International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 2 Issue 10, October 2015

ISSN: 2394-4404

In Vivo Evalution Of Neuroprotective Activity Of Manikya Pishti

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Abstract: ***Previously ratnas were used for parad bandhan. As the priority of Ras shastra is to attain a Body that is deha dharan , hence Ras shastra had used almost all the ratnas(gems) for the purpose of inducing the longevity of life in human body. Also internal use of the ratnas can cure the several diseases present on the earth.***

Neurodegeneration is the umbrella term for the progressive loss of structure or function of neuron including death of neurons. Neurodegeneration can be found in many different levels of neuronal circuity ranging from molecular to systemic. Neuron cells once died cannot be regenerated .Today the cases of neurodegenerative diseases are increasing day by day because of environmental haphazard, pollution, stress and sedentary life style of people

There are number of patients suffering from neurodegenerative disease in India.Treatment for the diseases is only symptomatic and treatment given is very costly. If neuroprotective drugs are used in such patients it may prove beneficial. As per ayurvedic text Manikya is medhya, vaat kapha har, bhutonmad naashak, vaatnaadi paushtikar.

Hence in the present study an attempt is made to carry out the experimental study of neuroprotective activity in swiss albino mice.

I. INTRODUCTION

Ayurveda being ancient Indian science its theme is to maintain the proper health and to cure the diseases from human being.

From the historical point of view, upto the seventh century herbal preparations were used for curative purpose and after this period the uses of minerals and metals for therapeutic purposes started .Since these metals and minerals could not be used orally in their crude form hence it was necessary to have specialised technical knowledge of converting minerals and metals into biologically effective form i.e deha satmya [5.] Hence the concept of separate branch of Rasa-shastra emerged in the field of Ayurveda. The word Rasa denotes numerous things including mercury,which has been extensively used in the preparation of potent bhasma.

Rasashastra (Iatrochemistry) had used almost all the gems for the purpose of inducing longevity of life in human body. (Vaidyopadhyaya, 1983)Also internal use of gems can cure several diseases (Vaidyopadhyaya, 1983). According to *Ayurveda, Manikya* has been grouped under *ratna varga. Manikya Pishti* preparation from precious gem *Manikya* is a famous Ayurvedic preparation. It is a versatile drug having properties like memory enhancing, aphrodisiac and specially recommended in erectile dysfunction, general debility, and it has best *rasayana* (antioxidant) as well as aphrodisiac property. So an attempt was made to do the experimental study of *Manikya Pishti* to facilitate its use in Ayurvedic therapeutics.

II. MATERIALS AND METHODS

MATERIALS

In ancient Ayurvedic classics, an emphasis has been placed on animal experimentation prior to its administration to human subjects. Sushruta states that man occupies a supreme position among all the living creatures. Hence for experimental trail, other animals should be utilized as experimental models.Present experimental study was carried outnat Haffkine institute.

Manikya pishti has been used in patients since ancient times. In an effort to correlate the ancient knowledge with the modern concepts of research in pharmacology, the effects of Manikya Pishti on some neuroprotective parameters in murine model were studied. Hence preclinical or Expermental study was conducted for Manikya Pishti.

ETHICAL COMMITTEE

Permission of Institutional Animal Ethics Committee (IAEC) was taken before conduction of experimentvide Letter

No.. The study was conducted at the Department of Toxicology, under guidance of expert.

Environmental and Housing conditions were maintained throughout the experiment for all the animals as follows:

HOUSING OF ANIMALS & ENVIRONMENTAL CONDITION

Temperature was maintained at 24°C to 27°C and humidity at 50% to 70%. The animals were maintained at controlled environmental condition with natural light and dark cycle.

Figure 1: Food & Water of Animals

HOUSING

- ✓ *CAGING:* Polypropylene Cages covered with stainless steel grid top with water bottle (300 mL capacity) were used.
- ✓ *BEDDING:* Clean corn husk.
- ✓ *WATER BOTTLE:* Polypropylene bottles with a stainless steel nozzle having capacity of 300 mL.
- ✓ *DIET:* Rodent pellet diet given to all the animals. 10 gm of these pellets were kept in feed tray every day.
- ✓ *WATER:* Drinking water filtered through Aqua guard water system was provided.

