Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

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An Experimental Study of *Draksha Avaleha* w.s.r. to *Pandu* (Iron Deficiency Anaemia)

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ABSTRACT

The present study is carried out to evaluate the haematological effect of *Draksha Avaleha* in iron deficiency anaemia *(Pandu)* as claimed in the texts and to assess the rodents were selected for the study because of various reasons viz. ready availability, economic feasibility and wide applicability for such studies. Among the various models, Phenylhydrazine induced iron deficiency anaemia *(Pandu)* model has been applied in the study. The drug dose was calculated following the normal procedures and the effect was significant found in mices.

KEYWORDS *Haematological effect, Pandu, Iron Deficiency Anaemia, Bioferon, Phenylhydrazine*

Received 19th September 22 Accepted 06th November 22 Published 10th November 2022

INTRODUCTION

Experimental study is heavily reliant on animal testing. Currently, non-human animals must be tested before any clinical study of a new medicine or formulation can be conducted. It is earlier also mentioned in our ayurvedic texts[1]. Medicine's goal is to alleviate suffering and maintain good health so that people can live longer. Drug, a vital tool in health care, are only used in therapeutics after being tested in the lab. This research is needed to assess and revalidate the efficacy of Ayurvedic pharmaceuticals in living creatures, as well as to determine, whether or not administering herbal, herbo-mineral and metallic prepared drugs have any negative consequences. Pharmacological investigations are also required to determine whether changes in a medicine's SOP result changes in its activity or toxicity profile.

MATERIALS AND METHODS

Trial drug: *Draksha Avaleha* (DA)[2]

Materials:

Chemicals and Equipment: Picric acid, Savlon, Spirit, Phenyl hydrazine, Syringe, Distilled Water, Digital balance, Rotary Shaker, Hot air oven, Hot plate, Anesthesia chamber, Halothane, Polypropylene cages, Water bottle, Oral feeding needles, Common glass wear etc.

Calculation of dose: According to the ayurvedic

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formulary of India, Part-2, the human dose of *Draksha Avaleha* is 6-12gm/day. The present study was designed into dose of 10 gm/day. The dose calculation for the animal was done on the basis of body surface ratio by referring the table of paget and barnes (1969). Thus, the dose conversion formula for animals, is human dose multiplied by 0.018 (conversion factor for rats) and the resulting product will be further multiplied by 5 to obtain to dose per kg body weight[3].

- For human dose10 gm/day
- Rat dose will be- 10gm×0.018 = 180mg (for wt. of 200gm.)
- Rat dose per kg. body weight= 180×5=900mg (rounded to 1gm)

Preparation of suspension

- The suspension of *Draksha Avaleha* was prepared by 1 gm of test sample dissolved in 5ml of 1% CMC (carboxymethyl cellulose) solution through proper mixing on magnetic stirrer.
- The suspension of standard drug was prepared by 1 gm of test sample dissolved in 5ml of 1% CMC (carboxymethyl cellulose) solution through proper mixing on magnetic stirrer.

Animal

- Strain: Albino wistar
- No. 18
- Sex: Both Sexes

Ethical Clearance: The experimental protocol was submitted to the animal ethics committee of the institute, and approval was obtained for conducting the place of work: - Animal house of Bilwal Med Chem And Research Laboratory Pvt. Ltd. H-9SKS Reengus Industrial Area, Reengus, Rajasthan.

Method:

1. Phenylhydrazine-induced Anemia in Albino Wistar rats: The temperature in the experimental animal room was maintained at 22ºC (+ 3ºC). Although the relative humidity was maintained at 30% not exceeding 70% other than during room cleaning the aim was to maintain humidity between 50-60%. Artificial lighting was used, the sequence being 12 hours light & 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

2. Marking of Albino Wistar rat for identification: -

The albino rat was marked with Picricacidin each group as H, B, T, HB, BT and HT where:

- H stand for head of albino rat
- B stand for Back of albino rat,
- T stand for Tail of albino rat
- HB stand for head and back of albino rat
- BT stand for Back and Tail of albino rat
- HT stand for head and tail of albino rat.

GROUP DESIGN-Eighteen healthy adult Albino Rats weighing between 120-250 gm were selected randomly and divided into 3 groups. Each group had 6 animals. Anemia was induced using 10mg/kg body weight of phenylhydrazine through oral route for 10 days in all 18 Albino wistar rats[4].

Group 1- Negative control group (Distilled water)

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Six Anemia induced Albino Wistar Rats were given distilled water in the dose of 5ml/kg/p.o. for 30 days.

Group 2- Trial drug group (*Draksha Avaleha*)

Six Anemia induced Albino Wistar Rats were given the test sample i.e., *Draksha Avaleha* 450mg/kg/p.o. for 30 days.

