibitory effect on Ca^{2+} entry through VDCs. This possibility was further tested and for this rat aortic rings were suspended in Ca^{2+} free/EGTA medium and $CaCl_2$ concentration–response curves were obtained in duplicate. Pre-incubation of the aortic rings with different concentrations of ASH induced a rightward shift with suppression of maximum response, in the $CaCl_2$, similar to verapamil, that showed ASH may also inhibits Ca^{2+} entry through VDCs.

High potassium has been reported, to lessen relaxation responses produced by opening of plasma membrane potassium channels by changing the equilibrium of K^+ ions across the membrane. Vascular K^+ channel activation has been known to play an important role in the regulation of vascular tone (López Canales *et al.,* 2015).The data showed us that TEA, a nonselective potassium channel blocker produced significant inhibitory effect on the vasorelaxantion of ASH. However, $BaCl^2$, a blocker of Kir channels and 4-aminopyridine, blocker of Kv channels not significantly inhibited relaxation of ASH. Moreover, K_{ATP} also have significant inhibitory effect as compared to control effect of ASH. These present findings on ASH stipulate that activation or opening of different potassium channels may also contributed to vasorelaxant of ASH.

HPLC analysis report showed the presence of naringenin and vanillic acid in ASH. Polyphenols have been reported for pharmacological activity against cardiovascular diseases and mainly in the regulation of high blood pressure. There have been reported various studies on flavonoids for their blood pressure lowering and vasorelaxant activity (Lombardo *et al.,* 2014). Naringenin has been reported for vasorelaxant effect through blockade of the Ca^{2+} channels and activation of potassium channels especially large conductance potassium channel (Sánchez-Recillas *et al.,* 2019). Consequently, our finding on the vascular mechanisms of ASH showed it to be novel treatment approach for control of high blood pressure by way of producing vasorelaxation by various pathways. Moreover, in present study of *Sorghum halepense* (L).Pers, many constituents of polyphenols and flavonoids determined, that thought to be a good source in the field of antihypertensive and vasorelxant drugs.

CONCLUSION

This study has identifies *Sorghum halepense* L as an important natural source for blood pressure lowering effect. On the basis of results of this study, we assume that aqueous soluble fraction of *S. halepense* produced hypotensive effect by various vascular mechanisms and its constituents as vanillic acid and naringinin may be responsible for its effects. Further electrophysiological studies would provide more insight into the cellular aspects of these mechanisms.

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