ORIGINAL ARTICLE

Protective Effect of Polyherbal Composition against Hemorrhagic Shock Condition in an Experimental Rat Model

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Abstract

Objective: Hemorrhagic shock (HS) is a condition which requires an immediate care and if not treated on time can result in death. Hence, we aim to study the protective role of fixed dose combination of polyherbal composition comprising of an equal amount of aqueous extracts of *Azadirachta indica* leaves, *Carica papaya* leaves, and methanolic extract of *Andrographis paniculata* against fixed volume bleed HS model. **Materials and Methods:** The study was conducted with 48 male and 48 female Wistar rats divided into 8 groups, normal control (G1), disease control (sublethal and lethal, G2 and G3) and 5 treatment groups (G4–G8). Treatment was initiated on the same day and continued for 14 days. At the end of 14 days, hemorrhage was induced by withdrawing 40% of circulatory blood volume for all groups and 60% of circulatory blood volume for G3. Treatment was continued further for 7 days. Complete blood count, serum levels of glucose, sodium, potassium, and rectal temperature were measured at before, 3 h, 24 h, 72 h, and 7 days post-hemorrhage, whereas interleukin (IL)-6, nuclear factor kappa B (NF-ƘB), and vasopressin levels were measured at 24 h time point. Serum lactate and bicarbonate levels were measured at 3 h and at the end of the study. Statistical analysis was done using one way ANOVA followed by Dunnett's test. $P < 0.05$ was considered statistically significant. **Results:** At 24 h, glucose levels were high and hematocrit levels were low compared to NC group, which were recovered to normal in all treatment groups at the end of the experiment except in disease control group. Our findings were further supported by serum level of electrolytes as well as IL-6, NF-ƘB, and vasopressin levels which were elevated in response to HS condition at 24 h post-hemorrhage. We have seen significant ($P < 0.0001$, $P < 0.001$, $P < 0.01$, $P < 0.05$) changes in the level of these parameters in disease control group compared to other groups. Recovery at the end of the experiment indicates that animals have responded to our treatments with maximum effect has been seen in G7 group. **Conclusion:** The polyherbal extracts used in this experiment have the potential to be used alone or in combination with available conventional medical therapy against HS condition to provide symptomatic relief, to improve quality of life and to promote the survival.

Key words: Hemorrhagic shock, fixed-volume hemorrhage, Hypovolemic shock, *Carica papaya*, *Azadirachta indica*, *Andrographis paniculata*, herbal extract

INTRODUCTION

Shock, an extremely critical and fetal condition arises when there is an imbalance between body's demand and supply of oxygen resulting in tissue hypo perfusion and hypoxia and circulatory failure.[1] There are four types of shock. (1) Hemorrhagic/hypovolemic shock (significant and continuous loss of blood or fluid, internally or externally). (2) Cardiogenic shock (due to underlying illness which leads to failure of heart to work as pump efficiently). (3) Distributive shock (due to sepsis, endocrinal or neurogenic abnormalities, anaphylaxis). (4) Obstructive shock (due to tension pneumothorax, pulmonary embolism, cardiac tamponade).[1,2] Hypovolemic shock, the most common and prevalent one can be the result of persistent hemorrhage is often reversible and having the mortality rate of 30–90% depending on its severity and intensity of organ failure.[3]

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It can be with or without the presence of trauma. Depleted intravascular volume has a consequence of decreased preload, leads to decreased stroke volume (SV) and decreased cardiac output (CO). In response to this compensatory mechanisms will get activated at both cellular and molecular level to restore the CO and oxygen delivery. This includes increase in systemic vascular resistance, increase in sympathetic nerve activity along with increased peripheral vasoconstriction, release of catecholamines, vasopressin to compensate blood pressure (BP), and CO.[3,4]

From ancient times, plants and plant-based products are being used for the treatment and management, control and prophylaxis of diseases such as cancer, AIDS, diabetes, hypertension, and many other infectious diseases.[5] With many countries such as India, China, USA, Nigeria, and even World Health Organization investing in traditional herbal medicine, the industry is having the annual global market of 60 billion US$.[6] Hence, in this study, we aim to study the protective effect of our Polyherbal extracts comprising of equal proportions of methanolic extract of whole aerial body of *Andrographis paniculata*, commonly known as "Kalmegh" or "King of bitters" (family: Acanthaceae), aqueous extracts of leaves of *Azadirachta indica*, commonly known as "Neem" (family: Meliaceae) and *Carica papaya* commonly known as "Papaya" (family: Caricaceae) in a fixed volume bleed hemorrhagic shock (HS) model in rats. All the three plants we have selected have an abundant growth in India and known for their wide range of medicinal properties. The LD_{50} values of these extracts are well documented.[7-14]

Fixed volume bleed out model is the type of model in which hemorrhage is induced in a controlled manner by withdrawing a predetermined percentage of circulating blood volume depending on bodyweight of an animal over a fix period of time followed by resuscitation and treatment. This model is preferred over other models as it offers an advantage to study shock-induced pathophysiological changes occur in carbohydrate and protein metabolism characterized by change in blood glucose and glycogen, lactate and bicarbonate levels, histopathological abnormalities, efficacy of different therapeutic intervention, as well as neuroendocrine compensatory mechanism reflected by changes in serum levels of vasopressin, cytokine release in response to the expression of protein transcription factor nuclear factor kappa B (NF-ƘB) and also to study the survival.[15,16]