ANIMAL IDENTIFICATION

Animals were marked on their body parts e.g. head, tail, head body tail, bodt tail and no mark using picric acid. Appropriate labels were attached to the cages indicating study, group, sex and cage number.

DESIGN OF PRECLINICAL WORK

Name of center	:	Dept. of Toxicology.
Type of study	:	In Vivo.
Type of animal	:	Swiss albino mice
Selection of animal	:	Ave. Wt.:- 20 to 25 gm Sex :-Female
Route of administration	:	Orally by gavage method
No. of animal	:	6 animals for Limit dose. 36 animals for neuroprotective study.
Drug vehicle	:	Ghrut and Madhu
Duration of study	:	3 weeks

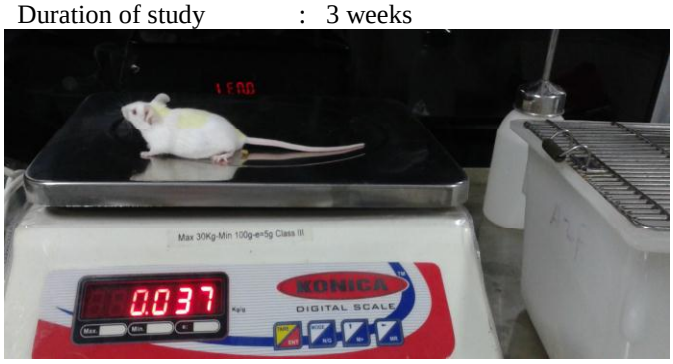

Figure 2

LIMIT DOSE TOXICITY STUDY

Before the actual study of neuroprotective activity of Manikya pishti the limit dose toxicity study of these test drug were done to see if the test drug shows any toxicity in its highest dose as per the OECD guidelines. 3 animals were used for the acute toxicity study.

DRUG DOSE

As per OECD guide lines, when available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/Kg body weight), then a limit test should be conducted.

A limit test at one dose level of 2000 mg/kg body weight was carried out with 3 animals per preparation.

MATERIAL

Test drug: Manikya pishti.

Vehicle control: Honey: 1 parts + Cow's ghee: 3 parts

Syringe with Oral canula: Tuberculin syringe with oral canula no.20 attached to it

ANIMAL SPECIES: Swiss albino mice. Animals were acclimated for seven Days prior to the experiment.

ANIMAL SEX: Female

ROUTE OF DRUG ADMINISTRATION: Oral

TEST DOSE: Single Dose

METHODOLOGY

PREPARATION OF COMPOUND

- ✓ The required amount of test drug i.e. Manikya Pishti were weighed on the Metler balance as per standard operating procedure.
- ✓ Weighed test compound was transferred into Petri dish.
- ✓ The vehicle was prepared freshly by taking 1 part of Honey and 3 parts of Cow's ghee (total 8 mL vehicle prepared by taking 2 parts of honey and 6 parts of Cow's ghee.)
- ✓ 1 mL of vehicle added to Petri dish containing Manikya pishti (one dose at a time), mixed it properly and transferred to tuberculin syringe syringe.
- ✓ Volume of administration is 1 mL.

- ✓ The test compound administered into oesophagus with the help of canula attached to syringe.
- ✓ Appropriate volume to be administered is made as per calculated dose which is given as follows.

NOTE: Acute toxicity of Manikya pishti was not seen in Swiss albino mice

NEUROPROTECTIVE ACTIVITY

Sr.No.	Group	No. of animal	Duration of study
1.	Negative Control Group	3	3 weeks
2.	Toxicity Control Group	6	3 weeks
3.	Vehicle Control Group	3	3 weeks
4.	Experimental Test GroupA 1	5	3 weeks
5.	Experimental Test Group A 2	5	3 weeks

Table 1: Grouping of animals for Neuroprotective activity was done as follows

All animals were given food and water *ad libitum***.** Materials and preparation of compound for Neuroprotective activity was same as limlit dose toxicity explained above.

