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TOXICITY STUDIES OF VANGA BHASMA (INCINERATED FORM OF TIN) PREPARED BY TWO DIFFERENT METHODS

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ABSTRACTS

Tin is used in a number of industrial processes throughout the world. Tinplate (sheet steel coated with a thin layer of tin) is the primary material used for food cans, and tin is also commonly used in bake ware and food storage containers. Vanga Bhasma (incinerated form of Tin) is one of the renowned Ayurvedic metallic preparations commonly prescribed by the physicians; mostly in Prameha Roga (Diabetes). In the present study Vanga Bhasma was prepared by two different methods and a comparative Acute as well as Chronic toxicity studies were done. In Acute toxicity study, up to the 2000mg/kg dose level no mortality and behavioral changes were observed. In Chronic toxicity study Ponderal changes, Bio chemical parameters, Hematological parameters and Histopathological parameters are studied. Mild fatty changes and cell infiltration was observed in Kidney and Jejunum in Charles foster albino rats at five times therapeutic Equivalent Dose.

KEYWORDS: Tin, Vanga Bhasma, Acute, Chronic, Toxicity.

INTRODUCTION

Tin occurs in nature in combination with Oxygen in the mineral cassiterite or Tin Stone, which is more or less a pure form of Tin Dioxide (SnO2). Tin has been known in India from ancient times. Its Vernacular name is Vanga in Sanskrit.[1] Tin has been used in the form of Bhasma (Incinerated form of Tin) which is prepared by number of process. Vanga Bhasma is a renowned medicine in use since 3600 A.D.It's main indication is in the treatment of Diabetes, Anaemia; various skin disease etc.It is believed to be a general tonic and alterative. It is used in the various diseases with different Vehicles (Anupana) usually in combination with other mineral or Herbal preparations or used alone.[2] But, simultaneously it is very specifically mentioned that improperly prepared Vanga Bhasma can cause harmful effects on the body.[3] Though Tin is a non toxic metal which is using in day to day practice. It is reported that prolonged use of it may cause benign Pneumoconiosis or some distinctive changes in the Lungs.[4] In this background the present study was undertaken to elucidate and compare the Toxicity profile of Vanga Bhasma prepared by two different methods to ascertain the safety aspects. The study was undertaken to assess the acute and chronic toxicity of Vanga Bhasma in Charles foster albino rats.

MATERIALS AND METHODS

Preparation of Vanga Bhasma: The following two procedures were adopted for this study.

In first method[5] (VB Group), Metallic Tin is heated in an Iron fry pan until it is melted and the molten mass is poured into the Lime water. This procedure was repeated for further six times. Tin is then re-melted with 1/4th of its weight of Apamarga panchanga (Achyrenthus aspera). The whole mass is stirred well with an Iron rod till it is reduced to a very fine powder. The powder is washed with distilled water. This Powder of Tin was levigated with Kumari Juice (Juice of Aloe) and small pellets were made. These pellets were enclosed in an earthen saucer and subjected for heat. This Putapaka (Incineration) procedure was repeated for six times more. In second method[6](VSVB Group), very thin folia of Tin was smeared with Oil of Bhallataka (Semicarpus anacardium) and placed in between the paste of Bhallataka and the mixture of powder of Chincha Twaka (Tamarindus indica), Pippal Twaka (Ficus religiosa)and Palash Twaka

(Butea monosperma). All these materials were packed and tied in the bundle of cloth and burnt in a closed room. After self cooling, white coloured chalk like Vanga was collected and powder it.

Dose selection: The classics of Ayurveda describe the dose of Vanga Bhasma to be from 1 Ratti to 2 Ratti(125mg to 250 mg).[7] Considering adult human dose of Vanga Bhasma i.e. 500 mg. For experimental study it was calculated by extrapolating the human dose to animal based on the body surface area ratio by referring to the table of Paget and Barnes (1969). The details of dosage have been given in respective experiments. Drug suspension was prepared by 5% gum acacia solution in distilled water and administered according to the body weight of the animals by oral route with the help of gastric catheter on to a syringe. Charles Foster strain of albino rats were obtained from the animal house facility attached to IPGT and RA., Gujarat Ayurveda University. The data generated during the study were subjected to student's't' test for unpaired data to assess the statistical significance. A 'p' value less than 0.05 is considered as statistically significant.

