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STUDIES ON THE EFFECTS OF ABHRAK BHASM ON HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF WISTAR RATS

Ayurveda

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ABSTRACT

The Ayurveda is the traditional system of medicine which is been used by Indian's. The science of life is the basic meaning of the word Ayurveda. The method of practicing had been a trusted and tested method in treating the illness. Abhrak Bhasma, an amorphous powder drug, is prepared by treating biotite (mica) with the juices of a number of reconstituent plants that make it a powerful cellular regenerator. It is commonly used ayurvedic drug against many diseases including hepatitis (hepatoprotective). It is also used as nervine tonic and is widely used in respiratory tract infections and megaloblastic anemia. It contains iron, magnesium, potassium, calcium and aluminium in trace amounts. It also contains silicates of iron, magnesium and aluminium.In this study a total of 30 adult albino Wistar rats of approximately 4 months age (approx. 150-200 gms) of either sex selected randomly to see the effect of Abhrak bhasm, an ayurvedic drug on hematological and biochemical profile of Wistar rats.The rats were weighed, marked and divided into 5 groups each consisting of six animals. In normal control group(Group E), no drug was administered and in rest of the four treated groups(Group-A,B,C,D), Abhrak bhasm @ 36 mg/kg B.wt. was administered orally once in each rat.All blood samples were collected from jugular veins by using 30 gauze needle in EDTA (Ethylene diamine tetraacetic acid) vials and in plain vials for hematological and biochemical investigations respectively at 0.5 hour,2 hours, 6 hours, 24 hours and at the end of study in Groups A, B, C, D and E respectively.A significant increase in Neutrophils percentage and a significant decrease in Lymphocytes percentage were observed in both group B and group C i.e. at 2 hours and 6 hours of drug administration respectively, as compared to control group. In two cases, in group B and group C i.e.at 2 hours and 6 hours of Abhrak bhasm drug administration, hypersegmented neutrophils were seen in blood smear. In three cases, two in group B and one in group D i.e. at 2 hours and 24 hours of drug administration, polychromasia were seen. The findings in the present study indicate the erythropoeisis stimulant action and also a significant phagocytic activity i.e. immunomodulatory activity of Sahastraputi Abhrak Bhasma drug. It consolidated the fact that Sahastraputi Abhrak Bhasma is highly regarded as Rasayan, thus leading to scope for exploring the mechanism of its activity through more animal and clinical studies.

KEYWORDS

Ayurvedic drug, Sahastraputi Abhrak Bhasma, Wistar rats, Immunomodulatory.

INTRODUCTION:-

The Ayurveda is the traditional system of medicine which is been used by Indian's. The science of life is the basic meaning of the word Ayurveda. The method of practicing had been a trusted and tested method in treating the illness. In Charaka and Sushruta age the treatment to illness was limited to the usage of medicinal herbs, but later in the 8th century AD Nagrjuna, an Indian alchemist suggest the combination therapy with the heavy metals and herbs as a medicinal agent. Based on his expertise he came to know that the formulation is so quick in action and effective in small dose and showed longer stability which later became the importance of the combination therapy.Mineral drugs (Rasa Yoga) formulated by the branch of ayurvedic medicine known as "Rasashastra" are the base of Ayurvedic Metallurgy. For the proper functioning of the human body specific amount of the heavy metals plays a crucial role as per the Veda. In Rasashastra, the mineralogy which is termed as "dhatus" and "updhatus plays a crucial role in the maintenance of the biological system. The lack of essential heavy metals on specific tissue on long term basis will cause undesirable problem or diseases to the body. The need for the required amount of the heavy metals in specific concentration is so essential to maintain the metabolic activity of the human system e.g.Hg,Au,Ag,Fe, Zn,Cu,Pb etc. Deficiency or excess amount of intake leads to imbalance in the biological system causes catabolic and anabolic grievance. The states of an equilibrium level of metals contribute the stronger immunity. The change in the balance of these metals could lead to fatal disease and affect the normal immune system **(Gopinath, H. and Shivashankar, M.2016, p.2374).**