III. METHODOLOGY

INDUCTION OF NEUROTOXICITY

The known and standard neurotoxic drug MPTP (1-methyl,4-phenyl 1,2,3,6 tetrahydropyridine) was used for the induction of neurotoxicity. The rout of administration of this drug was intraperitoneal (i.p). Except the healthy control group all the other 7 groups were induced by these MPTP drug at its standard dose of 40 mg/Kg body wt.

CONTROL GROUP (NEGATIVE CONTROL GROUP)

This group was the healthy control group. No test compound was given to the animals in this group. Total 3 animals were there in this group. On the last day of the study (21st Day) animals in this group were also sacrificed with the other groups and further studies were carried out.

TOXICITY CONTROL GROUP

Animals in this group were injected with MPTP (1-methyl,4-phenyl 1,2,3,6 tetrahydropyridine) i.p. drug at a dose of 40 mg/Kg body wt. A total of 6 animals were included in this group. On the last day of the study (21st day) animals in this group were also sacrificed with the other groups and further study was carried out.

VEHICLE CONTROL GROUP

In this group only vehicle i.e. ghee and honey were given to the animals. The group had 3 animals and on the last day of the study (21st day) animals in this group were also sacrificed with the other groups and further study was carried out..

EXPERIMENTAL TEST GROUP A 1

This group was administered Manikya pishti at a dosage of 2mg/Kg body weight. The group consisted of 5 animals and on the last day of the study (21st day) these animals were also sacrificed with the other groups and further study was carried out.

EXPERIMENTAL TEST GROUP A 2

This group was administered Manikya pishti at a dosage of 4 mg/Kg body weight. The group consisted of 5 animals and on the last day of the study (21st day) these animals were also sacrificed with the other groups and further study was carried out.

After the animals were sacrificed Whole Blood in EDTA bulb was collected used for hematological analysis along with the Brain tissues. The Brain tissue was segregated into two halves sagitally and one half was kept at -80°C this part was homogenized and used for Biochemical analysis, the other half was stored in formalin and later processed for histological analysis. Muscle tissue was also collected and used for histological analysis.

OBSERVATIONS AND RESULT OF EXPERIMENTAL STUDY

HEMATOLOGICAL PARAMETERS

Hematological parameters were seen after the collection of blood sample taken from each group of animals and there results were as follows:

RESULT

MICE CODE	H. B GM %	RBC x 10^6 / cmm	WBC X 10^3 /cmm	PLT X 10^5/ cmm	PCV %	MCV fl	MCH Pg	MCHC Gm/dl	N. %	E. %	L. %	M. %
A1	14.1	8.81	9.1	7.12	40.3	45.7	16.0	35.0	30	00	69	01
A2	14.5	8.34	5.8	1.95	40.6	48.7	17.4	35.7	17	00	83	00
MPTP Control	12.9	7.64	5.1	6.75	36.4	47.6	16.9	35.4	21	00	79	00
Healthy Control	13.6	7.97	7.1	5.77	39.6	49.7	17.1	34.3	23	00	77	00
Vehicle Control	15.1	8.21	7.1	4.86	43.7	53.2	18.4	34.6	27	00	73	00

Table 2

Hb : Hemoglobin g / dl
RBC : Red Blood Cells million / cmm
WBC : White Blod Cells thousands / cmm
PLT : Plaelet Count lakhs / cmm
PCV : Packed Cell Volume %
MCV : mean corpuscular volum
MCHC : mean corpuscular haemoglobin concentration
MCH : mean corpuscular haemoglobin

N. Neutrophilis %
E : Eosinophils %
L : Lymphocytes %
M : Monocytes %

RESULT

Both Manikya Pishti increases the haemoglobin but comparitively Manikya pishti is good increaser of haemoglobin.

BIOCHEMICAL PARAMETERS: Estimation of the biochemical parameters after the sacrifice of the animals was carried out by the brain –organ homogenate .One way Annova test was applied and the observations and results were as follows.