Experimental Design: The first step of any Drug research is to identify the toxic and therapeutic range. With the help of Animal experiments such data can be obtained. This is very important and also essential study for those drugs that contain metals, minerals etc.The Toxicity study has been carried out in two stages. In first stage acute toxicity of both the prepared Vanga Bhasmas were assumed in single large dose. In second stage chronic toxicity of prepared Vanga Bhasmas were given in five time more than Therapeutic effective dose.

A) Acute toxicity study: It refers to immediate harmful effects generated by the single dose of drug at several levels higher than the therapeutically equivalent dose.

Study Protocol
Charles Foster strain albino rats of either sex with an average body weight of 160-280g - were used in the study. Thirty animals were taken and each group comprising 3 animals. The animals were obtained from the animal house facility attached to IPGT and RA., Gujarat Ayurveda University. They were maintained on Pranav Agro Industries 'Amrut' brand rat pellet feed and water given ad libitum. The experiments were carried after obtaining the permission of the Institute's Animal Ethical Committee.

Dose fixation: In Acute toxicity study five dose levels were studied: i.e. TED (Therapeutic Equivalent Dose) × 10, TED× 15, TED × 20, TED × 30 and TED × 40.so, both the test drugs were given up to 2 g/kg for higher dose level. This is to confirm with the concept of the dose limit test as suggested by OECD- guidelines.[8] Frequency and duration of administration was once only.

Parameters studied – Cage side behavior/Gross behavior/ mortality/ autopsy of the dead animals.

During acute toxicity studies the animals were keenly observed for following signs and symptoms- general appearance, increased or decreased motor activity, convulsions, straub reactions, muscle spasm, catatonia, spasticity, ophisthotonus, hyperesthesia, muscle relaxation, anesthesia, arching and rolling, lacrimation, salivation, diarrhea, writhing, mode of respiration and changes in skin colour etc.

B) Chronic Toxicity Study: It refers to harmful effect of long term exposure to test drug. Charles Foster strain albino rats of either sex - average body weight - 160-280g; 6 animals in each group (3 male and 3 female). Other details as mentioned above. Chronic toxicity study was carried out at TED ×05 (**T**herapeutic **E**quivalent **D**ose) i.e. 250 mg/kg for Rats.

Study protocol: The animals selected as above were allotted to different groups. Group A i.e. Vanga Bhasma (prepared by First method) and Group B i.e. Vastra Puti Vanga Bhasma (Second method) were administered with same dosage i.e. 250 mg/kg. in the prescribed volume. A separate Group (C) which received the vehicle was kept for comparison. Both drugs were mix in distilled water and suspension was prepared. This suspension was administered orally through gastric catheter. The drug solution was administered once daily for 90 consecutive days as mentioned above. Body weight and behavioral pattern, food and water consumption pattern was recorded before starting drug administration. The rats were weighed again on the last day and sacrificed by severing jugular vessels. Blood was collected immediately in two different types of ampoules, one containing dilution fluid for cell counter and other in plain bulb for biochemical investigations. Further the rats were dissected and organs were separated and kept in normal saline (0.09%) carefully. All the organs were weighed with a monopan balance and transferred immediately to a glass bottle containing 10% formalin. These samples were sent to the laboratory to carry out histopathological studies.