MATERIALS AND METHODS

Collection of plant material

Dry powders of *C. papaya* leaves and whole aerial body of *A. paniculata* were obtained from Universal Agri Exports, Ahmedabad and Shree Shail Herbs Pvt. Ltd., Nagpur, respectively. We have collected *A. indica* leaves locally from Ahmedabad, washed to remove debris and dried under shed, powdered, and sieved. All the samples were sent to M.S. University, Baroda, for their identification. Our samples were evaluated by Department of Botany. (Specimen no. Bot/21617/aut.)

Preparation of plant extracts

Dry powders of all the three plants were subjected to extraction by cold maceration method.[17,18] Aqueous extraction was carried out for the leaves of neem and papaya with distilled water and chloroform (Merck, India) (97.5:2.5) in the ratio of (1:10 w/v) continuously for a period of 3 days. Dry powder of Kalmegh was extracted with absolute methanol (Merck, India) consecutively for 3 days in dark (1:5 w/v). After 3 days, they were filtered using Whatman filter paper, grade 1 and then concentrated at 45°C under reduced pressure and stored at −4°C in airtight containers till further use.

Animals

Forty eight male and 48 female healthy adult Wistar rats (VAB biosciences, Hyderabad) weighing 230–240 g were procured and acclimatized for 7 days under standard laboratory condition (12:12 h light and dark cycle, relative humidity 30–70% and temperature maintained at 22 ± 3°C). They were fed with standard laboratory pellet diet (Krishna Valley Agrotech LLP, Pune) and provided with reverse osmosis treated drinking water *ad libitum*. They were housed in clean and sterilized polypropylene cages (3 rats per cage). Entire protocol to conduct the study was thoroughly prepared, reviewed, discussed, and approved by Institutional Animal Ethics Committee (ARL/PT/1082/2019) prior commencing the experiment. All the experiments were executed in strict accordance with the rules and regulation of the CPCSEA guideline (Registration no. 1709/PO/Rc/S/13/CPCSEA).

Grouping and treatment

At the end of acclimatization period, animals were allocated to different groups based on their body weight in such a way that the difference in the body weight of animals in each group should minimum and not more than 20%. On the same day, treatment was initiated and continued for 14 days once in a day by oral route as follows.

Fixed volume bleed out HS model in rats

After receiving the treatments for 14 days [Table 1], all the animals except the animals in the NC group were anaesthetized using isoflurane (initially, 5% and then 1% to maintain in air by inhalation) and slowly and gradually 40% (sublethal) and 60% (lethal) of circulating estimated blood volume (EBV) was removed over a period of 5 min and 10 min, respectively, by retro-orbital puncture method. The EBV for each animal was calculated using: weight (g) * 0.06 (ml/g) + 0.77.[19] Lethal HS model was induced to check the survival of animals and

Table 1: Grouping and treatment of animals

Group	Group type	Treatment (p.o)	No. of rats	
			Male	female
G1	No hemorrhage, normal control (NC)	CMC (1%)	6	6
G2	DC sublethal (40% blood removal)	No treatment	6	6
G3	DC lethal (60% blood removal) to check survival	No treatment	6	6
G4	Sublethal hemorrhage with treatment	*Carica papaya* (C) extract (500 mg/kg)	6	6
G5	Sublethal hemorrhage with treatment	*Azadirachta indica* (N) extract (200 mg/kg)	6	6
G6	Sublethal hemorrhage with treatment	*Andrographis paniculata* (K) extract (200 mg/kg)	6	6
G7	Sublethal hemorrhage with treatment	Equal (33.33%) of all the three extracts (C,N,K) (300 mg/kg)	6	6
G8	Sublethal hemorrhage with treatment	Equal (33.33%) of all the three extracts (C,N,K) (500 mg/kg)	6	6

Where p.o= per oral route, CMC = Carboxymethyl cellulose, C = *Carica papaya* extract, N = Neem (*Azadirachta indica*) extract, K= Kalmegh (*Andrographis paniculata*) extract. DC: Disease control

it was monitored till the end of the study. Once hemorrhage was induced, animals were kept unresuscitated, but monitored carefully for a period of 60 min to induce shock. After 60 min, all animals were resuscitated with ringer's lactate solution (twice the volume of the blood shed). There were no deaths in this protocol except in Group 3 (G3), where 60% of EBV has been removed. G3 has reported 16% of mortality as one male and one female animal died 24 h and 72 h post-HS, respectively. All the treatments were continued after HS was induced, further for a period of 7 days as shown in Table 1. All the animals were observed daily for morbidity and mortality as well as any changes in skin color or texture.