Groups	Mean	Standard deviation
HC	5.813	0.814
TC (SC)	16.58	1.054
VC	15.66	0.873
A 1 (PLD)	11.58	1.114
A 2 (PHD)	6.05	0.747

	Sum of Squares	Df	Mean Square	F
Between Groups	794.2	6	132.4	168
Within Groups	27.57	35	o.7876	
Total	821.7	41	_	

Table 3: Effect of Manikya Pishti on Lipid peroxidase (LPO) levels in MPTP induced neurotoxicity

Significance at p<0.0001
# means compared to HC(healthy control)
$ means compared to VC (vehicle control)
* means compared to TC (toxicity /MPTP control)

Intra-peritoneal administration of MPTP significantly increased the concentration of lipid peroxides (16.58±1.054 µg/mg protein) in brain tissue, as measured by concentration of MDA when compared with healthy control (5.813±0.214 µg/mg protein). Treatment with Manikya Pisti dose dependently reduced lipid peroxide concentrations compared with Toxicity control and Vehicle control. However, Vehicle control (15.66±0.873 µg/mg protein) did not produce any statistically significant changes in the lipid peroxide concentrations when compared with Toxicity control.

RESULT: Pishti exhibited significant protection as compared to toxicity control.

Groups	Mean	Standard deviation
HC	15.42	0.214
TC (SC)	5.16	0.534
VC	4.12	0.631
A 1(PLD)	6.94	0.714
A 2 (PHD)	10.14	1.04

	Sum of Squares	Df	Mean Square	F
Between Groups	678.5	6	113.1	222
Within Groups	17.83	35	0.5094	
Total	696.4	41	–	

Table 4: Effect of Manikya pishti on reduced glutathione (GSH) levels in MPTP induced neurotoxicity

Significance at p<0.0001
# means compared to HC(healthy control)
$ means compared to VC (vehicle control)
* means compared to TC (toxicity /MPTP control)

Prominent oxidative stress in colonic mucosa was induced by MPTP in the saline control as shown by the decrease in brain GSH levels (5.16±1.054 µg/mg protein) when compared with healthy control (15.420±0.214 µg/mg protein). This oxidative abnormality in brain tissue was ameliorated by pishti and bhasma in dose dependent manner (PLD 6.94±0.714, PHD 10.14±0.747 & BLD 4.21±0.673, BHD 11.92±0.7 µg/mg protein; *P*<0.05) respectively, that is manifested as the significant increase in GSH levels when compared with toxicity & vehicle control (*P*<0.05). Further, vehicle control (4.12±0.631 µg/mg protein) did not significantly alter the GSH levels when compared with toxicity control.

RESULT: Pishti exhibited significant protection as compared to toxicity control.

Groups	Mean	Standard deviation
HC	0.173	0.02
TC (SC)	0.057	0.003
VC	0.06701	0.0014
A 1 (PLD)	0.061	0.001
A 2 (PHD)	0.741	0.001

	Sum of Squares	Df	Mean Square	F
Between Groups	0.06319	6	0.01053	142.6
Within Groups	0.002585	35	0.0000 7385	

Total	0.06577	41	_	

Table 5: Effect of Manikya Pishti on Superoxide dismutase (SOD) levels in MPTP induced neurotoxicity

Significance at p<0.0001
# means compared to HC(healthy control)
$ means compared to VC (vehicle control)
* means compared to TC (toxicity /MPTP control

Severe oxidative stress induced by intra peritoneal administration of MPTP showed a significant decrease in the SOD levels (0.057 ± 0.003 U/mg protein) in toxicity control as compared to healthy control (0.103 ± 0.02 U/mg protein). All the treatment groups failed to elevate the level of superoxide dismutase.

Groups	Mean	Standard deviation
HC	46.44	6.171
18.84		2.45
15.66		1.773
21.17		3.271
29.423		3.412

	Sum of Squares	Df	Mean Square	F
Between Groups	0.06319	6	655.1	51.43
Within Groups	445.8	35	12.74	
Total	4376	41	_	

Table 6: Effect of Manikya Pishti on Catalase levels MPTP induced neurotoxicity

Significance at p<0.0001
# means compared to HC(healthy control)
$ means compared to VC (vehicle control)
* means compared to TC (toxicity /MPTP control)

Intra-peritoneal administration of MPTP showed a significant decrease in the catalase levels (18.84±2.450 U/mg protein) in brain tissue of toxicity control when compared with healthy control (46.440±4.780 U/mg protein). Treatment with high doses of pishti and bhasma 4 mg/kg & 2 mg/kg (29.423±3.412 & 31.42±4.01 U/mg protein) significantly ameliorated the catalase levels as compared to toxicity and vehicle control. Nevertheless, vehicle control and low doses of pishti and bhasma (15.66±1.773, 21.17±3.271 & 22.01±1.8749 U/mg protein resp) did not produce any significant change in the catalase levels when compared with toxicity control.