Parameters studied
Haematological parameters like R.B.C count, W.B.C, Lymphocyte, Granulocytes, Eosinophil, Monocytes, and Hemoglobin were counted through cell counter. Blood sugar, S. total cholesterol, blood urea, S. creatinine, S. triglyceride, , S. Alkaline phosphates, S.G.O.T, S.G.P.T, Total protein, S.bilirubin, S. albumin, S. globulin and A/G ratio etc. Biochemical Parameters were studied. Examination of the viscera and general animal profile was done at the time of the sacrifice, involving external examination of the animal- different parts like head, body, limbs, nature of discharges from natural orifices like mouth, nose, vagina, anus, penis etc. Internal examination of buccal cavity of its organs; abdominal cavity and its organs, pelvic cavity with organs, limbs

were done. Histopathology of important organs like brain, spleen, thymus, lymph node, heart, lungs, liver, intestine, kidney, testis, uterus and ovary were also done.

RESULTS

A) Acute toxicity study

Effect of both the preparations of the Vanga Bhasma was studied after a single administration up to five dose levels with 2000mg/kg as the maximum dose. The animals were observed for 72 hrs periodically for general behavioral changes to screen its effect on CNS and toxic symptoms and mortality was observed up to 7 days. Even up to the 2000mg/kg dose level no mortality and behavioral changes were observed.

B) Chronic toxicity study

No behavioral changes (including cage side behavior) were observed in both the treated groups. No mortality was observed in any of the groups at 5 time's therapeutic effective dose. Initially food intake was decreased and gradually it came to normal level in both test drug treated groups in comparison to water control group. Fecal and urine output remained unaffected in both the groups.

Table -1 shows data related to effect of two different preparations of Vanga Bhasma on the body weight of albino rats after 90 days of treatment. Normal body weight gain was observed in control as well as test drug administered groups. The body weight gain was comparatively less in test drug administered rats in comparison to the control rats. However, the observed decrease in body weight is found to be statistically non-significant. An apparent decrease in weight of liver (Table 2) was observed in both the test preparation administered groups in comparison to control group. The observed decreased were found to be statistically highly significant for VB treated group and it was observed significant for VSVB treated group. Statistically significant decrease was observed in weight of spleen in both the test drug administered groups in comparison to control group. Administration of test preparation VB leads to an apparent but statistically non- significant decrease in the weight of kidney however in test drug VSVB group a statistically significant decrease in weight of the kidney was observed. Weight of other organs like Thymus, Prostate, Seminal Vesicle were found decreased but this decreased were found to be statistically non significant. Highly significant decreased were observed in Hb% and MCH count in comparison to control group. While, surprisingly significant increased were observed in RBC and HCT value VB and VSVB treated group. in Bio chemical parameters Blood Urea were found statistically Highly significant decreased in both the treated group of Vanga Bhasma in comparison to Control group. Alkaline Phosphatase activity were found decreased up to highly significant level in VB Group where it was observed significant decreased in VSVB group.A/G ratio was decreased up to significant level only with VSVB Group.

DISCUSSION

The first important parameter is body weight changes. Decrease in body weight indicates tissue loss and impairment of the nutritional status. In the present study no decrease in body weight was observed even after administration for 90 days. (Table 1) This indicates that the test drugs do not cause serious degenerative changes leading to tissue loss and they also do not interfere drastically with food absorption and assimilation. Of course there was comparatively slightly less degree of body weight gain in test drug administered groups in comparison to control group which did not reach statistically significant level. In both the groups significant decrease in liver and spleen weight was observed. (Table 2) To ascertain whether it is due to tissue degeneration the result of histological examination was taken into consideration. Histological examination did not reveal any drastic tissue degeneration in both the organs hence it can be suggested that the observed decrease do not reflect any toxic potential. In VB group a moderate but statistically significant decrease in kidney weight was observed. Concurrent histological profile analysis showed in cell infiltration in one rat and mild glomerular dilatation and hemorrhagic spots in another rat in VB group. Sections from VSVB exhibited odematous appearance in some sections; microfatty changes and glomerular dilatation were observed in the tubular epithelium. Thus the changes observed were more in VSVB group than VB group. This rule out drastic tissue degeneration as the cause of the decrease in weight observed in VB group. In VSVB group histological examination showed increase in the connective tissue proportion in the organ though the there were normal features in the epithelial lining the alveoli. This may be the reason for the observed weight decrease in the ventral prostate. The implication of this decrease is not clear since the testis was found to be normal in fact with higher degree of spermatogenesis.

