Research Article

Action of Abhraka Bhasma on Hemato-Biochemical Profile of Hyperthermic Male Wistar Rats

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ABSTRACT

Abhraka Bhasma is an ayurvedic formulation with multiple benefits bringing about the regulation of metabolic system. Heat-stress is accompanied by several hematological and biochemical alterations which are signs of physiological responses of an individual against heat stress. The present study was designed to evaluate the ameliorative potentials of Abhraka Bhasma on hemato-biochemical alterations evoked in the serum of exogenously induced heat-stressed male rats. Thirty two male Wistar rats were divided into four groups. G1 acted as control, G2 comprised dosed-only group, G3 as heat-treated group and G4 were subjected to combined heat and Abhraka Bhasma treatment. Sahastraputi Abhraka Bhasma was used as the test drug. G1 were fed with honey, G2 were administered orally with Abhraka Bhasma with honey, G3 were subjected to heat stress at 43ºC for 1 h daily for thirty days; were also given honey and Group G4 animals were resorted to heat stress-cum-Abhraka Bhasma administration with honey as vehicle. The sera of rats were extracted on day 31 and was analysed for hematological and biochemical indices. Heat caused significant decrease ($P<0.05$) in Hemoglobin concentration, RBCs counts, MCHC and Platelet values in G3 animals which were restored to their normal values by Abhraka Bhasma administration in G4 rats. No significant alterations ($P>0.05$) were observed in PCV, MCV, MCH, RDW-CV and WBC counts in G3 animals when compared to controls. Alterations in the values of serum (TC, HDL, LDL, SGOT, SGPT and albumin) activities induced by heat-stress were revealed in G3 animals which were ameliorated by co-administration with Abhraka Bhasma in G4 animals. However, there was no significant ($P>0.05$) differences in the changes in the level of BUN, creatinine, Na+, K+, Cl-and triglyceride levels in G3 animals as compared to controls. Abhraka Bhasma potentiates ameliorative responses to heat-evoked hemato-biochemical alterations in hyperthermic male rats.

Keywords: Ameliorative, exogenously, Sahastraputi, vehicle, hyperthermic.

***Key message**: Abhraka Bhasma has an ameliorating potential on heat-evoked hemato-biochemical alterations in heat-stroke male rats. The feasibility and efficacy of this herbo-mineral formulation should be considered as a possible alternative to conventional treatment in heat stroke patients. Further randomized and controlled studies are warranted to evaluate Abhraka Bhasma as a novel strategy to reduce the morbidity and mortality associated with heat stroke.*

INTRODUCTION

Abhraka Bhasma, a therapeutic form of mica (Biotite), is a herbo-mineral product of Ayurvedic pharmaceutical. It is made with a delicate set of repetitive steps during which, the zero valent metal state is converted to a higher oxidation state. This enhances the therapeutic rationality as well as applicability of it. It is shown to be composed of nano size particles made by a stringent processing and incineration of biotite in *gaja puta* (pit) reducing them to finest ash. When the process is repeated 1000 times, the Abhraka Bhasma so obtained is called "*Sahastraputi*" Abhraka Bhasma. It is a reguvenative substance for immunity and improves cellular metabolism due to its potency. Reduced mica cures *rajayakshma* (Pulmonary Tuberculosis, Koch's Infection) and removes the derangement of the *tridoshas* (three basic physical energies) to establish its equipoise. [1] It should be administered in the dose of one '*ratti*' and gradually increased by one *ratti* till the dose becomes one '*tanka*'. [2]

Heat beyond the tolerance limit results in hyperthermia due to generation of heat shock proteins (HSPs) that is associated with systemic inflammation, multiorgan dysfunction, modulation of metabolism leading to rapid loss of homeostasis and encephalopathy. Onset of heat-related changes can arise after fifteen minutes of exposure proven in a study of heat tolerance in rats where the temperature of the rats was increased to 42°C for 15 min. This level of heat stress had induced tissue injury in heat stroke rats and caused damages to rat liver and intestinal tissues. [3] Heat intensity as an important factor influencing metabolism was not extensively studied in rats, particularly the extremely high ambient temperatures.

Hematological and biochemical disorders are ailments of major public health concern and little is known about mechanisms and pathological aspects of hyperthermia. [4] Combined with hematology and urinalysis, the biochemical profile, being an effective sensitive index of physiological and pathological changes, forms the data base for most diagnostic investigations providing important information about the internal environment of a given organism. Hematological parameters serve as baseline data as well as reference point for future surveys. [5] This necessitates the analysis of blood indices in

heat-stroke individuals to provide reliable information on their blood parameters. This would effectively treat the metabolic disorders, deficiencies and chronic dysfunctions resulting from heat stress.

