ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(5):1250-1254

ORIGINAL RESEARCH ARTICLE

Toxicity Study of N*aga bhasma w.s.r. to* Ayurvedic Measure for Toxicity Eradication

Dr. Manoj Dash*[1], Dr. Namrata Joshi[2], Asst. Prof. R.S Gupta[3] and Prof. L.K. Dwivedi[4]

[1]*Govt.Ayurveda College, Raipur, Chatisgarh, India*
[2]*Rishikul P.G. Ayurvedic College, Haridwar (Uttarakhand), India*
[3]*Department of Zoology,Rajasthan University,Rajasthan,India*
[4]*Department of Rasashastra & Bhaisajya kalpana, National Institute of Ayurveda, Jaipur, India*

Received 19 May 2012; Revised 13 Oct 2012; Accepted 21 Oct 2012

ABSTRACT

Naga bhasma is a popular and effective dosage form prepared from lead metal in Ayurvedic practice. Since modern literature attributes certain toxicity to lead salts. An attempt is made to screen the acute and sub-acute toxicity of Nagabhasma in the form, dose and route as is in the practice of Ayurveda. In this paper, dose-effect relation of Nagabhasma on Digestive System (G.I.T., Liver and Pancreas) genitourinary system (testes, seminal vesicle, cauda) has been presented. Analysis of the data related to body weight changes in different groups reveal highly significant increase in body weight. The test drug at the dose of 10mg/kg studied does not produce any significant degenerative changes. From toxicological point of view the test drug does not produce any serious tissue damage at therapeutic dose and intermediate dose level. On histopatholgical study in highest dose the stages of Spermatogenesis was reduced, the structure of graffian follicles shows separation in their wall, the stages of spermatocytes look degenerative. But after giving Swarna Bhasma with Haritaki Churna mixed with sugar for 3 days to the highest dose Albino rats, the primary and secondary spermatocytes are properly devloped, all the stages of Spermatogenesis are seen on histopathological study, the wall surrounding the semineferous tubule looks intact.

Key words: Naga Bhasma,Swarna Bhasma,Haritaki churna,Testis.

INTRODUCTION

As regards the Toxicity Study of the drug, previous animal experiments conducted on rabbit, dog and rats have shown hypertension, atherosclerosis and increased vascular permeability in CVS when administered with lead acetate (Graffith, 1944; Bechmann, 1925; Makasav and Kriudin, 1972). In chronic lead poisoning in children cedema , interstitial fibresis and inflammatory changes in the heart have been mentioned (Anderson, 1977; M. Singh *et al*, 1983). Haematological changes in the form of anaemia and basophilic stippling are established findings of chronic lead poisoningIn GIT non-specific inflammation have been reported by many workers in predominantly in stomach, jejunum and colon. Wide-spread mucosal necrosis with sloughing in acute poisoning resulting hypovolumic shock has also been noted.

MATERIALS AND METHODS

DRUG

Further, to check the toxicological effect of the trial drug NAGA BHASMA, we prepare it according to the procedure described in A.F.I. As many of the pharmaceutical companies and vaidya's are using the A.F.I. mentioned procedure to manufacture the proposed drug "NAGA BHASMA'.Here an attempt has been made to know the toxic effect of this drug.

EXPERIMENTAL DESIGN

ACUTE Toxicity study

Total 15 wistar albino rats of male sex, weighing 150 g to 250 g were taken and divided randomly into 5 groups, each containing 3 animals. All the animals in group I were treated with Naga bhasma orally at the dose of 10 times more than therapeutic dose of Likewise Group II were given 20 times, group III were given 40 times, Group IV were given 80 times, and Group V were treated by 100 times more than therapeutic dose of Naga Bhasma. Single dose of drugs were administered orally according to the stated dosage

***Corresponding Author:** Dr. Manoj Dash, **Email:** drmanojkumardash@gmail.com

schedule. Gross behaviour and exitus (death) were recorded for 7 consecutive days.

Grouping for Acute Toxicity Study

Group	Drug	Dose (mg/100g)	Relation to T.D.
I	Suspension of *Naga bhasma*	41.60	10 Times
II	"	83.20	20 Times
III	"	166.40	40 Times
IV	"	332.80	80 Times
V	"	416.00	100 Times

Observations

Animals were observed for a period of seven days to check any gross behavioral change and exitus noticed. Mild behavioural hyperactivity was noticed in all the groups treated with Naga Bhasma.Sign and symptoms include alertness, spontaneous activity in the cage after drug administration up to 5 minutes. The awareness and alertness was recorded by visual measure of the animal's response when placed in a different position in cage. The effect may be due to the action of *Naga bhasma* on CNS. No exitus was observed in any of the experimental animal.