Blood sampling and parameter measurement

Blood samples were collected before the hemorrhage was induced on 14th day and considered as "0" h (before) sampling. Then, hemorrhage was induced and again blood samples were collected at 3 h, 24 h, 72 h, and at the termination of experiment which is on 7th-day post-hemorrhage by retro-orbital plexus method under diethyl ether anesthesia. Serum and plasma were separated and stored in pyrogen free tubes at −20°C till further use. Complete blood count was determined using automated blood cell counter (Nihon Kohden, MEK-6420p), serum parameters such as serum sodium and serum potassium were determined by colorimetric method (Accucare, Lab-Care Diagnostics India Pvt. Ltd.) and serum glucose was determined by Trinder's end point method (Erba, Transasia Biomedical Ltd.) for all the five time points. Rectal temperature and body weight of all animals were also recorded. Serum lactate and bicarbonate levels were also measured at 3 h and at termination using radiometer (ABL 800 FLEX).

ELISA

NF-KB and interleukin (IL)-6 levels were determined in serum samples and vasopressin level was determined in plasma sample using commercially available ELISA (Kinesis Dx, CA, USA (K11-5141, Rat NF-KB), Krishgen Biosystems (KB3068 Ver 5.0, Rat IL-6 GENLISA, and KLR0385 ver 2.0, Rat Vasopressin GENLISA) kits as per the instructions given by the manufacturer at 24 h post-hemorrhage.

Statistical analysis

All data were depicted as mean ± SEM. Data were analyzed for statistical significance using GraphPad Prism 8 and it is carried out using one way ANOVA followed by Dunnett's multiple comparison tests. Values of $P < 0.05$*, 0.01**, 0.001***, and 0.0001**** were considered statistically significant.

RESULTS

Effect on plasma vasopressin and serum sodium level

The ELISA was carried out 24 h after the HS was induced to see the effect of HS on different groups. Figure 1 indicates the plasma concentration of vasopressin 24 h post-HS. Apart from the NC group, all the other groups have shown a significant increase in plasma vasopressin level post-HS. In the NC group, the average vasopressin levels in plasma were 3.56 ± 1.23 for male group and 5.78 ± 0.83 for female group, respectively, and in comparison to NC group remaining all groups have shown increased level of vasopressin ranging from 8.51 pg/ml to 33.54 pg/ml.

Serum Na^+ levels were within the normal range in all groups before the HS was induced (120 ± 6.18–149.54 ± 5.40 mmol/L). However, 24 h after HS, there is a sharp increase in sodium levels and remained higher even after 72 h except in the NC group. At the end of the experiment serum

sodium levels were returned to normal range in all treatment groups but prevailed higher in DC group [Table 2]. At the end of the experiment, serum sodium levels were significantly ($P < 0.0001$, $P < 0.001$) less in all treatment groups compared to DC group [Figure 2].

Effect on NF-ƘB and IL-6 levels

NF-ƘB and IL-6 levels were measured in serum samples of all animals 24 h after the HS was induced by ELISA. Compared to the NC group both the levels of NF-ƘB and IL-6 were significantly higher in DC group in males as well as in females ($P < 0.0001$, $P < 0.001$) [Figures 3 and 4]. All treatment groups have responded in a different manner. Group 5 (G5) has received *A. indica* treatment and NF-ƘB in both males and females was below the detection level in G5. Similarly females in Group 4 have shown NF-ƘB level below detection point. Although the NF-ƘB level in all other treatment groups was either equal or higher than the NC group, they were considerably low than the DC group. As in case of serum IL-6 concentration, all animals in treatment groups have low levels of IL-6 compared to DC group, even lower than the NC group except in case of Group 4. Females have shown low level of these two parameters compared to males in all treatment groups.

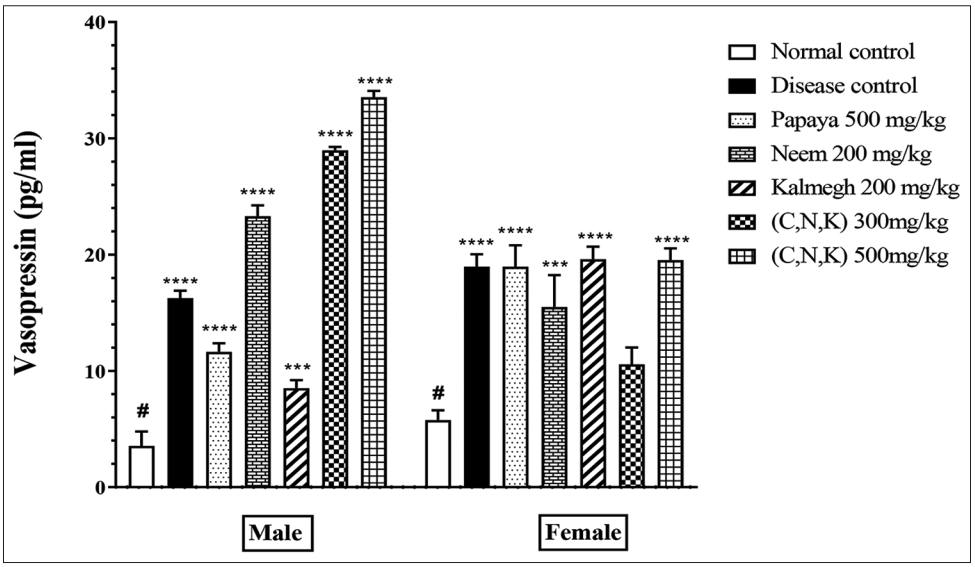

Figure 1: Average of plasma vasopressin concentration (pg/ml) of all groups at 24 h (1 day) post-HS depicts the effect and extent of HS on plasma vasopressin concentration. Values are represented as mean ± standard error of mean. ($n = 6$). Asterisk denotes that treatment groups are statistically different from (#) normal control, ****$P < 0.0001$, ***$P < 0.001$