Group 3- Standard drug group (Bioferon)

Six Anemia induced Albino Wistar Rats were given the standard drug i.e., Bioferon 230mg/kg/p.o. for 30days.

Administration of doses: The test substance was administered by gavage using an oral feeding needle. Animals had been fasted prior to dosing. Following the period of fasting the animals were weighed and the test substance was administered. After the substance was administered, food may be withheld for a further 3-4 hours in rats.

Evaluation: Blood sample was withdrawn with the help of capillary from orbital sinus site, collected in EDTA coated blood sample collection vial and hematological analysis such as Hb, RBC, MCHC, MCV was carried out in Automatic Hematology Analyzer

Statistical analysis-

The results are expressed as mean ± SEM, Comparison between before and after treatment are done using Student t paired test and Comparison in between the treatment groups and control group are performed by applying analysis of variance (ANOVA) test followed by Dunnet's multiple tests[4].

OBSERVATION AND RESULTS

Table 1 Hemoglobin levels in all groups

Hemoglobin	GROUP 1		GROUP 2		GROUP 3	
	0DAY	30DAY	0DAY	30DAY	0DAY	30DAY
H	9.32	9.83	9.43	12.32	8.34	13.43
B	10.23	9.78	10.23	11.34	9.32	14.32
T	8.54	9.32	10.22	13.23	8.94	13.84
HB	9.45	9.12	8.93	14.67	10.14	14.32
BT	9.54	9.34	9.34	13.96	10.43	14.34
HT	9.65	9.78	9.12	14.76	9.34	14.94

As the results shown in table no. 1, the models of group A (where mice were treated with distilled water) did not show any significant and relatable changes before and after treatment, rather models of group B (where *Draksha Avaleha* was administered in mice) have shown significant changes in Hb. Group C, the group of standard drugs has also shown a good raise in Hb.

Table 2 Red Blood Cell (RBC) levels in all groups

RBC	GROUP 1		GROUP 2		GROUP 3	
	0DAY	30DAY	0DAY	30DAY	0DAY	30DAY
H	4.12	4.46	4.45	6.45	3.98	6.56
B	3.45	4.12	3.29	5.65	4.14	6.97
T	3.65	3.94	3.94	5.96	3.78	6.43
HB	3.12	3.66	4.27	5.73	3.76	6.56
BT	4.36	4.85	3.29	6.14	3.59	6.37
HT	3.76	4.14	3.02	6.12	3.72	6.83

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As the results shown in table no.2, the models of group A (where mice were treated with distilled water) did not show any significant and relatable changes before and after treatment, rather models of group B (where *Draksha Avaleha* was administered in mice) had shown significant changes in values of RBC. Group C, the group of standard drugs has also shown a good raise in RBC count.

Table 3 Mean Cell Hemoglobin Concentration (MCHC) Levels in all groups

MCHC	GROUP 1		GROUP 2		GROUP 3	
	0DAY	30DAY	0DAY	30DAY	0DAY	30DAY
H	54.43	52.34	51.43	45.32	53.56	31.34
B	51.34	52.83	58.43	41.35	55.65	35.54
T	56.76	55.93	56.84	41.45	58.54	36.64
HB	59.54	57.84	59.32	43.56	54.56	37.43
BT	56.54	55.29	56.43	35.78	54.56	34.54
HT	61.33	59.32	54.56	39.54	53.56	31.35

As the results shown in table no.3, the models of group A (where mice were treated with distilled water) did not show any significant and relatable changes before and after treatment, rather models of group B (where *Draksha Avaleha* was administered in mice) have shown significant changes in values of MCHC. In Group C, the group of standard drugs had also shown a good result in MCHC count.

Table 4 Mean Corpuscular Volume (MCV) in all groups

MCV	GROUP 1		GROUP2		GROUP 3	
	0 DAY	30 DAY	0 DAY	30 DAY	0 DAY	30 DAY
H	82.34	81.24	87.32	51.34	85.43	41.23
B	84.56	83.45	84.83	54.54	87.25	45.54
T	85.93	83.45	81.34	48.34	84.43	48.95
HB	87.43	81.84	84.09	41.85	85.32	51.34
BT	85.42	83.45	87.32	56.54	82.83	48.32
HT	81.35	79.32	85.82	51.35	81.39	45.32

As the results shown in table no. 4, the models of group A (where mice were treated with distilled water) did not show any significant and relatable changes before and after treatment, rather models of group B (where *Draksha Avaleha* was administered in mice) have shown significant changes in values of RBC. Group C, the group of standard drugs had also shown a good in MCV count.