The use of heavy metals in the formulation of Ayurvedic medicine is in practice for ages with potent efficacy and safety, nevertheless there are concerns over the toxicity of the heavy metals used in it **(Kumar, G. and Gupta, Y. K., 2012, p.569).** Abhrak Bhasma, an amorphous powder drug, is prepared by treating biotite (mica) with the juices of a number of reconstituent plants that make it a powerful cellular regenerator. It is commonly used ayurvedic drug against many diseases including hepatitis (hepatoprotective). It is also used as nervine tonic and is widely used in respiratory tract infections and megaloblasticanemia. It contains iron, magnesium, potassium, calcium and aluminium in trace amounts. It also contains silicates of iron, magnesium and aluminium (**Pal, D.K. et. al., 2014, p.10**).

Abhrak Bhasma is like heavenly medicine and it destroys vata (air), pitta (fire) and disease ksaya (phthisis).Since ancient times Abhrakbhasma were used as an Ayurvedic medicine to cure various diseases such as Asthama, Tuberculosis, Cancer, Hepatic dysfunction, Diabetes etc. The toxicity of the minerals has been shown only when excess dose of material is given. Abhrakbhasma the oxide form of the minerals are poorly soluble and hence not in free form which has been shown by acute toxicity study 5000 mg/kg B.wt. is safe in the wistar rats of both sex **(Gopinath, H. and Shivashankar, M.,2016, p.2375)**. A number of Rasashastra texts including Rasa Tarangini advocate the fact that Abhrak when given 20-100 Putas, helps in Rognivrutti (curing diseases) but when Putas are increased from 100-1000, it acts like a Rasayan (prevents disease) **(Tamhankar et al.,2015,p.22).**

Allopathic medicines involve rigorous research and clinical trials before they are allowed to be marketed and used. In contrast, traditional medicines rarely go through such procedures and are made freely available in the market without any stringent regulations. Though, various traditional literatures like Charaksamhita, Susruthasamhita that describe the use, mode and method of feeding exist, these barely address to the mechanism of action at physiological and molecular levels **(Subedi, et.al.,2017,p.238).** The present study is designed to see the effect of Abhrak bhasm on different hematological and biochemical parameters in Wistar rats.

MATERIALS AND METHODS:-

Animals:-In this study a total of 30 adult albino Wistar rats of

approximately 4 months age (approx. 150-200 gms) of either sex selected randomly to see the effect of Abhrak bhasm, an ayurvedic drug on hematological and biochemical profile of Wistar rats. Rats were procured from the Animal Husbandry department of UP University of Medical Sciences(UPUMS), Saifai, Etawah**(CPCSEA registration no.-1087/GO/ReBi/S/ReBi/L/CPCSEA)** and the experiment was started after taking the approval from Institutional Animal Ethic's Committee **(IAEC protocol approval no.-IAEC/02/AH/2019-20 dated 07/03/2020)**of UPUMS. Rats were housed under normal conditions and received a standard food and water ad libitum. An hour prior to experimentation, animals were brought and kept in the experimental room. The rats were weighed, marked and divided into 5 groups each consisting of six animals. In normal control group(Group E), no drug was administered and in rest of the four treated groups(Group-A,B,C,D), Abhrak bhasm @ 36 mg/kg B.wt. was administered orally once in each ra **(Table-1).**

The behavior and the physical appearance of animals such as exploratory activity, cuddling and sleeping, consumption of water and food, appearance of fur, cleanliness of the coat and body, appearance of faeces were observed throughout the study to see whether there are any deviations from the normal behavior of rats.