Significant decrease in Hb concentration (Table 3) was observed in both the treated groups, may be due to several causes like haemolysis, decrease in the formation and release of RBC's and formation and release of immature RBC'S. If we analyze different RBC related parameters it could be observed that there was no significant decrease in RBC count, instead a moderate increase was observed, since MCV and RDW valued did not increase, the decrease due to formation and release of immature RBC can be ruled out. Then remains the possibility of incorporation of Hb to the RBC's. In this regard if we analyze MCH value an explanation can be found. MCH represents weight of hemoglobin in pictograms of a single RBC. In this parameter a significant more than 50% decrease was observed. Thus it can be suggested that the decrease in Hb may be due to interference with the incorporation of Hb in to the RBC. HCT increases when there is an increase in the RBC count especially in polycythemia. Since a moderate increase was observed in RBC count it can be suggested as the reason for the observed HCT. This may be a reflex

effect due to decrease observed in Hb concentration. On the whole it can be suggested that the test drug do not have marked hemotoxicity related to RBC parameters. Whatever changes that have been observed were only of moderate magnitude.

In VB treated group none of the studied parameters were found to be affected, where as significant decrease in lymphocytes and significant increase in Granulocytes were observed in VSVB treated group.(Table 3) All forms of granulocytes are produced in the bone marrow and are termed 'myeloid series'.[9] Decrease of the Lymphocytes count i.e. known as Lymphopenia is uncommon and occurs in most acute infections, severe bone marrow failure, and immunosuppressive therapy. Further when there is increase in granulocyte, especially of low intensity, lymphocyte percentage tends to be on the lower side. If we analyze the taking all the four parameters it emerges that though not significant a moderate increase in WBC count was observed in both the groups. This may be due to low level of inflammation produced by the drugs in certain organs like jejunum and kidney.

Significant decrease was observed in Blood urea and S.Alkaline phosphates in both the treated groups.(Table 4) In normal healthy person the blood urea level depends on the rapid and efficient excretion from kidney. Elevated levels are normally seen when the kidney function is affected. Decrease may be seen when liver function is affected.[10] In this study both the administered group showed significant decrease in weight of liver, hence the observed decrease in serum urea level can be better judged by considering changes in histopathological study of the liver. Histological examination of liver showed normal cytoarchitecture in VSVB group and in VB group only minor changes were observed which can be considered responsible for the observed decrease. The activity of S.Alkaline phosphates is considered as the most valuable index of osteoblastic activity. In adults the serum enzyme level is mainly derived from bone marrow and some portion from intestine. Its serum level is elevated in the following pathological conditions like biliary obstruction, diseases of the bone and during pregnancy. It may be reduced due to the capacity of the liver to synthesize and release this enzyme. In the present study weight of liver was observed to be decreased in comparison to control group, hence the observed decrease may be due to drug induced moderate alteration in the liver function. . However, since decrease is observed in both blood urea and alkaline phosphates activity it can be suggested that it may be reflecting mild to moderate interference with the liver function and has no serious toxic implications.

The fatty acids and glycerol are derived from hydrolysis of ingested fat (after emulsification) by lipase in the lumen. Fat digestion and absorption occur mainly at the duodenum- jejunal junction. Decrease in serum triglycerides(Table 4) may be due to less intake of fat diet or interference with its absorption. Another possibility is the increase in peripheral utilization and decrease in its metabolism. If no functional impairment is involved this effect may rather be considered as favorable effect for reducing the elevated triglyceride levels. The proteins are the chief solids of the plasma, out of which, albumin is the most abundant and fairly homogenous. Hypo-albuminaemia may occur in liver disease having destruction of hepatocytes while hyperglobulinaemia may be present in chronic inflammatory disorders. In the present study significant decrease was observed only in the VSVB treated group. This decrease is mainly due to slightly higher value of serum globulin level and slightly lower value of serum albumin. Individually they did not amount to any significant change however, when A/G ratio was calculated it got converted to significant decrease in A/G ratio. Thus it may not be of any pathological significance.