RESULT: Pishti exhibited significant protection as compared to toxicity control.

Groups	Mean	Standard Deviation
HC	23.73	1.03
VC (SC)	2.73	0.26
TC	2.8	0.33
A1 (PLD)	4.35	0.25
A2 (PHD)	8.6	0.29

	Sum of Squares	Df	Mean Square	F
Between Groups	997.4	6	166.2	0.9954
Within Groups	4.615	14	0.3296	
Total	1002	20	_	

Table 7: Effect of Manikya Pishti on CNS Dopamine levels MPTP induced neurotoxicity

Significance at p<0.0001
# means compared to HC(healthy control)
$ means compared to VC (vehicle control)
* means compared to TC (toxicity /MPTP control)

Intra-peritoneal administration of MPTP showed a significant decrease in the Dopamine levels in brain (2.73±0.26 ng/mg protein) in brain tissue of toxicity control when compared with healthy control (23.73±1.02 ng/mg protein). Treatment with high doses of pishti and bhasma 4 mg/kg & 2 mg/kg (8.6±0.29 & 6.4±0.36 mg/kg protein) significantly improved the dopamine levels as compared to toxicity and vehicle control. Nevertheless, vehicle control and low doses of pishti and bhasma (2.8±0.33, 4.35±0.25 & 4.06±0.12 ng/mg protein resp) did not produce any significant change in the dopamine levels when compared with toxicity control.

RESULT: Pishti high dose is most effective on Dopamine. Also at lower doses Bhasma and Pishti exhibited significant protection as compared to toxicity control.Null Hypothesis was rejected since all the groups showed signicant changes. And comparision within the groups was subjected to Bonfoerons multiple test.

HISTOPATHOLOGICAL PARAMETERS

Histopathology of the organ brain was studied and result were as follows:

Lesion Grading : Minimal (1) , mild (2) , moderate (3) , marked (4).
Distribution of Lesions : Focal (a) , Multifocal (b) , Diffuse (c).
No abnormalities detected : NAD.

OBSERVATION

Code	Skeletal Muscle	Brain
A1	NAD	NAD
A2	NAD	NAD
MPTP control	NAD	Mild degree degenerative and necrotic changes with moderate degree gliosis
Normal	NAD	NAD

control		
Vehicle control	NAD	Minimal degree diffuse malacia

Table 8

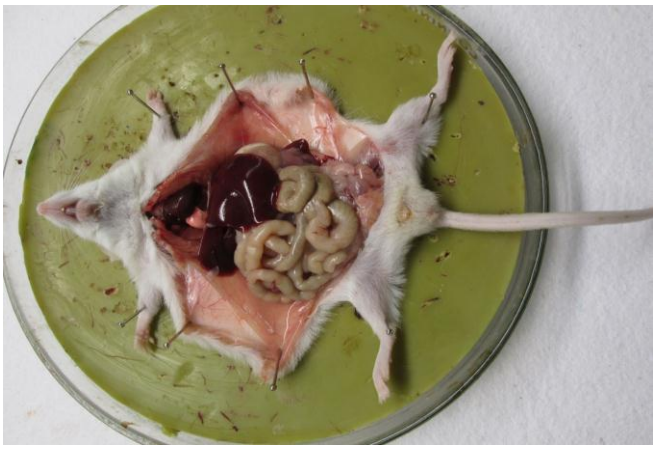

Figure 3

Figure 4

RESULT

Histopathological studies of brain viewed under light microscope in control and experimental animals. Hematoxylin /Eostin staining paraffin sections.