In the acute toxicity study, the animals group did not manifest any signs of toxicity and no exitus (death) was observed up to 100 times more than therapeutic dose. There was only mild behavioral hyperactivity noticed in all groups treated with both the drugs. From this it can be mentioned that the approximate LD_{50} value is more than 416mg/100g of the weight of the experimental animal.

Sub acute toxicity study

- ☑ The animals were obtained from the animal house of Department of Zoology, Rajastan University. They were exposed to natural day and night cycles, with ideal laboratory conditions in terms of ambient temperature and humidity. They were fed ad libitum with amrut brand rat pellet and Portable tap water. Solution of *Naga bhasma* was prepared in 10 mg/0.5 ml concentration of distilled water. Total 36 wistar rats of male sex weighing between 150g to 250 g were taken and divided randomly into 4 groups, each containing 9 male animals. All 36 rats were treated by naga bhasma. After induction of Naga bhasma for 2 months. 6 animals from group I, II, III, IV were autopsied and Histology of GIT, Kidney, Liver, testes and other reproductive organs were studied .After studying the Naga bhasma effect the rest 12 animals were divided into 4 groups. Group were received only madhu and Ghrita measuring by Volume, Group II, III, IV were received Haritaki Churna 1 gm. with Swarna Bhasma 15.6 mg. mixed with sugar.
- ☑ The suspension of Test drug was prepared in 10 mg/0.5 ml concentration of Madhu & ghrita measuring by volume. The test drug Haritaki Churna with Swarna Bhasma were administered for 3 days. The schedule was continued for 3 days with daily doses of test drugs and vehicle. Gross behaviour was observed throughout the study period. After Completion of Experiment, on 4th day, body weights were recorded. To know its Vrisya (Aphrodisiac) effect the mating behaviour test was carried out.

SUB ACUTE TOXICITY STUDY

It refers to the harmful effect of long-term exposure to test drugs. The sub acute Toxicity Study has been carried out for a period of 60 days.

Table 1: Grouping for Sub acute toxicity study

Group	Drug	Dose (mg/kg)
I (Control)	Vehicle	0.5 ml
II	Suspension of *Therapeutic dose of Naga bhasma*	10
III.	Suspension of five times to Therapeutic dose of *Naga bhasma*	50
IV	Suspension of ten times to Therapeutic dose of *Naga bhasma*	100

Table 2: Grouping for Vikara Shanti Upaya

Group	Drug	Dose (mg/kg)
I (Control)	Vehicle	0.5 ml
II	Suspension of *Therapeutic dose of Naga bhasma* with suspension of Haritaki Churna with Swarna Bhasma mixed with sugar	10
III.	Suspension of five times to Therapeutic dose of *Naga bhasma* with suspension of Haritaki Churna with Swarna Bhasma mixed with sugar	50
IV	Suspension of ten times to Therapeutic dose of *Naga bhasma* with suspension of Haritaki Churna with Swarna Bhasma mixed with sugar	100

RESULTS

Table 3: The summary of histopathological changes with *Naga bhasma*

S. No.	Tissue	Toxic changes present or absent			
		Group-I	Group-II	Group-III	Group-IV
1.	Liver	NE	NE	NE	Fatty degenerative changes with mild hepatocytes damage were seen under microscope

2.	Kidney	NE	NE	Cloudy swelling with Granular deposits are visible	Degeneration and necrosis in proximal convoluted tubules were visible under microscope
3.	Small intestine	NE	NE	Focal mucosal necrosis with oedema were seen	NE
4.	Testis	Increase in spermatogenesis	Increase in spermatogenesis	Atrophic leydig cell	Semineferous tubule with no sperm, decrease size of alveoli
5.	Seminal vesicle	Filled with fluid	Filled with fluid	Filled with fluid	Fluid does not seen
6.	Cauda Epididymes	NE	NE	NE	The structural epithelium has been changed.

Table 4: The summary of histopathological changes with Swarna Bhasma & Haritaki Churna

S.No	Tissue	Toxic changes present or absent			
		Group-I	**Group-II**	**Group-III**	**Group-IV**
1.	Liver	NE	NE	NE	The necrosed hepatocytes regenerate. The Kupfer cells regenerate. The sinusoidal dilatation and congestion of the liver cells are decreased
2.	Kidney	NE	NE	Cloudy swelling with Granular deposits are visible	Epithelium necrosis of proximal convulated tubules looks regenerated.
3.	Small intestine	NE	NE	Oedema has been subsided	NE
4.	Testis	Increase in spermatogenesis	Increase in spermatogenesis	Leydig cells regenerate and all the structure are properly visible	The tubule filled with sperm
5.	Seminal vesicle	Filled with fluid	Filled with fluid	Filled with fluid	Filled with fluid
6.	Cauda Epididymes	NE	NE	NE	Epithelium has been intact.