In animals and humans, some physiological and biochemical adaptations could occur to protect essential cell functions against the heat stress and to permit a rapid recovery from hyperthermic damage. In order to explore the ameliorative effect of Abhraka Bhasma on alterations in hemato-biochemical indices evoked from heat stress, this study was conducted using rat as an experimental model. The results of this research will provide the knowledge of the characteristics of these early biomarkers in heat-stress as well as combined heat and Abhraka Bhasma treated rats. Furthermore, development of biochemical science can provide tools to strengthen its usage.

The current study aims to create greater awareness amongst the science fraternity as a whole to derive maximum benefit from the development of this pharmaceutical product and perhaps assist in the development of new treatment methodologies for people living in tropical and sub-tropical regions. A study on the effect of 10 and 20 putti Abhraka Bhasma on hemato-biochemical parameters has been documented but no literature of the Sahastraputi Abhraka Bhasma was observed and hence its selection. [6]

MATERIALS AND METHODS

Test Drug (Abhraka Bhasma)

Sahastraputi Abhraka Bhasma (subjected to 1000 putas) was procured from a renowned organization, Shree Dhootpapeshwar Ltd, Khetwadi, Mumbai, India, to study its potency on hematological and biochemical parameters in heat-stroke rats.

Dose determination

The dose was calculated by extrapolating the therapeutic dose of humans to rat on the basis of BSA ratio (conversion factor 0.018 for rats) by referring to the table of "Paget & Barnes" (Paget & Barnes, 1964). [7] Therapeutic dose of Sahastraputi Abhraka Bhasma is 15-60 mg of human per day.

Rat dose = (a) total clinical dose x (extrapolation factor (b)) 0.018 = (c) mg/200g of rat per day

Total clinical dose considered was 60 mg/kg of human per day

60 mg/kg of human per day x 0.018 = 1.08 mg/200g of rat/day

Or, 1.08 x 5 = 5.04 g/kg of rat/day

The actual dose administered to the animal was 1.08 mg/200g of rat/day;

Or, 5.04 g/kg of rat/day

Vehicle

The classical method of administration of the test compound Bhasma/Rasakalpas was adopted wherein honey was used as vehicle control (CCRAS). For the experiment 3 parts of honey was diluted with 4.5 parts de-ionized water and the volume of administration of this freshly prepared diluted honey was 0.5 ml.

Caring of animals

The current experiment was carried out on 32 healthy adult male albino Wistar rats (150 - 200 gms live body weight) which were housed in polypropylene cages. The ambient temperature maintained was 32+/- 2^0C with relative humidity 60-80% and kept on photoperiod of 12:12 h' light and dark conditions. Animals had free access to standard laboratory pellet diet and water was allowed *ad libitum*. The animals were kept under the principles of laboratory animal care and use as given in the reference study. Prior to starting of the experiment the animals were acclimatized for seven days. Before conducting the experiment, the animal ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) approved by Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

Experimental design

Rats were divided into four groups of eight rats each. After markings the division into groups were done as follows:

- Group G1; served as normal control (n=8)
- Group G2; treated with Abhraka Bhasma (n=8)
- Group G3; subjected to heat only (n=8)
- Group G4; simultaneously given heat and Abhraka Bhasma (n=8)

G3 and G4 were subjected to heat stress keeping the animals in a self designed incubator at 43^0C for 1 hr daily (in the early morning after overnight fast) and then returned to their normal cages (at 33^0 C) for 4 successive weeks. Rectal temperature was measured immediately after heat exposure, using a ^{0} C thermometer. After heat treatment the animals of G1 and G3 were fed orally with 0.5 ml of honey while animals of G2 and G4 were administered Abhraka Bhasma once daily (by oral gavage) using 0.5ml honey as a vehicle for 30 days. The rats were fed with basal diet 4 hrs after dosing to get maximum effect of the test drug (OECD guidelines).

Incubator

A specially self-designed 8 inch x 8 inch x 24 inch heating apparatus based on modern technology was fabricated and manufactured by Hindustan Apparatus Mfg. Co., Kurla, Mumbai. It was divided into three compartments, the inner chamber being made of stainless steel and outer of mild steel with powder coating (Fig. 1). Inside the chamber a safety thermostat, that cuts off if the incubator overheats to maintain a constant temperature

of 43°C, was strategically placed along with a blower and an air ventilator at the top. A state-of-the-art microprocessor that can be programmed to maintain different temperatures for varying intervals was also installed in it. The inner chamber has an electrical heater mounted on an inside wall and covered by a perforated protective panel. Mounted in the chamber wall just above the heater is a fan whose motor extends through the chamber wall into the control area of the case and whose blades face inward.

Figure 1: Incubator to maintain optimal temperature of 43°C and humidity of the atmosphere inside the compartments.