Table:-1

S.No.	Group	Treatment	No. of animals	Sample collection time after drug administration (in hours)
1-	A	AbhrakBhasm	06	0.5
2-	B	AbhrakBhasm	06	2.0
3-	C	AbhrakBhasm	06	6.0
4-	D	AbhrakBhasm	06	24
5-	E	No drug(Control)	06	At the end of study

Test drug:-In this study, highest purified form of Abhrak Bhasm drug i.e. Sahastraputi Abhrak bhasm (subjected to 1000 PUTAS) which is considered to be of finest quality **(Subedi, et.al., 2017, p.238)** was used as the test drug. The drug was given along with 0.5 ml honey**. (Fig.1)**

Animal equivalent dose calculation based on body surface area:
Dose of Abhrak Bhasm in adult human=375mg/60-70Kg Body weight **(Singh, J., 2014.)**

Animal Equivalent Dose (mg/kg) =Human Dose (mg/kg) X Conversion factor Conversion factor for rats is 6.2 **(Nair, A. B. & Jacob, S., 2016, p.29 and Shin et. al. 2010, p.353)**

Therefore, AED (for rats) = 36mg/kgB.wt. orally once.

Abhrak Bhasm was weighed as per the body weight of different rats by using milligram digital balance and a suspension of Abhrak Bhasm was prepared with honey for administration in rats **(Fig.-2).**

Blood samples:-All blood samples were collected from jugular veins **(Fig.3 & Fig.4)** by using 30 gauze needle in EDTA (Ethylene diamine tetraacetic acid) vials and in plain vials for hematological and biochemical investigations respectively as per **Table-1**. The blood samples collected in plain vials were then centrifuged at 3000 rpm for 10 minutes and serum was collected.

Fig.1Drug administration; Fig.2-Abhrak bhasm drug with honey; Fig.3 & Fig.4- Blood collection from jugular vein.

Hematological studies:-
Blood samples in EDTA vials were used to assay hematological parameters. These were sent to the Central Lab. Hematological investigations for the parameters WBC(White Blood Cells { $X10^3/\mu l$}),RBC(Red Blood Cells{ $X10^6/\mu l$}), HGB (Hemoglobin{ g/dl}), Hematocrit (%), MCV(Mean Corpuscular Volume{ fl}), MCH (Mean Corpuscular Hemoglobin { pg}), MCHC (Mean Corpuscular Hemoglobin Concentration{ g/dl}) were done by using 3-Part Automated Hematology Analyzer.

For determining the number of platelets (PLT{ X103/µl}) and the Differential Leucocyte Count (DLC) i.e. the percentage of Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils, the blood smear was made and stained with May-Grunwald-Giemsa (MGG) stain. Finally, the Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils were counted under light microscope.

Biochemical studies:- B
lood samples were sent to the Central Lab within an hour of collection and after collecting serum, serum biochemical parameters-Random blood sugar(RBS) (mg/dl), Urea(mg/dl), Creatinine (mg/dl), Total Cholesterol (mg/dl), Triglycerides (mg/dl), HDL (mg/dl), Total Billirubin(mg/dl), Direct Billirubin (mg/dl), Total proteins (g/dl), Albumin (g/dl), Globulin (g/dl), Serum Albumin Globulin Ratio(SAGR), Aspartate Amino Transferase (AST in IU/L), Alanine Amino Transferase (ALT in IU/L), Alkaline Phosphatase (ALP in IU/L) were determined by using Fully Auto Biochemistry Analyzer SelectraProXL.

STATISTICALANALYSIS:-
Forstatistical analysis, data were analyzed by one-way analysis of variance(ANOVA) using Statistical Package of Social Sciences (SPSS) software version 25 for windows. The results were expressed as mean± standard error(SE). Statistical significance was accessed at 95% confidence interval and $P<0.05$ was considered as statistically significant.

RESULTS:-
Hematological studies:-The results of hematological studies are listed in Table-2. There were no significant changes in WBCs, Eosinophils, Monocytes and Basophils values after Abhrak Bhasma administration @36mg/kg B.wt. orally once in treatment groups as compared to control group. A significant decrease in hemoglobin concentration in group B and group D i.e. after 2.0 hours and 24 hours of drug administration respectively and in MCV value were observed in group D i.e. at 24 hours of drug administration as compared to control group was observed.

A significant increase in Neutrophils percentage and a significant decrease in Lymphocytes percentage were observed in both group B and group C i.e. at 2 hours and 6 hours of drug administration respectively, as compared to control group.