Table 5: Effect of *Naga bhasma* preparations on body weight in albino rats

Group	Body weight Before treatment Mean ± SEM % Change	Body weight After treatment Mean ± SEM % Change	Average food intake g/rat/day 1st month	Average food intake g/rat/day 2nd month	Weight of Liver (g) Absolute Mean ± SEM % Change	Weight of kidney (g) Absolute Mean ± SEM % Change	Weight of Testes (g) Absolute Mean ± SEM % Change
Control	**154.17 ± 7.79**	**167.50 ± 7.93**	**38.05**	**41.75**	**3.73 ± 0.21**	**1.39 ± 0.00**	**1.41± 0.01**
Therapeutic dose	173.33 ± 4.94*↑	188.33 ± 4.77*↑	43.32	47.02	4.64 ± 0.23**↑	1.4 ± 0.16↑*	1.57 ± 0.05**
Intermediate dose	160.83 ± 2.71*↑	168.33 ± 4.77*↑	40.27	42.08	4.31 ± 0.36*↑	1.42 ± 0.01↑*	1.42 ± 0.01*
Highest dose	155.00 ±2.58*↑	160.83 ±2.39*↑	38.75	40.20	4.37 ± 0.24*↑	1.41 ±0.01↑*	1.42 ± 0.07

↑ = Increase, ↓ = Decrease * =non significant **= Significant

DISCUSSIONS

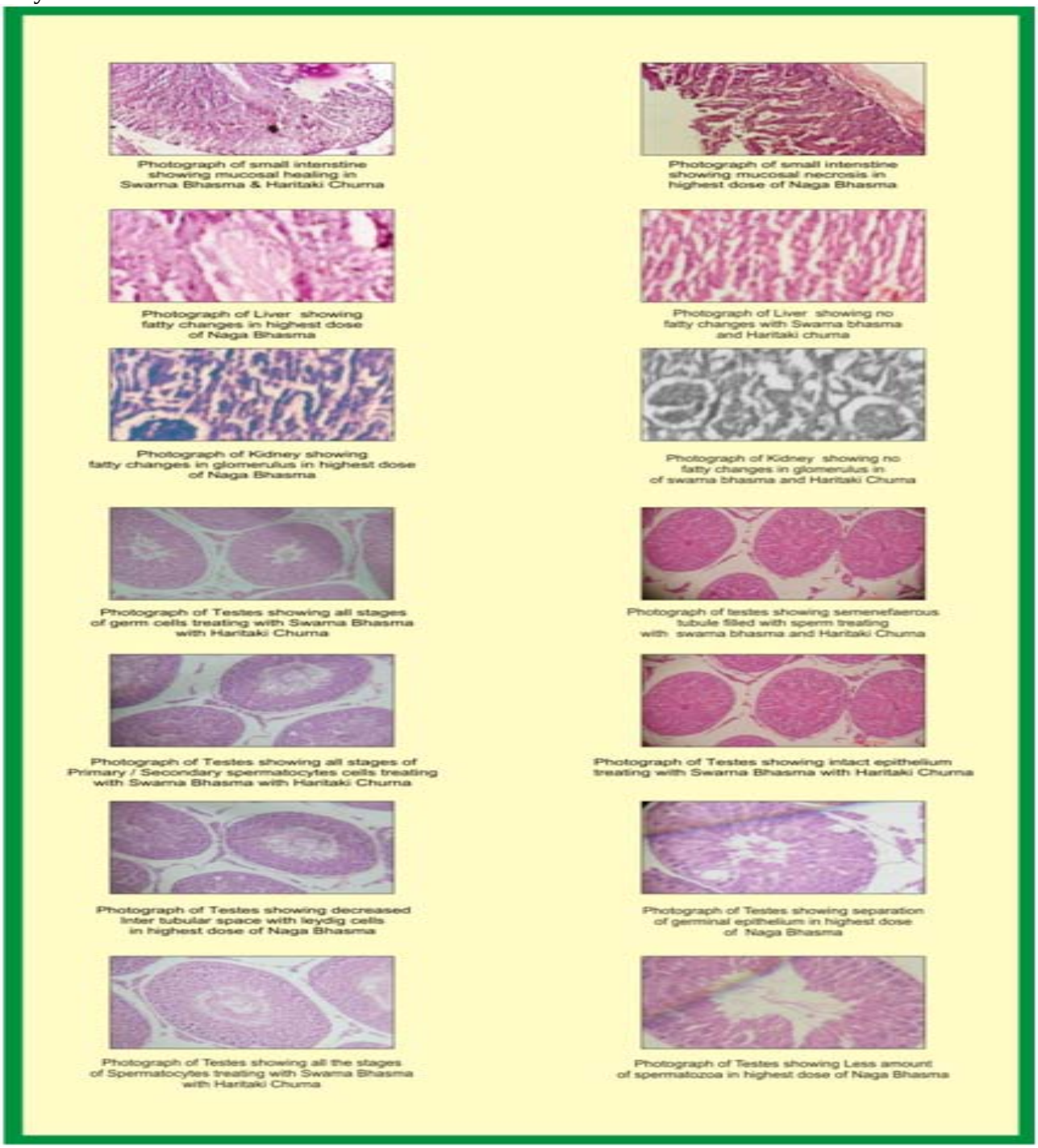
ACUTE TOXICITY STUDY

After giving 100 times to human therapeutic equivalent dose there was no such significant toxicity seen. No exitus (death) was observed after 7 days of acute toxicity study. It indicates that the Bhasmas was safe in such a high dose. As this dose clinically are unlikely to be given, but the pharmacodynamic of this preparation was safe. Some changes like irritability while giving medicine, slow movement during morning time was predominantly seen in such a higher dose.

SUB ACUTE TOXICITY STUDY

- ☑ Mild hyperactivity was observed in all the rats at therapeutic dose(4 mg/kg) intermediate dose(20 mg/kg) as well as at highest dose (40 mg/kg). So the preparation may be considered as reflex stimulant of the central nervous system. The stimulation may be due to the methods of administration of drugs or due to the irritation of the GI tract.
- ☑ On analyzing of the data pertaining to the body weight in different groups at varying test doses, a considerable non-significant increase in body weight was evident. Because body weight is indicative of increase appetite and food intake, thus it shows normal well being status of the experimental animals. Thus it can be presumed that any of the three test drugs does not cause any deleterious effect on total body function as a whole.
- ☑ In the present study microscopic examination showed focal area of mucosal necrosis of small intestine along with oedema was noted in two animals in higher dose. In one animal submucosal lymphoid hyperplasia of small intestine was observed in long term therapy. It appears that small intestine is more susceptible to the toxic effect of lead than the stomach, esophagus and large intestine. But after giving the haritaki churna with Swarna Bhasma for 3 days there has been remarkable changes observed in small intenstine. The oedematous pathological finding has been subsided.