Hemato-biochemical analysis

After administration of a drug to treat the heat related diseases, differences in the biochemical and hematological variables will indicate a response of an individual to the adverse physiological stress. The effect of heat can be observed after short time exposure but this case does not hold true for Abhraka Bhasma. To explore the possible effect of slowly affecting trial drug on blood profile, groups were continuously treated with the test drug for 30 days. Next day six animals from each group were anesthetized using ketamine hydrochloride 24 mg/kg body weight. 3-4 ml of blood was collected from each animal through retro-orbital plexus. About 1 ml of blood of each animal was collected in K_3EDTA tube (anticoagulating tube) and 2-3 ml in Gel Vacutainer assay tube .

The K_3EDTA samples were immediately subjected to analysis to determine the complete hemogram using automated Cellenium 19 Hematology Cell Counter. After complete hemolysis, the serum in the vacutainer tube was obtained through centrifugation at 3,000 rpm for 10 min. The serum of all the animals was collected and biochemical parameters were analysed on VITROS DT 60 II Dry Chemistry Analyser.

Statistical analysis

The results of hematological and biochemical analysis were presented as the mean ± SD of mean of six rats per group and are also rounded off to nearest digit. Statistix 0.9, version 3, Beta was used for descriptive statistics and differences between groups were analyzed by Analysis Of Variance (ANOVA) calculator. [8] Differences were considered to be of statistical significance at an error probability of less than 0.05 ($P<0.05$).

RESULTS

Hematological parameters analysis

Heat caused significant effects on some of the hematological indices of the experimental animals. The effects of administration of Abhraka Bhasma at dose level of 5.04 g/kg bw on haematological parameters of male albino rats on day 31 from the start of the experiment are shown in Table 1. Hematologic Profile Analysis of the experimental animals showed that administration of the the drug produced significant alterations ($P<0.05$) in the Hb, RBC, PCV, WBC and platelet count of the Abhraka-cum-heat treated (G4) animals in comparison to G3 rats at the end of the investigation period (Table 2).

Table 1: Sera concentration of hematological indices in male Wistar rats on day 31 after the start of the experiment

Hematological indices	Experimental groups			
	Group G1 (Control + Vehicle)	Group G2 (Abhraka + Vehicle)	Group G3 (Heat + Vehicle)	Group G4 (Heat + Abhraka + Vehicle)
Haemoglobin(Hb)	16.57 ± 0.63^{a}	17.12 ± 1.35^{b}	15.77 ± 0.59^{abc}	16.90 ± 0.66^{c}
Packed Cell Volume (PCV)	48.60 ± 1.86^{a}	48.73 ± 2.1^{b}	45.48 ± 2.04^{abc}	48.97 ± 0.91^{c}
RBC Count	9.21 ± 0.32^{a}	9.58 ± 0.38^{b}	8.25 ± 0.77^{abc}	9.12 ± 0.52^{c}
MCV	52.18 ± 2.06	50.90 ± 0.89	51.15 ± 1.25	51.97 ± 1.35
MCH	18.18 ± 0.72	18.28 ± 0.43	18.20 ± 0.46	18.60 ± 0.80
Mean Corpuscular Haemoglobin Concentration (MCHC)	34.85 ± 0.47^{ab}	36.03 ± 0.73^{a}	35.81 ± 0.53^{b}	35.53 ± 0.74
RDW-CV	16.30 ± 0.74	15.57 ± 0.88	15.90 ± 0.97	16.45 ± 0.46
Total White Blood Cell Counts (WBC)	12900 ± 3183.7	12300 ± 1718.1	10950 ± 3462.2^{a}	16950 ± 5194.1^{a}
Platelets Count	963667 ± 76954^{abc}	821333 ± 122379^{a}	718667 ± 122181^{bd}	872167 ± 35863^{cd}

*Values are mean of 6 replicates ± SD; **Each parameter is significantly different at P<0.05; ***Values with same superscript are significant to each other.

The Hb concentration in the Abhraka-cum-heat treated group was significantly higher (*P*<0.05) compared to heat-treated group. Interestingly, heat-treated groups also showed significant difference (*P*<0.05) in the Hb count as compared to controls (Table 1), the value being towards the lower side. The Abhraka-only group of animals showed a good level of haemoglobin concentration. The RBC concentration in the heat-treated group was significantly lower (*P*<0.05) as compared to the rest of the experimental animals. The RBC concentration in the control group was marginally higher than those in the Abhraka-heat treated group but it was not significant (*P*>0.05). The mean RBC concentration was notably higher in Abhraka-only rats than the rest of the experimental animals (Table 1).

The data shows a significant decrease (*P*<0.05) in the packed cell volume of the heat-treated groups as compared to rest of the three groups which did not show any significant differences in PCV concentration among themselves. The highest PCV value was noted in Abhraka-heat treated group and lowest in heat-treated group. Interestingly, G1, G2 and G4 animals showed marginal difference in packed cell volume level amongst each other. The values obtained for MCV, MCH and RDW-CV were not significantly different (*P*>0.05) between the groups (Table 1). The MCHC count in the Abhraka-only treated group was significantly elevated as compared to the control group. The MCHC count in the heat-treated group did not significantly vary (*P*>0.05) from those obtained in the Abhraka-heat treated groups but showed significant alterations from that of controls.