- ✓ Section of brain from mice of healthy control group showed normal architecture.
- ✓ Section of brain from mice treated with dose of Manikya pishti (2 & 4 mg/kg body wt) for 21 days showed normal texture .
- ✓ Section of brain from mice treated with MPTP for 21 days showed pathological changes like cell swelling, vascular degeneration and cytoplasmic vacuolation.
- ✓ Section of brain from mice treated with MPTP for 21 days along with Manikya Pishti showed marked reduction of degeneration and vacuolation.

RESULT OF EXPERIMENTAL STUDY (ANIMAL STUDY)

Hence from these study it can be concluded that Manikya Pishti is effective neuroprotective formulations.

IV. DISCUSSION

The Neuroprotective activity of Manikya Pishti was seen in the Present study.

First of all, the animals were given the drug dose that is Manikya Pishti and on the 7th day the Neurotoxic drug MPTP was given and after the induction of this neurotoxic drug the test dose that is Manikya Pishti was again administered orally to the animals for further 14 days. After 21 days the animals were sacrificed and the organ that is brain is collected and further study was carried out with the organ homogenate. Statistically One way Annova test was applied.

EFFECT OF MANIKYA PISHTI ON MALONEDIALDEHYDE (MDA) LEVELS

Intra-peritoneal administration of MPTP significantly increased the concentration of lipid peroxides (16.58±1.054 µg/mg protein) in brain tissue, as measured by concentration of MDA when compared with healthy control (5.813±0.214 µg/mg protein). Treatment with Pisti dependently reduced lipid peroxide concentrations compared with toxicity (MPTP) and Vehicle control. However, Vehicle control (15.66±0.873 µg/mg protein) did not produce any statistically significant changes in the lipid peroxide concentrations when compared with toxicity control.

EFFECT OF MANIKYA PISHTI ON REDUCED GLUTATHIONE (GSH) LEVELS

Prominent oxidative stress in colonic mucosa was induced by MPTP in the saline control as shown by the decrease in brain GSH levels (5.16±1.054 µg/mg protein) when compared with healthy control (15.420±0.214 µg/mg protein). This oxidative abnormality in brain tissue was ameliorated by pisti in dose dependent manner (PLD 6.94±0.714, PHD 10.14±0.747 & BLD 4.21±0.673, BHD 11.92±0.7 µg/mg protein; $P<0.05$) respectively, that is manifested as the significant increase in GSH levels when compared with saline & vehicle control ($P<0.05$). Further, vehicle control (4.12±0.631 µg/mg protein) did not significantly alter the GSH levels when compared with Toxicity control. Studies conducted by *Sriram et al.* demonstrated that in the midbrain of experimental mice treated with MPTP and GSH agonists, Mitochondrial Complex I was inhibited only 18 h after MPTP dose and no change was observed at the early time points examined. This implied that the depletion of GSH and increased ROS formation preceded the inhibition of the mitochondrial enzyme in the midbrain. Thus in the present study the pisti may protect the neurons by interacting with pathways pertaining to generation of GSH.

EFFECT OF MANIKYA PISHTI ON SUPEROXIDE DISMUTASE (SOD) LEVELS

Severe oxidative stress induced by intra peritoneal administration of MPTP showed a significant decrease in the SOD levels (0.057 ± 0.003 U/mg protein) in saline control as compared to healthy control (0.103 ± 0.02 U/mg protein). All the treatment groups failed to elevate the level of superoxide dismutase.

EFFECT OF MANIKYA PISHTI ON CATALASE LEVELS

Intra-peritoneal administration of MPTP showed a significant decrease in the catalase levels (18.84±2.450 U/mg protein) in brain tissue of saline control when compared with healthy control (46.440±4.780 U/mg protein). Treatment with high doses of pisti 4 & 2 mg/kg (29.423±3.412 & 31.42±4.01 U/mg protein) significantly ameliorated the catalase levels as compared to Toxicity and vehicle control.

EFFECT OF MANIKYA PISHTI ON CNS DOPAMINE LEVELS

Intra-peritoneal administration of MPTP showed a significant decrease in the Dopamine levels in brain (2.73±0.26 ng/mg protein) in brain tissue of saline control when compared with healthy control (23.73±1.02 ng/mg protein). Treatment with high doses of pisti 4 & 2 mg/kg (8.6±0.29 & 6.4±0.36 ng/mg protein) significantly improved the dopamine levels as compared to toxicity (MPTP) and vehicle control. Araki T. et al. have demonstrated in the past that increased levels of dopamine correlate with inhibition of MPTP toxicity.