Table 2: Average serum sodium level of all groups at different time points

			Sodium (mmol/L)			
Group	**Treatment**	**Sex**	**0 h**	**24 h**	**72 h**	**7 days**
G1	NC	M	126.48±2.56	129.64±3.27	133.27±4.03	132.70±3.05
		F	133.11±4.96	135.68±3.38	134.29±2.84	134.27±3.61
G2	DC	M	134.77±6.58	183.67±6.58	180.18±16.91	176.49±5.76
		F	134.22±5.16	198.28±4.33	198.99±10.41	179.82±7.95
G4	Papaya (C) (500 mg/kg)	M	132.88±5.86	185.82±5.40	157.58±7.24	144.13±5.08
		F	143.63±3.51	211.57±4.92	166.93±6.54	144.01±2.84
G5	Neem (N) (200 mg/kg)	M	120.57±6.18	174.74±4.52	168.76±2.83	133.31±5.50
		F	149.54±5.40	208.30±4.15	159.12±14.36	150.48±7.95
G6	Kalmegh (K) (200 mg/kg)	M	140.56±4.86	173.66±2.72	173.23±7.90	143.26±6.14
		F	142.75±3.36	192.55±4.69	160.65±7.43	143.60±9.14
G7	All equal (C,N,K) (300 mg/kg)	M	143.15±5.17	174.04±2.98	160.94±3.88	127.52±8.04
		F	140.32±4.36	193.55±3.20	151.81±6.13	134.09±3.42
G8	All equal (C,N,K)(500 mg/kg)	M	143.37±4.71	170.64±8.43	188.66±9.15	134.20±7.47
		F	132.79±3.26	186.32±6.74	153.83±9.89	138.78±2.99

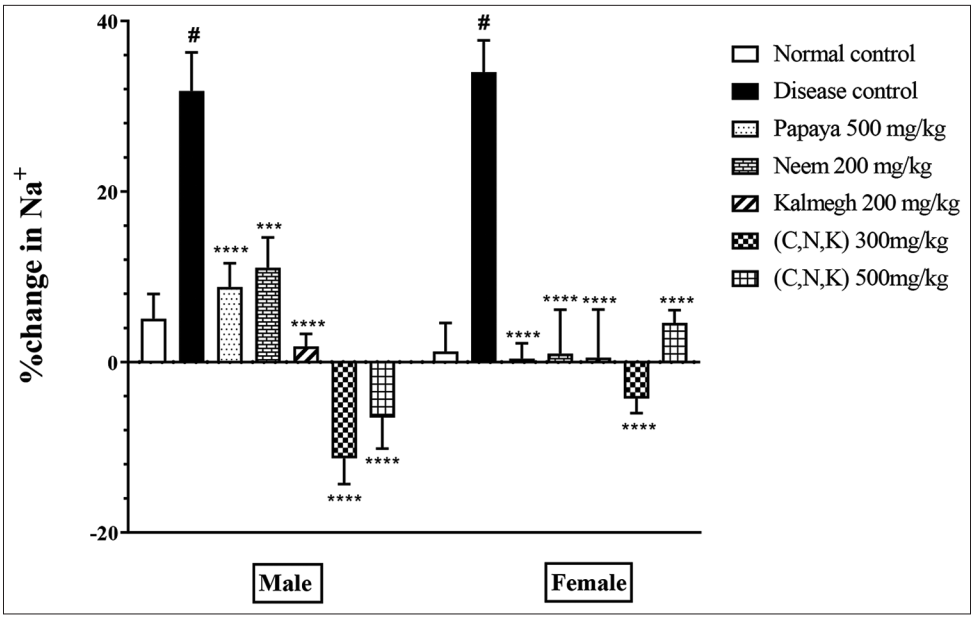

Figure 2: Graph depicts the % change in serum sodium (Na^+) level in all groups in 7 days post-HS. Data were assessed by one-way ANOVA followed by Dennett's multiple comparison tests. Values are represented as mean ± standard error of mean. (n = 6). Asterisk denotes that treatment groups are statistically different from (#) disease control, ****P < 0.0001, ***P < 0.001