There was a significant increase (*P*<0.05) in the white blood cell count of the Abhraka Bhasma-cum-heat treated group as compared to the heat-treated group. However, rest of the animals did not show any significant differences in WBC count amongst themselves. Platelet count decreased significantly (*P*<0.05) in heat-treated animals when compared to Abhraka-heat treated rats and controls. The platelet count increased notably (*P*<0.05) following the administration of the drug in heat-cum-Abhraka treated G4 rats. The platelet count of the controls were markedly high as compared to the rest of the animals (Table 1).

Table 2: *P* values of the four experimental groups of male Wistar rats as recorded on day 31 from the start of the experiment

Hematological Indices	P Values					
	G1-G2	G1-G3	G1-G4	G2-G3	G2-G4	G3-G4
Haemoglobin (Hb)	0.387	0.047	0.396	0.049	0.727	0.011
RBC Count	0.098	0.018	0.726	0.004	0.111	0.045
Packed Cell Volume (PCV)	0.912	0.02	0.671	0.045	0.802	0.003
MCV	0.193	0.32	0.839	0.698	0.136	0.301
MCH	0.776	0.955	0.362	0.762	0.408	0.313
Mean Corpuscular Haemoglobin Concentration (MCHC)	0.008	0.007	0.087	0.581	0.266	0.453
RDW-CV	0.153	0.443	0.685	0.551	0.055	0.238
Total White Blood Cell Counts (WBC)	0.693	0.371	0.134	0.465	0.064	0.044
Platelets Count	0.037	0.002	0.025	0.177	0.352	0.014

**P<0.05 is considered to be significant*

Biochemical Profile Analyses

Results obtained for the effect of Abhraka Bhasma on the biochemical parameters in experimental rats are depicted in Table 3. Statistically, there was no significant (P>0.05) difference in urea (BUN), creatnine, sodium, potassium, and chloride levels between all the animals when compared to each other.

An approach to value of total cholesterol showed that heat affects the cholesterol content in blood by notably elevating it while administration of Abhraka Bhasma administration showed marked reduction in the value of cholesterol content in G4 animals. However, Abhraka Bhasma significantly (P<0.05) decreased the elevated levels of LDL-C which was altered due to heat treatment. The drug raised VLDL-C and HDL-C levels to higher level which were significantly decreased (P<0.05) due to heat exposure in G4 (Table 2). Heat as well as Abhraka failed to produce any marked changes in the serum triglyceride content.

The liver enzyme activities are shown in Table 3. There was a significant decrease (P<0.05) in the liver marker enzymes ALT and AST but significant increase (P<0.05) in ALP and AcP in the Abhraka-heat treated groups compared with the heat-treated animals. There was statistical difference (*P*<0.05) in ALT activity between experimental groups other than between control and

Abhraka-heat treated ones (Table 4). Maximum ALT activity was observed in heat-treated groups on 31st day of the start of the experiment. Though the mean value of ALT in G4 rats decreased inspite of giving heat-stress, it remained higher than that of the Abhraka-only group but lower than that of the controls. Significantly (P<0.05) increased AST values were observed in heat-treated animals. In heat-exposed group G3, the ALP activity was significantly higher as compared to values of rest of the groups of animals (Table 4). Results presented in Table 3 show that the Abhraka Bhasma administration was found effective to restore the normal activity of ALT and AST enzyme.

Table 3: Sera concentration of biochemical indices in male Wistar rats on day 31 after the start of the experiment