- ☑ In present study apart from the cloudy swelling which was observed in highest dose treated group, only in two animal showed focal areas of tubular cell necrosis and colloid cost in the lumina which may be attributed to the toxic effect of lead. But after giving

haritaki churna with Swarna Bhasma for 3 days the necrosed tissue of the tubulear area has been regenerated.



- Highly significant decrease in weight was observed in testes at highest dose group. As the process of spermatogenesis is androgen dependent, decreased androgen level reflect in reduced number & volume of mature Leydig cell, nuclear diameter, degenerating semineferous tubule and their functional status. But here remarkable change has been observed after giving haritaki churna with Swarna Bhasma. The process of spermatogenesis with mature leydig cell has been increased. The semineferous tubule is filled with sperm.
- Decrease in the spermatogenesis in highest dose is the other finding which requires discussion. It is to be pointed out here that the test drugs did not produce any disturbance in the cytoarchitecture of the testis. Only the sperm presence indicated decrease. The germinal layer remained intact in all the groups and also differentiation of spermatids. Thus the observed decrease may be due to decrease in the maturation of spermatids. The mechanism remains to be determined. In cases of severe oligospermia the interstial cells of Leydig show hyperplasia and hypertrophy. No

such changes were observed in any of the groups. It may be due to general cytolytic effect of the test preparations.

- ☑ On the basis of histopathological study highest dose seems to possess higher toxic potential in comparison to other two groups..

CONCLUSION

From this study we can say that *Naga bhasma* has got no acute toxic effects on G.I.T, Liver, testis, kidney, and seminal vesicle in the dose of up to 416 mg/ 100 gm. body weight. Whereas for a period of 60 days study shows , no toxic effects has been observed on G.I.T. Liver,testes,seminal vescicle,kidney in therapeutic dose and intermediate dose. But in higher doses significant toxicity has been attributable to *Naga bhasma*. However, further giving Haritaki churna with Swarna Bhasma to the highest dose animals seems lesser amount of toxicity. So the concept of Bhasma Vikara Shanti Upaya described by different authors in Different rasa shastra litreture proves significance. However, further study with larger groups of animals may prove conclusive.