In Histopathology of important organs like brain, spleen, thymus, lymph node, heart, lungs, liver, intestine, kidney, testis, uterus and ovary were carried out. Out of which in VB treated group changes were observed in Heart, Kidney, Jejunium and Seminal vesicle. In case of VSVB Group, changes were observed in three organs i.e. Kidney,Jejunium and Seminal vesicle The observed changes were increased proliferation in both the groups, changes in kidney and jejunum in both the groups and mild cell infiltration and fatty changes in some rats in VB group. Among the observed changes none were severe. The increased epithelial proliferation observed with respect to seminal vesicle cannot be considered as toxic change since it represents stimulation of the activity not any degenerative changes. The exact reason for this stimulation is not known. Interestingly very good spermatogenesis was evident in testis sections. Both the drugs have the predilection for producing mild to moderate pathological changes in kidney. Since kidney related biochemical parameters blood urea and serum creatinine level were not elevated it can be considered as not serious. However, this fact should be borne in mind while prescribing these drugs in persons with pre-existing renal insufficiency in which it is better to avoid them. Further these changes are observed with 5 x TED dose and may not be evident at the TED dose level. Inflammatory changes were observed in jejunum- they may not be evident at TED dose level or can be avoided with suitable Anupana (Vehicle) like honey.

Effect on ponderal parameters

Table 1: Effect of both the Vanga Bhasma test preparations on body weight in Chronic Toxicity study.

Group	Dosage (mg/kg)	Body Weight (g)					
		Initial	Final	Actual change (g)	% change	% change of body weight	% change
Control (n=6)	Distilled water	218.00 ± 10.67	249.67 ± 17.75	31.67 ± 07.75	----	13.94 ± 2.93	----
VB (n=6)	250	220.00 ± 21.17	238.33 ± 20.06	18.33 ± 04.83	42.12 ↓	09.14 ± 3.04	34.43 ↓
VSVB (n=4)	250	237.50 ± 02.50	258.50 ±15.67	21.00 ±16.46	08.93 ↓	08.93 ± 6.93	35.93 ↓

Table -1 shows the Effect of both the Vanga Bhasma test preparations on body weight of Charles foster Rats in Chronic Toxicity study. The Data presented by Mean±SEM where 6 animals in each group. A 'p' value less than 0.05 is considered as statistically significant. A 'p' Value more than 0.001 is considered as statistically highly significant. * P < 0.05, ** P< 0.01, *** P< 0.001.

Table 2: Showing the effect of both the Vanga Bhasma on Relative weight of different organs of Charles foster rats in Chronic Toxicity study (mg/ 100 g body wt.).

Organ Weight	Control Group	VB Group	VSVB Group
Thymus	231.38 ± 10.22	194.02 ± 22.70	189.73 ± 19.21
Liver	3272.80 ± 88.58	2655.4 ± 101.20***↓	2918.90 ± 111.19*↓
Spleen	301.40 ± 22.23	227.60 ± 18.17*↓	232.70 ± 24.98*↓
Kidney	657.30 ± 08.35	598.2 ± 12.51**↓	633.40 ± 27.52
Testis	587.8 ± 32.59	777.86 ± 314.72	757.90 ± 172.93
Seminal Vesicle	438.90 ± 38.89	350.66 ± 02.90	356.90 ± 34.44
Prostate	136.30 ± 07.83	118.50 ± 34.39	110.25 ± 04.63

Data: Mean±SEM ↓: Decrease, * P < 0.05,** P< 0.01,*** P< 0.001

Table – 2 Shows the effect of both the Vanga Bhasma on Relative weight of different organs of Charles foster rats in Chronic Toxicity study (mg/ 100 g body wt.) The Data presented by Mean±SEM where 6 animals in each group. A 'p' value less than 0.05 is considered as statistically significant. A 'p' Value more than 0.001 is considered as statistically highly significant. * P < 0.05, ** P< 0.01, *** P< 0.001.