Biochemical Indices	Treatment Groups			
	Group G1 (Control + Vehicle)	Group G2 (Abhraka + Vehicle)	Group G3 (Heat + Vehicle)	Group G4 (Heat + Abhraka + Vehicle)
BUN (Blood Urea Nitrogen)	16.333 ± 3.0768	17.333±3.4448	15.333 ± 2.5820	16.833±1.4720
Creatinine	0.7167±0.0753	0.7667 ± 0.1211	1.0667±0.4719	0.6833 ± 0.0983
Sodium	143.83 ± 10.028	147.67 ± 9.7502	135.83 ± 11.669	147.00±10.469
Potassium	7.2000±0.9338	6.8167 ± 0.4916	7.7333±0.9092	7.0667 ± 0.4546
Chloride	104.00± 8.2462	102.50 ± 10.173	104.50 ± 9.6488	106.67±11.039
Total Cholesterol	128.67 ±10.191^{a}	125.83 ± 5.7764^{bc}	145.50 ± 10.015^{abd}	133.00±4.8990^{cd}
HDL Cholestrol	49.167 ± 8.4004^{a}	54.167 ± 6.4317^{b}	36.667±7.1461^{abc}	46.667±5.2409^{c}
Low density lipoprotein cholesterol	63.000± 16.248^{a}	40.500 ± 19.987^{b}	86.000±10.450^{abc}	57.333 ± 17.862^{c}
Very low density cholesterol	16.500 ± 5.3944^{a}	28.500 ± 13.248	16.167±4.3551^{b}	30.667 ± 11.039^{ab}
Triglycerides	160.17±52.849	162.67±35.001	160.67±63.349	155.17±43.041
SGOT	246.17±29.600^{ab}	202.50 ± 21.154^{acd}	300.33 ± 44.572^{bce}	239.83 ± 22.004^{de}
SGPT	191.50 ± 17.717^{a}	182.50 ± 16.956^{b}	235.83 ± 35.414^{abc}	172.50±35.602^{c}
Alkaline Phosphatase (ALP)	358.00 ± 84.423	434.17±61.516^{a}	296.33 ± 45.746ab	407.83 ± 78.563^{b}
Acid Phosphatases (AcP)	8.9167±1.9702	10.050 ± 1.9552^{a}	7.4167±1.4359^{a}	9.0000 ± 1.9860
Total Proteins	6.8667 ± 0.6772	7.0500 ± 0.7314	6.7000 ± 0.3688^{a}	7.2500 ± 0.3017^{a}
Albumin	3.8333±0.2582^{a}	3.9333 ±0.4502^{b}	3.0333 ± 0.6501^{abc}	4.0667±0.2733^{c}

**Values are mean of 6 replicates ± SD; **Each parameter is significantly different at P<0.05; ***Values with same superscript are significant to each other.*

Table 4: *P* values of the four experimental groups of male Wistar rats as recorded on day 31 from the start of the experiment; P<0.05

Biochemical Indices	P Values					
	G1-G2	G1-G3	G1-G4	G2-G3	G2-G4	G3-G4
BUN (Blood Urea Nitrogen)	0.607	0.556	0.727	0.281	0.75	0.244
Creatinine	0.416	0.102	0.462	0.161	0.188	0.075
Sodium	0.517	0.232	0.604	0.086	0.911	0.112
Potassium	0.397	0.342	0.764	0.056	0.379	0.142
Chloride	0.785	0.925	0.645	0.734	0.512	0.725
Total Cholesterol	0.566	0.016	0.37	0.002	0.043	0.043
HDL Cholestrol	0.274	0.02	0.55	0.001	0.051	0.02
Low density lipoprotein cholesterol (LDL-C)	0.058	0.015	0.578	0.001	0.155	0.007
Very low density cholesterol (VLDL-C)	0.067	0.909	0.018	0.056	0.764	0.014
Triglycerides	0.925	0.988	0.861	0.947	0.747	0.864
SGOT	0.015	0.033	0.683	0.001	0.013	0.014
SGPT	0.39	0.021	0.269	0.008	0.548	0.011
Alkaline Phosphatase (ALP)	0.104	0.147	0.307	0.001	0.532	0.013
Acid Phosphatases (AcP)	0.343	0.163	0.946	0.025	0.382	0.146
Total Proteins	0.668	0.602	0.239	0.32	0.549	0.018
Albumin	0.648	0.019	0.148	0.019	0.528	0.005

**P <0.05 is considered to be significant*

Significant (*P*<0.05) increase was also noted in serum ALP activities of G4 animals where Abhraka Bhasma ameliorated the alterations produced in alkaline phosphatase enzyme levels due to heat exposure on 31st day of the experiment . Notably high mean ALP values were observed in G2 animals where only Abhraka Bhasma was fed orally to the rats . However, G3 animals showed significant difference from G2 animals but not from controls. Repeated intake of Abhraka Bhasma post heat treatment for 30 days significantly increased (P<0.05) serum AcPase activity in G4 animals (Table 4). The ALP concentration was significantly (*P*<0.05) reduced in the heat-treated group and significantly elevated in the Abhraka Bhasma-heat treated group when compared to each other as illustrated in Table 4. The ALP value of heat-treated rats was significantly different from controls and Abhraka-only given rats as well. However, AcP values of heat-treated animals showed no significant changes from controls and Abhraka-cum-heat treated rats but differed from Abhraka-only group. The highest mean ALP and AcP indices were observed in Abhraka-cum—heat stressed animals and Abhraka-only rats respectively.

We also observed a significant decrease (*P*<0.05) in the total protein level of the heat-stressed animals as compared to the animals given both heat and Abhraka treatment but showed no significant differences from controls and Abhraka-only rats (Table 4). There was a significant decrease (*P*<0.05) in the albumin level of the heat-treated rats when compared either of the controls, Abhraka-only or Abhraka-heat stressed group of the experimental animals. However, the other three groups did not show any significant differences amongst themselves. The highest albumin level was observed in Abhraka-heat treated group of animals. The mean level of albumin values of heat-treated animals were exceptionally lower than the rest of the animals (Table 3).