नाग भस्म की विषाक्तता का अध्ययन एवं विकार शान्ति उपाय

इस वैज्ञानिक शोधकार्य में नाग भस्म की विषाक्तता के बारे में जानकारी करने की कोशिश की गई है । आयुर्वेद के रसाचार्यों के मतानुसार किसी भी औषधि का प्रयोग करने से पहले जानवरों, (पशु–पक्षियों) पर उसका प्रयोग देखा जाना आवश्यक है । आधुनिक मतानुसार नाग भस्म का अधिक सेवन करने से शरीर में विषाक्तता का वर्णन भिन्न–भिन्न वैज्ञानिक जर्नल में मिलता है । इस मत को आधार बनाकर चूहों पर नाग भस्म की विषाक्तता का वैज्ञानिक अध्ययन करने की कोशिश की गई । ए.एफ.आई. में वर्णित नाग भस्म के निर्माण करने के उपरान्त इसकी तीव्र एवं जीर्ण विषाक्तता का प्रभाव जानने की कोशिश की गई । सभी सबंधित तथ्यों पर विचार करने पर यह पाया गया कि भिन्न–भिन्न वर्धक मात्रा में, नाग भस्म का सेवन करने पर शरीर के वजन में बढ़ौतरी पाई गई । नाग भस्म 10 मि.ग्राम. प्रति कि. ग्रा. शरीरभार मात्रा का सेवन करने पर शरीर में कोई भी विषाक्तता मुख्य अवयवों में देखने को नहीं मिली । नाग भस्म की वर्धक मात्रा (5 गुणा एवं 10 गुणा सामान्य मात्रा से) 60 दिन तक सेवन करवाने पर शरीर में कोई भी गम्भीर विषाक्तता नहीं पाया गया । प्रयोग के तौर पर चूहों पर नाग भस्म की वर्धक मात्रा (10 गुणा) का लम्बे समय तक सेवन करवाने पर उनमें स्पर्माटो जैनेसिस प्रक्रिया कम होती परिलक्षित की गई । प्राप्त विषाक्तता तथ्यों में यह पाया गया कि 10 गुणा थैरापैटिक मात्रा में नाग भस्म का इस्तेमाल करने पर सिमाईनल फलिकिल एवं पौरूष ग्रन्थी में सामान्य बदलाव देखने को मिला । 10 गुणा थैरापैटिक मात्रा में नाग भस्म का प्रयोग करने पर यकृत एवं गुर्दों में सामान्य बदलाव, देखने को मिला। रसशास्त्र में वर्णित विकार शन्ति उपाय के मतानुसार 10 गुणा थैरापैटिक मात्रा दिया गया नाग भस्म समूह को स्वर्ण भस्म एवं हरितकि चूर्ण प्रयोग करने के उपरान्त सिमाईनल फलिकिल एवं पौरूष ग्रन्थी में सुधार पाया गया

REFERENCES

1. Graffith, Bechmann, Makasav and Kriudin, Environmental Health criteria series 15, Lead and organotin compounds, W.H.O., 1980, 62-68, 84-86.
2. Cudamani, Rasakamadhenu, ed. By Yadavji Trikamji, Ayurveda Granthamala, No. 15, Bombay, 1925, 2/462.
3. Madhava, Ayurveda Prakasa, ed. By Gulraja Sharma Mishra, 2nd ed. Chowkhamba Sanskrit Series, 1962, 2/154.
4. Sadananda, Rasatarangini, Com. By Kashi Nath Shastry, 12th ed. Motilal Banarsidas, Varanasi, 1979, 17/6-7.
5. Bhavamishra, Bhavaprakasha, Com. By K.C. Chunekar, 5th ed. Chowkhamba Sanskrit Series, Varanasi, 1977, Dhatwadi, Kande 132.
6. Culling, C.F.A. : A hand book of histopathology techniques reprinted 2nd ed. Butterweth & Co., London 1966.
7. Goodman & Gillman. The Pharmacological basis of therapeutics 6th ed., MacMillan Pub. Co., New York, 1980, 1602.
8. Barnes & Stones, 1959, Cheftel, 1967; Biles, 1974 – National Academy of Sciences, Washington, as quoted in E.H.C. 15, W.H.O., Geneva, 1980, 62-63.