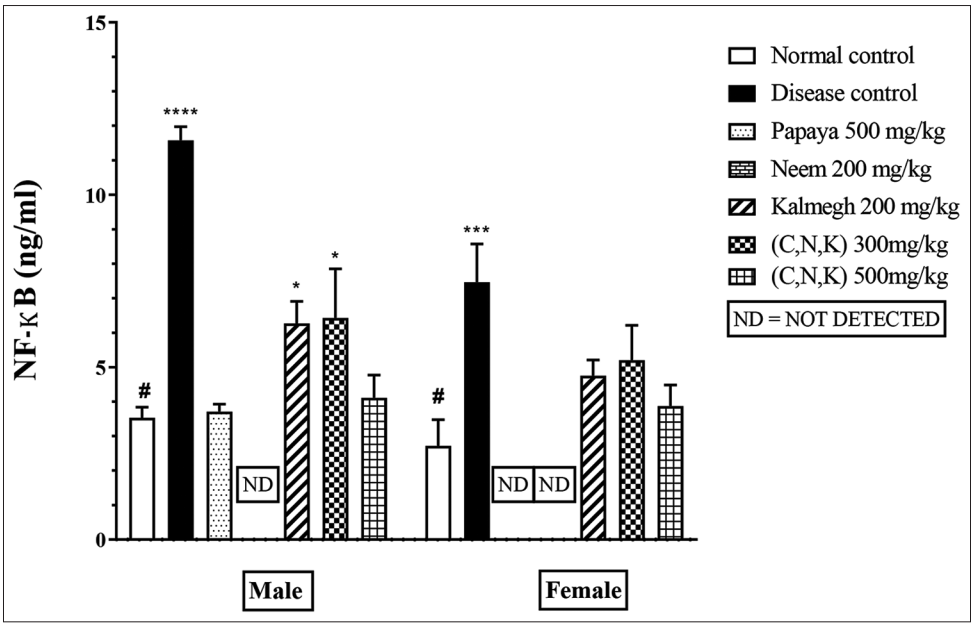

Figure 3: Average of serum NF-ƘB concentration (ng/ml) of all groups at 24 h (1 day) post-HS depicts the effect and extent of HS on serum NF-ƘB concentration. Values are represented as mean ± standard error of mean. (n = 6). Asterisk denotes that treatment groups are statistically different from (#) normal control, ****P < 0.0001, ***P < 0.001,*P < 0.05

Effect on serum glucose and hematocrit level

Serum glucose and hematocrit levels were determined at 0 h, 3 h, 24 h, 72 h, and at the end of the experiment which is at 7 days. There is no major change in these parameters in the NC group at any time point and they were within the normal range in both males and females. In case of hematocrit, there is a significant decrease (P < 0.0001, P < 0.001, P < 0.01) at 24 h in all other groups [Table 3]. The decrease was constant even at 72 h, and after that a recovery has been seen in all treatment groups except in DC group. The highest recovery was seen in G4, G7, and G8.

Serum glucose level remains within the range throughout the experiment in the NC group but a steep increase has been observed in all other groups at 24 h and 72 h (maximum). After 3 days, they were returning to normal in all treatment groups but not in DC group [Table 4]. Even at the end of the experiment, glucose

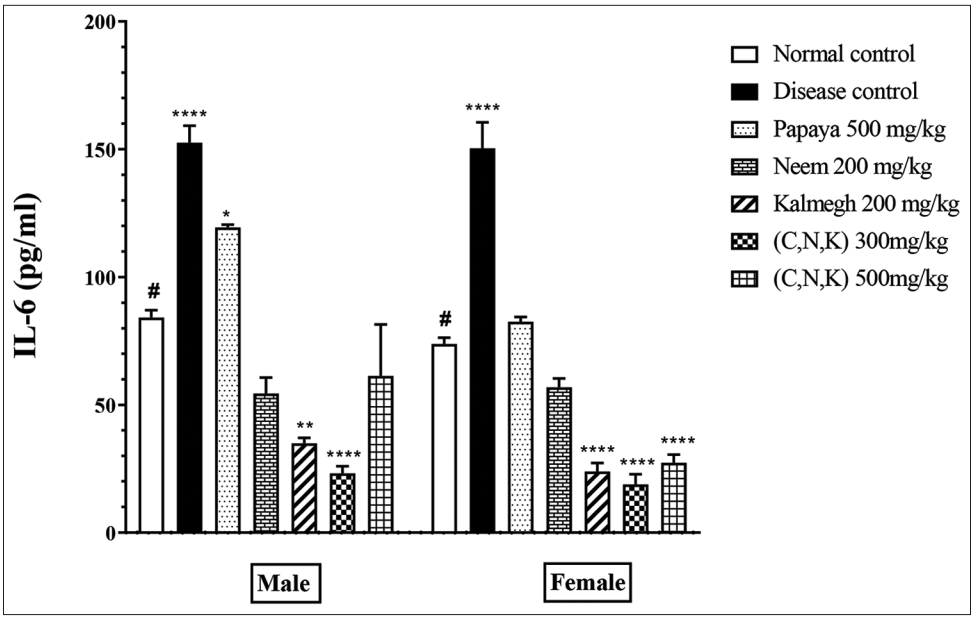

Figure 4: Average of serum interleukin-6 (IL) concentration (pg/ml) of all groups at 24 h (1 day) post-HS depicts the effect and extent of HS on serum IL-6 concentration. Values are represented as mean ± standard error of mean (n = 6). Asterisk denotes that treatment groups are statistically different from (#) normal control, ****P < 0.0001, **P < 0.01,*P < 0.05