DISCUSSION

The extreme limits of core temperatures (Tc) compatible with life appear to fall above 24°C and below 45.6^{0}C.[9] Marked hyperthermia (up to 45ºC [113ºF]) occurs minutes to hours later; core body temperature tends to rise 1ºC every 5 to 60 minutes.[10] The heterogeneous and ubiquitous nature of heat induced illnesses has presented a timeless challenge to healthcare providers. Heat stroke is a life-threatening disease characterized by hyperpyrexia (elevated core body temperature exceeding 40°C), a variety of tachyarrhythmias, acidosis, distributive shock and coagulopathy commonly occur during heatstroke. There is growing evidence that endotoxemia and cytokines may be implicated in its pathogenesis. In a 2002 review in the NEJM, the definition was expanded to include an understanding of the pathophysiology of this condition.

Literature survey reveals that short term studies on hemato-biochemical parameters have been conducted in sheep, goat, broilers and chickens but no long term studies done on blood parameters in rats or humans. However, long term studies up to 90 days have been done to assess the effect of hyperthermia on testes of rat. Assessment of haematological and biochemical parameters can be used to determine the extent of deleterious effects of unfavourable conditions on the blood. Literature has shown that ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters. The various biochemical and haematological parameters investigated in this study are useful indices of evaluating the blood relating functions of Abhraka Bhasma.

The results of our study indicate that there were significant differences in hemoglobin and RBC content but not in MCV and MCH content between all the groups. Thermoregulatory shifts due to heat-exposure is known to decrease the life span of erythrocytes was found by Meyerstein, 1975 and the red cell volume recorded by Jones, 1976, while increase the packed cell volume and the plasma loss due to body dehydration was observed by Khalil and Kotby, 1982.[11] The lower levels of hemoglobin and RBC due to heat-stress may be an indication that imbalance occurs between the rate of production (erythropoiesis) and destruction of the blood due to heat stress which is corrected by administration of Abhraka Bhasma in G4 animals. Significant increase in the volume of the erythrocytes, as well as hemoglobin (p<0.05) of G4 animals was probably due to adequate availability of aminoacids for the synthesis of the proteins that make up this cellular type i.e. erythropoietin production and/or secretion. This is perhaps as an adaptive mechanism, triggered by Abhraka Bhasma, to the heat-stress. The lack of significant change in the MCV in all the groups shows that heat and Abhraka Bhasma does not alter erythrocyte size.

Regarding PCV levels the findings in our study In another investigation with clinical trials, subjects were treated at 50^{0}C for 5 min. Dramatic elevations in PCV and MCV values were observed in thermally treated group.[12] It was thought that decreased PCV levels were due to an increase rate of breakdown of red cells which is prevented by administration of G4 rats with Abhraka Bhasma.

Our study recorded significant increase in MCHC in heat-treated group as compared to controls as well as G1 and G2 animals. However, no changes were observed regarding MCH and MCV concentration. In another experiment, heatstroke was induced by partially submerging the animals in the three experimental groups into waterbaths at supranormal temperatures of 41^0, 42^0 and 43°C. The results indicated that the mean corpuscular haemoglobin concentration (MCHC) were slightly increased during hyperthermia.[13] However, no significant effect of Abhraka Bhasma was found on MCHC concentration suggesting that the trial drug does not affect this parameter at all.

No significant differences were found while analyzing RDW-CV parameter amongst all the animals. This rules

out the possibility of occurrence of anisocytosis (increased variation in red cell size).

Though there was no significant difference (P>0.05) in heat-treated rats as compared to controls the mean leucocyte count was higher in heat-cum-Abhraka than heat-treated ones. The increase in WBC count in Abhraka Bhasma treated rats may be as a result of increased secretion of the leukocytes due to the activation of the animal's defense mechanism and immune system against heat stress and/or decreased rate of removal of them. Furthermore, the protective effect of Abhraka Bhasma on WBC may be attributed to their antioxidant properties either as a vital component of enzymatic antioxidant Cu-Zn SOD or due to their ability to antagonize the catalytic properties of the redox-active transition metals iron and copper with respect to the promotion of hydroxyl radicals formation from hydrogen peroxide and superoxide.[14] From the observed significant elevated values of WBC in G4 animals, it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes. The study showed that doses of Abhraka Bhasma, when given for a longer time to heat-stroke rats, led to a significant increase in white blood cell (WBC) count indicating a boost in the immune system in them as compared to controls . Such effects may also be due to increase in vascular permeability.