Table 5 Standard Error of The Mean

Parameters	GROUP 1 Mean ± SEM		GROUP 2 Mean ± SEM		GROUP 3 Mean ± SEM	
	0DAY	30DAY	0DAY	30DAY	0DAY	30DAY
Hb	9.46±0.224	9.53±0.124	9.55±0.226	13.38±0.554	9.42±0.314	14.20±0.210
RBC	3.74±0.183	4.20±0.169	3.71±0.214	6.01±0.120	3.83±0.081	6.62±0.095
MCHC	56.66±1.454	55.59±1.116	56.17±1.163	41.17±1.351	55.07±0.763	34.47±1.067
MCV	84.51±0.932	82.13±0.683	85.12±0.924	50.66±2.110	84.44±0.847	46.78±1.442

As per results shown in table no. 5, the mean score of Hb, RBC, MCHC and MCV respectively group A, B and C got deviated after treatment. Group A did not show any significant and relatable changes, rather the values of group B were altered significantly, like mean score of Hb

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was increased by 3.83, RBC count was also increased by 2.3 after administration of drug, MCHC which is the marker of average amount of Hb in a single RBC got decreased by 15 and MCV (measurement of Red Blood Cell size) in anemic rats also got decreased by 34.46.

Table 6 Dunnett's multiple comparisons of Haemoglobin

Hemoglobin					
Dunnett's multiple Comparison test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P Value
Group1vs.Group2	-3.852	-5.057to-2.646	Yes	****	<0.0001
Group1vs.Group3	-4.670	-5.876to-3.464	Yes	****	<0.0001

Table 7 Dunnett's multiple comparisons of RBC

RBC					
Dunnett's multiple Comparisons test	Mean Diff.	95.00%CIof diff.	Significant	Summary	Adjusted P-Value
Group1vs.Group2	-1.813	-2.268to-1.358	Yes	****	<0.0001
Group1vs.Group3	-2.425	-2.880to-1.970	Yes	****	<0.0001

Table 8 Dunnett's multiple comparisons of MCHC

MCHC					
Dunnett's multiple Comparisons test	Mean Diff.	95.00% CI of diff.	Significant	Summary	Adjusted P-Value
Group1vs.Group2	14.43	10.34 to 18.51	Yes	****	<0.0001
Group1vs.Group3	21.12	17.03 to 25.20	Yes	****	<0.0001

Table 9 Dunnett's multiple comparisons of MCV

MCV					
Dunnett's multiple comparisons test	Mean Diff.	95.00 % CI of diff.	Significant	Summary	Adjusted P-Value
Group1vs.Group2	31.47	26.20 to 36.73	Yes	****	<0.0001
Group1vs.Group3	35.34	30.07 to 40.61	Yes	****	<0.0001

From the above table no. 6,7,8 and 9 we can conclude that the results are significant enough after the trial and mean differences are shown in each table.

RESULTS

Anemia was induced in 18 Wistar Albino Rats using 10mg/kg phenylhydrazine orally for 10days and then divided into 3 groups. Each group contains 6 rats. Group1 received 5ml/kg distilled water. Group 2 received test sample 450mg/kg and 3rd group received standard drug 230mg/kg orally for 30days. After 30 days statical comparison was done with help of ANOVA followed by Dunnett's multiple comparison tests. The findings are: - treatment with distilled water created no changes in hemoglobin level (0 day-9.46) and (30 days -9.53). In group 2 (treated with test sample, dose of 450 mg/kg) changes in levels of hemoglobin were observed (9.55 to13.35). In group 3 treatment with standard drug dose of 230 mg/kg, changes in level of hemoglobin was observed (9.42 to 14.20). Statistical comparison showed that group 2 and group 3 both samples had similar biological response in increase level of Hemoglobin (p value-<0.0001).

DISCUSSION

The experimental study was planned to reassess the haematological effect of *Draksha Avaleha* in

iron deficiency anaemia as claimed in the texts and to assess the rodents were selected for the study because of various reasons viz. ready availability, economic feasibility and wide applicability for such studies. Among the various models, Phenylhydrazine induced iron deficiency anaemia model has been applied in the study. The drug dose was calculated following the normal procedures and the effect was evaluated in the dose of 450mg/kg/p.o. for *Draksha Avaleha* and for Bioferon 230 mg/kg/p.o. dose was taken and the results were compared.

CONCLUSION

Draksha Avaleha have significant hematinic activities on models. *Draksha Avaleha* have reverse symptoms of *Pandu Roga* and have significant results on IDA (Iron Deficiency Anemia).In the above study comparatively Group-C and Group- B had shown better result as compared to Group-A. In observational, parameters like HB%, RBC, MCV, MCHC had shown statistically significant change at various stages of the trial. Above study concludes that *Draksha Avaleha* can be easily utilized as an effective medicine for the treatment of *Pandu*.

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