Table 3: Average hematocrit (HCT) level of all groups at different time points

			Hematocrit (%)			
Group	**Treatment**	**Sex**	**0 h**	**24 h**	**72 h**	**7 days**
G1	NC	M	44.17±1.04	43.60±1.66	42.87±0.78	42.70±0.43
		F	39.50±0.80	40.70±0.75	40.43±0.77	39.33±1.05
G2	DC	M	44.92±1.21	30.40±1.96	25.32±1.05	34.40±1.10
		F	41.36±1.05	33.68±2.16	31.28±2.74	32.24±1.24
G4	Papaya (C) (500 mg/kg)	M	43.08±0.44	32.72±0.73	29.30±1.08	40.28±0.90
		F	42.38±1.12	33.24±1.43	34.16±2.48	38.82±0.99
G5	Neem (N) (200 mg/kg)	M	42.16±1.28	33.02±1.62	29.82±1.18	37.20±1.07
		F	39.72±1.80	30.30±1.56	32.62±1.50	35.36±1.65
G6	Kalmegh (K) (200 mg/kg)	M	44.43±2.16	31.07±1.36	33.52±1.03	38.73±0.81
		F	45.70±0.43	31.77±0.92	33.33±0.67	37.68±0.42
G7	All equal (C,N,K) (300 mg/kg)	M	46.87±1.34	30.42±0.97	36.42±1.76	43.53±1.11
		F	43.10±1.32	31.88±1.20	33.03±0.92	41.50±1.96
G8	All equal (C,N,K) (500 mg/kg)	M	45.77±1.75	33.82±1.87	33.45±1.23	43.00±1.08
		F	45.27±1.39	36.62±4.47	30.32±1.58	41.68±0.27

levels were too high in DC group, whereas in comparison to DC group all the treatments groups have significantly less change in glucose level post-HS [Figure 5]. Although glucose levels were returning to normal range, they were still higher in all groups that what it was before HS was induced.

Effect on other hematological and biochemical parameters

HS condition has no effect on body weight of animals. At the end of the study, all the groups irrespective of their disease state, sex, and treatments received have shown an increase of 1.85–3.30% in body weight. Throughout the experiment, there was no change in color or texture of the skin of animals. No sign of hypothermia or hyperthermia was observed. All animals have recorded rectal temperature in normal range. Serum lactate and bicarbonate levels were measured at 2 time points: 3 h post-HS and at the end of the experiment. Both the levels were almost same at both the time points in the NC group (serum lactate [at 3 h male: 1.28 ± 0.04, 7 days: 1.22 ± 0.08] and [female: 1.38 ± 0.03, 7 days 1.36 ± 0.09]). In all other groups, lactate levels were in the range of 1.45–1.76 mmol/L

Table 4: Average serum glucose level of all groups at different time points

			Glucose (mg/dl)			
Group	**Treatment**	**Sex**	**0 h**	**24 h**	**72 h**	**7 days**
G1	NC	M	115.63±4.42	111.02±8.64	129.21±3.17	124.04±3.13
		F	125.29±2.99	121.87±1.71	126.15±1.55	129.94±0.28
G2	DC	M	138.34±9.73	228.47±9.11	241.06±25.36	211.45±19.42
		F	122.34±3.04	185.02±8.40	181.13±5.33	180.58±4.38
G4	Papaya (C) (500 mg/kg)	M	137.41±2.99	232.61±8.10	201.68±9.83	171.44±9.85
		F	118.97±3.80	175.58±11.40	176.40±13.69	112.38±3.40
G5	Neem (N) (200 mg/kg)	M	127.83±9.68	161.52±6.95	332.58±4.77	157.84±7.01
		F	121.64±3.15	166.93±10.95	168.46±20.24	130.97±6.31
G6	Kalmegh (K) (200 mg/kg)	M	128.98±5.31	160.79±5.76	356.77±13.54	162.81±4.20
		F	127.97±3.39	184.54±9.04	181.53±26.46	157.33±9.42
G7	All equal (C,N,K) (300 mg/kg)	M	115.30±4.20	143.54±6.97	231.84±11.52	126.62±4.53
		F	125.38±3.43	169.12±5.37	181.73±9.53	131.39±6.23
G8	All equal (C,N,K) (500 mg/kg)	M	123.44±3.86	218.89±9.18	327.16±20.23	164.24±3.50
		F	125.01±2.65	179.20±9.59	208.94±35.76	160.04±6.72

at 3 h and in the range of 1.33–1.46 mmol/L at the end of the experiment. In case of bicarbonate levels in the NC group (male [3 h: 21.17 ± 0.21, 7 days: 21.63 ± 0.25] and female [3 h: 21. 60 ± 0.44, 7 days: 22.53 ± 0.87]). 3 h post-hemorrhage bicarbonate levels fall below 20 mmol/L in DC group as well as in males of G4, G5, G6, and G7, but at the end of the experiment their levels were above 20 mmol/L in all groups. WBC count was found to be considerably higher post-hemorrhage at 24 h and continued to be on higher side than what it was before HS was induced till the end of the study and at the same time opposite effect has been seen on RBC count which were much lower than what it was before the beginning. At 24 h post-HS, hemoglobin count in some groups was found to be decreased while in some groups it was increased except in the NC group where no such effect was seen but at 72 h post-HS, all the groups except NC have shown a remarkable decrease in hemoglobin levels which was also recovered at the end of the experiment.