In two cases, in group B and group C i.e.at 2 hours and 6 hours of Abhrak bhasm drug administration, hypersegmented neutrophils were seen in blood smear. In three cases, two in group B and one in group D i.e. at 2 hours and 24 hours of drug administration, polychromasia were seen.

In the present study, as per Table-2,it was observed that the difference in means of RBC, HCT & MCH of groups of rats after half an hour, 2 hours, 6 hours and 24 hours after Abhrakbhasm intervention was found to be statistically significant (i.e $P<0.05$). On the other hand, when comparision within the group was done using Bonferroni correction, it was observed that the difference in variance was not statistically significant.

Table-2:-Hematological values in Wistar rats in different groups after Abhrak Bhasm drug administration as compared to the control group.

S.No.	Hematological parameters	Normal range[1,2]	Control group	Treatment groups				Statistical Value	
				A	B	C	D	P value	Sum of squares(R^2)
1.	WBC	5-13 ($X10^3/\mu l$)	12.25±2.23	9.80±1.60	12.55±1.48	11.92±0.81	12.75±1.71	0.716	34.042
2.	RBC	6-10 ($X10^6/\mu l$)	7.04±0.13	6.57±0.35	6.12±0.59	6.91±0.15	6.24±0.27	**0.049**	3.877
3.	HGB	11-17 (g/dl)	13.50±0.27*	12.65±0.51	11.55±0.43*	12.33±0.28	11.63±0.30*	**0.006**	15.39
4.	HCT	37.9-52.5 (%)	46.38±1.58	42.35±3.48	38.50±1.56	44.32±1.63	37.38±1.47	**0.026**	348.235
5.	MCV	48.9-58.3(fl)	65.82±1.17*	61.73±0.83	62.92±0.91	64.08±1.84	59.95±0.59*	**0.015**	119.827

6.	MCH	17.1-20.9 (pg)	19.20±0.4.	19.33±0.44	18.88±0.26	17.83±0.71	18.73±0.43	**0.045**	8.341
7.	MCHC	32.9-37.9 (g/dl)	29.27±1.04	31.30±0.42[a]	30.03±0.32	27.93±0.82[a c]	31.20±0.57[c]	**0.010**	47.555
8.	PLT	638-1200 ($X10^3/\mu l$)	1050.5±61.99	827.17±75.71[a]	632.33±75.37[b]	1205.8±85.23	1343.8±126.93[a b]	**0.001**	1245822.533
9.	Neutrophil	6.2-33.2 (%)	15.0±1.46*	17.60±3.35	26.67±2.20*	27.67±2.70*	22.17±1.51	**0.003**	732.688
10.	Lymphocyte	62.2-90.3 (%)	83.33±1.60*	80.95±3.64[a]	72.00±2.31*	70.17±2.51*[a]	75.83±1.70	**0.002**	806.219
11.	Eosinophil	0.2-4.5 (%)	0.83±0.31	0.73±0.35	0.83±0.17	1.50±0.22	1.17±0.17	0.217	2.421
12.	Monocyte	0.8-3.9 (%)	0.33±0.21	0.72±0.18	0.50±0.22	0.67±0.33	0.83±0.40	0.751	0.919
13.	Basophil	0.0-0.8 (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	0.000

All values are expressed as Mean±SE. Starmark (*) shows the significant association between means of respective group when compared with control at 95% confidence interval (when P<0.05)

a-indicates significant difference (P<0.05) in the values in between groups as compared to group A.
b-indicates significant difference (P<0.05) in the values in between groups as compared to group B.
c- indicates significant difference (P<0.05) in the values in between groups as compared to group C.

1-Bhardwaj, K.R. and Upadhyay, D.S., 1994 (p. 30).
2-Gilkinis,M.L.A. and Clifford,C.B.,2008 (p. 6 & 7).

Biochemical studies:-The results of biochemical studies were listed in Table-3. No significant changes were observed in values of Urea, Total cholesterol, Triglycerides, Total Billirubin, Direct Billirubin, AST and ALT in treatment groups when compared with control group. However, a significant increase was observed in HDL, SAG ratio and ALP in group C i.e. after 6 hours of Abhrak Bhasm administration as compared to control group. There was significant decrease in Total protein, albumin and globulin values in groups A & B i.e. after half an hour and 2 hours of drug administration as compared to control group.