Table 3: Showing the effect of both the Vanga Bhasma on Hematological parameters in Chronic Toxicity study.

Parameters	Control Group	VB Group	VSVB Group
Lymphocyte (%)	66.85 ± 02.92	61.31 ± 01.50	55.70 ± 04.40** ↓
Hemoglobin (g/dl)	11.40 ± 00.30	06.15 ± 00.34*** ↓	06.45 ± 00.33*** ↓
RBC (10^6/µl)	05.65 ± 00.23	06.43 ± 00.10** ↑	06.40 ± 00.10** ↑
HCT (%)	45.58 ± 01.81	55.10 ± 01.8** ↑	54.50 ± 02.3* ↑
MCH (pg)	20.30 ± 00.73	09.30 ± 00.35*** ↓	08.60 ± 00.33*** ↓
RDW (%)	06.68 ± 00.20	05.91 ± 00.30* ↓	06.10 ± 00.40 ↓

Table 3 shows the effect of both kinds of Vanga Bhasma preparations on Haematological parameters of Charles foster rats in chronic toxicity study. The Data presented by Mean±SEM where 6 animals in each group. A 'p' value less than 0.05 is considered as statistically significant. A 'p' Value more than 0.001 is considered as statistically highly significant. * P < 0.05, ** P< 0.01, *** P< 0.001.

Table 4: Showing the effect of both the Vanga Bhasma on Biochemical parameters in Chronic Toxicity study.

Parameters	Control Group	VB Group (250mg/kg)	VSVB Group (250mg/kg)
Blood Glucose(mg/dl)	92.00 ± 02.60	93.50 ± 04.20	94.00 ± 04.90
S.Cholesterol (mg/dl)	58.50 ± 05.10	76.80 ± 08.50	57.50 ± 05.30
S.Triglycerides(mg/dl)	218.10 ± 47.40	124.50 ± 33.25	95.75 ± 28.00* ↓
Blood Urea(mg/dl)	48.50 ± 01.60	34.30 ± 02.18*** ↓	36.20 ± 02.59*** ↓
S.creatinine(mg/dl)	00.65 ± 00.02	00.63 ± 00.02	00.67 ± 00.02
Total Bilirubin(mg/dl)	00.60 ± 00.08	00.60 ± 00.08	00.47 ± 00.06
Alkaline Phosphatase activity(IU/L)	253.40 ± 42.20	073.60 ± 13.60*** ↓	112.00 ± 19.10** ↓
S.G.O.T. activity(IU/L)	401.20 ± 62.40	361.50 ± 73.60	302.50 ± 26.84
S.G.P.T.(IU/L)	116.60 ± 11.34	99.33 ± 21.76	86.20 ± 06.96
A/G Ratio	01.48 ± 00.07	01.35 ± 00.08	01.10 ± 00.07** ↓

↓: Decrease, ↑: Increase, * P < 0.05, ** P< 0.01, *** P< 0.001

Table 4 shows the effect of both kinds of Vanga Bhasma preparations on various 10 types of Biochemical parameters of Charles foster rats in chronic toxicity study. The Data presented by Mean±SEM where 6 animals in each group. A 'p' value less than 0.05 is considered as statistically significant. A 'p' Value more than 0.001 is considered as statistically highly significant.

CONCLUSION

In acute toxicity study, the animals in both the test drug groups did not manifest any signs of toxicity up to 40 times (2000 mg/ kg) human therapeutic equivalent dose (50mg/kg). In chronic toxicity study, no serious toxicity was found in both the test drug groups. Only cell infiltration and fatty changes in the Kidney were observed in VB group and VSVB group respectively. Though no marked degenerative changes were observed in any of the organ studied, there are indications that especially kidney and jejunum may be affected if higher dose levels are used over a prolonged period.

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