DISCUSSION

Vasopressin, also known as antidiuretic hormone, plays an important role in maintaining sympathetic vasomotor activity through its hormonal, osmoregulatory, and pressor actions during HS condition. In HS condition, there will be a loss of blood volume which will cause a sharp fall in BP, SV, and CO as well as altered blood gases. Hence, the body activates its compensatory mechanisms to restore BP, SV, and CO to its normal level through activation of baroreceptors and chemoreceptors which causes cardiac stimulation, systemic vasoconstriction, and volume redistribution by releasing catecholamines, vasopressin, and activating Renin-Angiotensin-Aldosterone System.[20,21] This is the reason we have seen a significant ($P < 0.0001$, $P < 0.001$) increase in plasma vasopressin level in all HS-induced groups compared to NC [Figure 1]. Thus, released vasopressin increases the renal reabsorption of sodium and water to restore the blood volume.[22] Such changes were also reflected in this experiment. Significantly high ($P < 0.0001$, $P < 0.01$, $P < 0.05$) serum sodium level in response to increased level of vasopressin was detected at 24 h post-hemorrhage [Table 2]. The serum sodium levels were remained high at 72 h as well but then returned to normal (135–155 mmol/L) at the end of the experiment in all the groups that have received the treatment but remained higher in DC group [Figure 2]. When compared to DC group a significant decrease ($P < 0.0001$, $P < 0.001$) has been seen at the end of the experiment indicating the recovery with the highest effect was seen in males and females of G7. The serum sodium levels remained high by 31.79% in DC male and 34.01% in DC females at the end of the experiment and were outside the normal range.

The amount and duration of blood loss in HS condition is a major determinant factor of its consequences and can even result in circulatory collapse and multiple organ failure in 20–30% of HS patients. One of the major causes of this fatal situation is volume resuscitation which is also recommended in the guideline to treat HS patients to fasten the recovery, but this resuscitation causes a secondary ischemia-reperfusion (IR) injury. IR injury has multiple complications such as production of reactive oxygen species, which releases nuclear factor-kappa B (NF-ĸB) which triggers the production and release of pro-inflammatory cytokines such as IL-6 and tumor necrosis factor α.[23-25] We have also observed a significant ($P < 0.0001$) increase in NF-ĸB and IL-6 levels post-hemorrhage in DC group compared to NC group. At the same time in treatment groups, the effect of HS after 24 h is less prominent as denoted by low levels of NF-ĸB and IL-6. In fact, we were not able to detect NF-ĸB in G5 as well as

in females of G4. In all treatment groups, IL-6 levels were considerably low compared to DC and in G5, G6, G7, and G8, IL-6 level was even lesser that what it was in the NC group [Figures 3 and 4].

Another two major manifestations of HS are a rise in blood glucose level and a fall in hematocrit level. There exists a very strong inverse relationship between these two parameters in HS. In the NC group, these two parameters remained almost unchanged throughout the experiment, but in all other groups a sharp rise in blood glucose and a sharp fall in hematocrit are observed at 24 h post-HS and the effect remained the same even at 72 h. This is because hypovolemia in HS cause a decline in capillary pressure which in turn causes a protein shift from interstitium to plasma and draws in the fluid to compensate the intravascular loss. The first 24–48 h

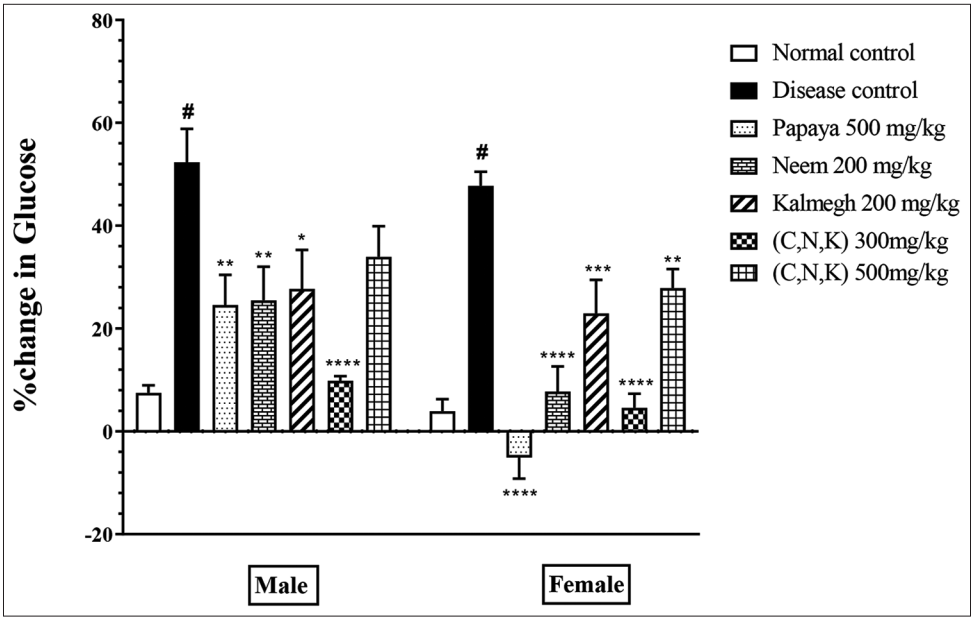

Figure 5: Graph depicts the % change in blood glucose level in all groups in 7 days. Data were assessed by one-way ANOVA followed by Dennett's multiple comparison tests. Values are represented as mean ± standard error of mean (n = 6). Asterisk denotes that treatment groups are statistically different from (#) disease control, ****P < 0.0001, ***P < 0.001,**P < 0.01, *P < 0.05