As per Table-3, it was observed that the difference in means of blood glucose of groups of rats after half an hour, 2 hours, 6 hours and 24 hours after Abhrak Bhasm intervention was found to be statistically significant (i.e. P<0.05) and progressive increase in the mean values of blood glucose were seen in half an hour, 2 hours, 6 hours and 24 hours as compared to the control group. On the other hand, when comparision within the group was done using Bonferroni correction, it was observed that the difference in variance was not statistically significant.

Table-3:-Biochemical values in Wistar rats in different groups after Abhrak Bhasm drug administration as compared to the control group.

S. No.	Biochemical parameters	Normal range[1,2]	Control group	Treatment groups				Statistical Value	
				A	B	C	D	P value	Sum of squares(R^2)
1.	RBS	70-208 (mg/dl)	83.33±8.50	115.50±8.45	126.00±16.56	144.83±15.24	145.00±20.69	**0.037**	15596.200
2.	Urea	12.3-27.1 (mg/dl)	46.63±2.81	40.73±3.63	40.85±5.40	40.70±3.68	39.62±2.87	0.705	188.009
3.	Creatinine	0.2-0.6 (mg/dl)	0.66±0.17*	0.77±0.05	0.49±0.033[b]	0.66±0.06[b]	0.98±0.11*[b c]	**0.001**	0.782
4.	Total Cholesterol	24-85 (mg/dl)	82.67±5.10	67.67±7.42	62.50±2.55	93.33±9.19	96.67±8.19	**0.005**	5516.533
5.	Triglycerides	14-114 (mg/dl)	95.33±8.89	92.17±14.84	66.50±4.23	95.17±8.75	95.00±11.83	0.239	3781.667
6.	HDL	36.36-72.73 (mg/dl)	23.17±5.78*	31.33±3.13	44.17±2.61*[b]	22.00±5.76[b]	24.50±3.94[b]	**0.009**	2032.467
7.	Total Billirubin	0.05-0.18 (mg/dl)	0.34±0.10	0.35±0.09	0.26±0.05	0.23±0.04	0.30±0.11	0.892	0.048
8.	Direct Billirubin	0.03-0.06 (mg/dl)	0.19±0.07	0.18±0.03	0.15±0.04	0.14±0.03	0.17±0.05	0.948	0.009
9.	Total Protein (g/dl)	5.2-7.7 (g/dl)	8.15±0.41*	5.57±0.17*[a]	5.13±0.31*[b]	7.69±0.62[a b]	7.41±0.29[a b]	**0.000**	43.727
10.	Albumin	3.4-5.5 (g/dl)	4.63±0.22*	3.61±0.13*[a]	3.44±0.12*[b]	4.65±0.34[ab]	3.96±0.28	**0.002**	7.667
11.	Globulin	1.5-2.5 (g/dl)	3.51±0.25*	1.96±0.16*[a]	1.69±0.21*[b]	3.04±0.33[ab]	3.45±0.23[ab]	**0.000**	17.456
12.	SAG Ratio	1.58-3	1.34±0.08*	1.91±0.15	2.27±0.38*[b]	1.57±0.09	1.18±0.13[b]	**0.005**	4.640
13.	AST	65-203 (IU/L)	235.33±17.66	244.00±28.70	249.33±26.61	192.00±24.37	238.83±11.90	0.417	12613.200
14.	ALT	16-48 (IU/L)	103.50±9.64	71.00±4.35	110.00±3.35	122.33±17.63	94.17±7.95	**0.016**	8915.133
15.	ALP	26-230 (IU/L)	238.00±34.95*	231.67±14.13*[a]	601.50±25.51*[ab]	369.83±22.50[b]	258.50±54.38[b]	**0.000**	588327.533

All values are expressed as Mean±SE. Starmark shows the significant association between means of respective group when compared with control at 95% confidence interval (when P<0.05) .

a-indicates significant difference (P<0.05) in the values in between groups as compared to group A.
b-indicates significant difference (P<0.05) in the values in between groups as compared to group B.
c- indicates significant difference (P<0.05) in the values in between groups as compared to group C.