Abhraka Bhasma supplementation has been shown by the present study to normalize the heat-evoked thrombocytopenia. Although, the mechanism responsible for this is not clear, it may be due to splenic sequestration which is trapping platelets or decreased production of thrombopoietin by the liver due to liver failure. Increased destruction due to immunologic infections, increased utilization due to disseminated intra-vascular coagulation or impaired production due to anaemia and/or infections like sepsis can also be the cause of depressed platelet count. [15] Administration of Abhraka Bhasma post heat treatment ameliorates these alterations and helps in maintainance of homeostasis. A study on the ambiguities of heat stress along with the administration of Abhraka Bhasma on spleen and liver is called for which would substantiate the role of this trial drug in maintaining homeostasis. The significant thrombocytosis in control animals as compared to other groups of animals implicates that the platelet count was may be abnormally high due to active production of platelets within bone marrow cells or due to decreased removal of platelets from the blood by spleen.

The results of the current study implicate that there exists no significant difference in BUN, creatinine, sodium, potassium and chloride values between all the experimental group of animal.. No significant change in blood urea indicates normal tissue protein catabolism, controlled breakdown of blood protein and sufficient excretion of urea. No significant changes in creatinine amongst the experimental animals is an indicator of normal glomerular filtration of creatinine. One could expect to see hyponatremia from dehydration and water ingestion and hypokalemia thought to be associated with sweat losses from heat exposure.[16] Interestingly the levels of these electrolytes were brought to near normal levels by increasing sodium and potassium reabsorption. No significant alterations in chloride ion in sera of rats rules out the possibility of alkalosis and acidosis, that is the pH balance in rat's blood is well maintained even after 30 days of heat exposure at 43^0C.

Heat stress in the present study resulted in elevation of serum cholesterol levels. High cholesterol level accounts for the increase in levels of transaminases, marker enzymes important in heart and liver damage.[17] High cholesterol related to heat exposure suggests an altered lipid metabolism due to renal and liver dysfunction . Our results reveal that the Abhraka Bhasma is associated with decrease in ranges of circulatory cholesterol as compared to heat-treated rats . The hypocholesterolemic effect may be accompanied by a increase in cholesterol catabolism, inhibit HMGcoA reductase enzyme and/ or excitation of lipoprotein activity. Hence further study for its use for reduction of CVD risks is called for.

The hypolipidemic effects of Abhraka Bhasma found in current study may be related to its antioxidant property. Furthermore, hepatic normalization by administration of Abhraka Bhasma despite the continuation of heat exposure reaffirms that the trial drug is a novel drug in managing hepatic dysfunctions. However, the mechanism underlying this effect of Abhraka Bhasma needs further investigation.

No significant alterations in serum triglyceride level of the experimental rats were observed. This implicates that lipoprotein lipases, special enzymes on the walls of the blood vessels, have not been affected or denatured due to heat exposure in G3 or G4 animals and hence working unarduously. However, significantly decreased sera concentrations of LDL but increase in high-density lipoproteins by Abhraka Bhasma administration in heat combined with Abhraka Bhasma group of animals is worth mentioning.

Since in our study we found no significant difference (P>0.05) between heat-treated rats and controls hepatic toxicity due to depletion in total protein content is ruled out. However, the slight increase of total serum protein in heat-cum-Abhraka treated rats as compared to only heat-treated animals is caused by the elevation of more of serum albumin level as compared to serum globulin level. This is remarkably noted in G4 animals where Abhraka Bhasma administration significantly increased albumin level. Thus it can be proposed that Abhraka Bhasma aids in remarkably increasing the albumin level after heat-treatment. Albumin is synthesized in the liver hence, it is possible that initial inflammation of the liver due to heat stress may decrease its production and then Abhraka Bhasma influences the production of this globular protein in G4 rats. As the major function of albumin is to establish the plasma colloidosmotic

pressure so as to prevent plasma filtration through the capillaries edema possibly did not form in heat-treated animals because the decrease of this protein was slight.[18] Rats seldom develop edema, which forms only when the amount of protein in the food is largely reduced, as demonstrated by Enwonwu & Sreebny, 1970 which supplied a 0.5%-protein chow to Sprague-Dawley rats and observed edema in 25% of the animals after 10 weeks of treatment. Notable increase in sera total protein level in G4 animals inspite of heat treatment suggests steroidal function of the drug.

A significantly lower level of ALP was also encountered in this study in the heat-treated rats as compared to dosed and heat-dosed animals however, not when compared to controls. Abeni et al. (2007) confirmed this enzyme as a quick and reliable blood-marker for heat stress. Niu et al. (2007) observed a similar reduction in serum ALP, but increases in serum creatinine, urea nitrogen, aspartate aminotranferase, alanine aminotransferase in rats exposed to 43ºC for 20 min. Alkaline Phosphatases are a group of enzymes found primarily the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). What is measured in the blood is the total amount of alkaline phosphatases released from these tissues into the blood. The higher level of ALP in the blood serum of G2 and G4 animals might be due to slightly higher activity of these isoenzymes due to trial drug administration. However, investigation on thyroid, parathyroid and hypophosphotasia and vitamin C and B_6 will definitely throw some light on ALP activities in rat or humans.