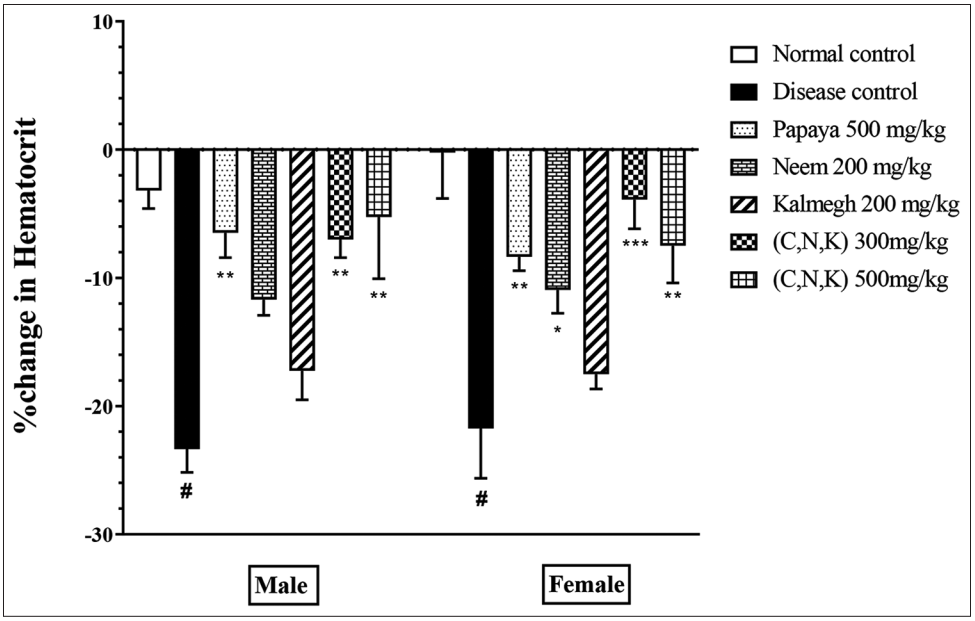

Figure 6: Graph depicts the % change in Hematocrit (HCT) level in all groups in 7 days. Data were assessed by one-way ANOVA followed by Dennett's multiple comparison tests. Values are represented as mean ± standard error of mean (n = 6). Asterisk denotes that treatment groups are statistically different from (#) disease control, ***P < 0.001, **P < 0.01, *P < 0.05

are therefore very critical as up to 2 L of fluid can shift to maintain the fluid balance and blood glucose also increases as a result of this osmotic effect as it draws in approximately 17 ml of fluid per 1 mmol/L rise in glucose to the vascular area.[26] However, after 72 h recovery has been seen and the levels became normal at the end of the experiment in all treatment groups with maximum effect has been seen in G7 but in DC they still remained higher [Figures 5 and 6].

Lactic acidosis, another major complication of HS is defined as when blood L-lactate level is greater than 5 mmol/L, blood pH is lesser than 7.35, and a plasma bicarbonate concentration is <20 mmol/L.[27] It happens as loss of blood volume also decreases the oxygenation to vital organs and in absence of ample amount of oxygen body shifts to anaerobic glycolytic condition which produces lactate. This can be very fatal. In this experiment, no such metabolic acidosis or alkalosis has been seen.

The choice of selecting 40% of EBV removal to induce HS was also appropriate as no animal died in G2 but at the same time 1 female and 1 male died in G3 which was created to check the survival post removal of 60% (lethal) of EBV.

It is evident from the experiment data that at the end of treatment period of 14 days with our polyherbal extracts we were able to induce HS condition in all animals. ELISA results of vasopressin, NF-ĸB, and IL-6 as well as rise in serum sodium, blood glucose, and fall in hematocrit at 24 h evidently indicate the presence of HS condition in all groups compared to normal control group, in which all of these parameters remained almost unchanged throughout the experiment. However, as the time progress, we have observed that animals in treatment groups have started recovering from HS condition which is clearly being reflected in the data measured at the end indicating that they have responded to our treatments, with the maximum effect is seen in G7 which is an equal combination of all three extracts at the dose of 300 mg/kg.

CONCLUSION

We can conclude that our polyherbal extracts have a protective effect in HS condition by keeping the metabolic and biochemical changes that happen after HS in check and thereby promoting the recovery and survival in HS condition. They can be used alone or in combination with conventional treatments to provide symptomatic relief and thereby improving the quality of life. However, we strongly emphasize on conducting more rigorously designed large scale pre-clinical studies and clinical trials to carry forward these observations.

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