1-Gilkinis, M.L.A. and Clifford, C.B., 2008 (p.8&9).
2-Ilhedioha et.al., 2013 (p.95 & 96).

DISCUSSION:-

In this study, no significant change in the values of RBC and a significant decrease in the level of hemoglobin at 2 hours and 24 hours of Abhrak Bhasm treatment as compared to control group is different with the findings of **Gigi Mathew, 2010 (p.140)**, who conducted an experimental study to ascertain the haematinic action of Guda Marita Abhrak bhasma. In this study, Abhrak bhasm has been selected for haematinic effect, because it has been indicated in Panduroga and even Guda is a dravya, prepared out of herbal drug called ikshu, which will increase the amount of blood. So as the hypothesis says when two or more drugs having similar properties, combined together would give enhanced effect. Thus both of them have been selected for haematinic effect. In that study, the RBC and Hb increase due to GudaMaritaAbhrakbhasma which may be due to the drug effect at the level of erythropoeisis and the stimulation of erythropoeisis and enhancement of Hb level. There in this study it was conferred that the drug Guda Marita Abhrak bhasm is possessing haematinic activity and Pro RBC synthesis. However in the present study, the findings of polychromasia i.e. increased number of immature RBCs or reticulocytes were indicative of increased erythropoeisis and were in agreement with the findings of **Gigi Mathew,2010 (p.141)**.

A significant increase in percentage of Neutophils was observed in group B and group C i.e. at 2 hours and 6 hours after Abhrak bhasm drug administration as compared to control group which is in agreement to the findings of **Tamhankar et al., 2015 (p.24)**. In the study done by Tamhankar, at al., 2015, shataputi Abhrak bhasm was subjected to In-vitro screening to assess its immunomodulatory effect using the Nitroblue Tetrazolium (NBT) assay. In this study, it was concluded that Shataputi Abhrak Bhasma brings about stimulation of Leucocytes in concentration dependent manner.5% and 10% solution of Shataputi Abhrak Bhasma stimulated 93% and 93.5% leucocytes respectively, which is an indicator of highly significant phagocytic activity. This study revalidated the reference of Shataputi Abhrak Bhasma as a Rasayan and hence also established it as an immunomodulator.

In the present study, there was no significant change in the levels of

AST & ALT as compared to control group and a significant increase was observed in ALP level in the group B i.e. at 2 hours after Abhrakbhasm drug administration as compared to the control group. However in the toxicity study conducted by **Buwa, S.K., 2000 (p.251 & p.252)**, a decrease in the level of all serum enzymes AST, ALT & ALP were observed in four groups of rats (consisting of 10 animals in each group) in which the Abhrakbhasma was administered orally @ 10mg/kg B.wt., @ 20 mg/kg B.wt., @ 30 mg/kg b.wt. and 40 mg/kg B.wt. respectively for a period of 15 days. In the present study, the increase in ALP level may be the result of alterations in metabolism **(Buwa, S. K., 2000, p.253)**.

In the present study, the significant changes in Hemoglobin, Total proteins, Albumins, Globulins, Serum Albumin Globulin ratio and HDL as compared to control group were within the normal laboratory limit and were considered as incidental. These findings were in agreement with the findings of **Tenpe, C. and Yeole, P., 2012 (p.51)** who observed significant changes in high dose treatment group (Diabecon, an ayurvedic formulation containing 10 mg Abhrak Bhasm along with other ayurvedic drugs was administered @500 mg/kg B.wt.PO once daily for 90 days in rats) in hematology and serum chemistry parameters compared to control group but were within the normal laboratory limit and were considered as incidental.

CONCLUSION:-

The findings in the present study indicate the erythropoeisis stimulant action and also a significant phagocytic activity i.e. immunomo dulatory activity of Sahastraputi Abhrak Bhasma drug. It consolidated the fact that Sahastraputi Abhrak Bhasma is highly regarded as Rasayan, thus leading to scope for exploring the mechanism of its activity through more animal and clinical studies.

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