No significant changes were observed in AcPase activity in all the animals except G2 and G3 animals where $P<0.05$. A quantitative histochemical assay has been used to investigate the effects of hyperthermia on lysosomal acid phosphatase activity in mouse spleen. In an experiment of heat exposure, animals were kept at 32^0C for 3 weeks. The AcPase enzyme activity assayed in pituitary and hypothalamic tissues in the heat-exposed rats showed no change in the activity.[19] This result is in consistent with our study. In contrast to our study are the results of an experiment where twenty-four Wistar strain rats were kept at 34°C for 5 weeks. Acp-ase activities were found to be lower in these animals. [20] Effects of hot, The increase in the mean acid phosphatase activities in G2 animals as compared to G3 rats may be as a result of activation of the enzyme or repair of lysosomal membrane which might be damaged due to heat stress and consequently preventing the leakage of the enzymes from the lysosome into the extracellular fluid. We considered it more of enzyme activation since there was corresponding increase in the acid phosphatase activity in the serum of Abhraka-only rats. This slight higher activation of AcPase activity was not visible in G4 animals hence it did not show significant difference from heat-stressed rats.

Heat stroke was found to be associated with increase in concentrations of serum transaminases may be due to inflammation of hepatocytes in G3 rats. Release of enzymes 144 by the parenchymatous cells from the liver which causes above-normal levels of AST and ALT in blood are indicative of hepatic dysfunction.[21] Marked reduction in serum transaminases revealed in G4 rats indicated that Abhraka Bhasma given orally at the doses and duration investigated in this study possibly would be lowering the level of transaminases after heat treatment by remedying cell injury occurring in advance of gross hepatic pathology. The trial drug possibly improve hepatic functions by making conditions conducive so that no changes in membrane permeability of hepatocytes can cause a generalized release of enzymes from the cell. Potent antioxidant and free radical scavenging activities of Abhraka Bhasma could counteract the free radical generation responsible for heat-induced oxidative stress and may contribute to the very high potency of Abhraka Bhasma. The significant difference in AST indice between control and Abhraka-only as well as Abhraka-heat treated groups indicates that Abhraka Bhasma is very effective in controlling negative alterations in AST values.

These results in totality confirm the use of Abhraka Bhasma as safe and potent traditional medicine capable of normalizing other biochemical and hematological abnormalities associated with heat stress. This herbo-mineral formulation thus could be prescribed as adjunct to other drug therapies used currently for treating heat stroke individuals. The administration of Abhraka Bhasma may be bringing about significant changes in the structure, function, metabolic transformation and concentration of biomolecules, enzymes and even metabolic pathways. The drug in addition to its other actions may favour an improvement in renal function. Abhraka Bhasma may be working by the alteration in taurine homeostasis a hypothermic modulator during heat stroke. Ahraka Bhasma possibly prevents a selective loss of compensatory splanchnic vasoconstriction which trigger cascade of events that characterize heat stroke. Abhraka Bhasma may suppress the direct thermal effect of heat on tissue cells, which is likely to occur at Tc 42°C, preventing proteins to get denatured. Enzymatic and genetic study of Abhraka Bhasma will go a long way in curing heat-stress in affected individuals.

A majority of the world's domestic animal populations are in regions where environmental stressors, including heat insult, adversely influence productive efficiency. Currently there is a paucity of data supporting the efficacy of current clinical treatments for heat stroke and there is a dire need for more efficacious therapeutics. The use of novel biotechnologies, including radiotelemetry, genomic, and proteomic analyses, will be critical in advancing our knowledge of heat stroke pathophysiology. These technologies combined with novel in vivo, in vitro, and in silico models will be critical to enhancing our understanding of the SIRS and developing of novel strategies to reduce the morbidity and mortality

associated with heat stroke. This study proved that Abhraka Bhasma can be considered as additional drug in suppressing clinical symptoms of heat-stroke in humans and animals.

CONCLUSION

In conclusion, this study implicates that Abhraka Bhasma have no adverse effects on normal serum biochemical profiles in heat-stressed conditions. Hence it can be tested for their beneficial effects in other stressful conditions too. The present study has shown that Abhraka Bhasma has an ameliorative effect on heat-induced alterations in hematological and biochemical parameters in rats by extension man. Therefore, it can be proposed that individuals and animals who are constantly exposed to heat-stroke may benefit from toxicity protection offered by Abhraka Bhasma supplementation. Abhraka Bhasma has an ameliorating potential on heat-evoked hemato-biochemical alterations in heat-stroke male rats. The feasibility and efficacy of this herbo-mineral formulation should be considered as a possible alternative to conventional treatment in heat stroke patients. Further randomized and controlled studies are warranted to evaluate Abhraka Bhasma as a novel strategy to reduce the morbidity and mortality associated with heat stroke.

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