HISTOPATHOLOGICAL PARAMETER

Histological examination of Brain section of mice treated with MPTP(1 methyl, 4 phenyl,2,3,4,6 tetrahydropyridine) revealed degenearative changes in the parenchyma of the brain .No infiltratiting polymorphs were seen, therefore the involvement of inflammation as a cause of the degeneration is ruled out.

Animals who were administered Manikya Pishti in addition to induction of neurotoxicity by MPTP(1 methyl, 4 phenyl,2,3,4,6 tetrahydropyridine) exhibit normal brain histology.Hence from these study it can be concluded that Manikya Pishti is effective neuroprotective formulations.

Though Al_2O_3 is a known neurotoxic compound. However Manikya with its chief component as Al_2O_3 exhibits neuroprotection in the present experimental study and also as per the Ayurvedic texts Manikya pishti are medhya, rasayan, vaatkaphahar, balya and madhur rasatmak. This can be attributed to the fact that Manikya is a complex structure with many known and unknown chemical compositions. So Manikya is not to be considered as Al_2O_3 only .Analytical tests like ICPAES have confirmed the prescence of iron, copper, magnesium, calcium, sodium, potassium. These elements have important role in neuroprotection. Thus as awhole , Manikya with all its chemical composition exhibits neuroprotection as observed in experimental study.

The results found in preclinical or experiment study are encouraging which will definitely form the base line for upcoming researchers.

PROBABLE MODE OF ACTION OF DRUG

Neurodegeneration generally occurs due to decreased level of dopamine, decreased level of reduced glutathione, super oxide dismutase, and catalase,increased level of lipid per oxidation,brain injury, increased oxidative stress, exposure to neurotoxic drugs ,increasing age etc. Neurodegeneration means death of neurons and neuron cells once died cannot be regenerated but they can be protected and this protection of neurons is termed as neuroprotection.

For the the protection of neurons it is necessary to stop the causes of the neurodegeneration. From the present study it can be said that Manikya Pishti increases the dopamine level also this pishti due to there antioxidant property increases the level of glutathione, super oxide dismutase, catalase and decreases the level of lipid peroxidase.

Also Mankiya is said to be rasayan that is it does the vayahsthapan and also it is jaravyadhi har hence neurodegeneration due to geriatric reason can also cured by the Manikya Pishti .Manikya increases the immunity that is saptadhatu poshak and vardhak which can protect the neurons from viral infections like encephalitis.

Ayurveda is of the view that Pragyaparadh is the basic cause of all mental and physical health (Cha.sha 1/102) .Medha which is a synonym of Pragya(saanskrit hindi kosh) has a great role in prevention of mental diseases and maintenance of good health and the references of various medhya drugs in Ayurvedic literature confirm the Importance of Medha which incorporates dhee,dhruti and smruti. Manikya is medhya hence it can be used in the condition like pragyapradh that is dhee, dhruti, smruti vibrashtha .Also in the diseases like unmaad, apasmaar and atatvabhinivesha all the three doshas are prakupit and due to it raja and tama gunas are also increased .There sthan sansraya occurs in head and heart ,sangyavaha strotas and smruti is effected .These effected smruti causes the above mentioned diseases.Manikya due to its medhya and rasayan property increases smruti also it is tridosha shamak and balya to brain and heart hence can cure the diseases like unmaad, apasmaar and atatvabhinivesha.

Manikya Pishti both has been proved to be a nanomedicine. Nanomedicines have good functions in cell alteration and also nanomedicines cross the blood brain barrier which have the good role in neuroprotection because dopamine itself cannot cross this blood brain barrier.Hence Pishti as nanomedicine can also be used as neuroprotective

After the induction of Neurotoxic drug i.e MPTP , it gets converted into MPP+ through biotransformation. This MPP+ is highly toxic and it hampers the cellular respiration chain which induces apoptosis.This have high affinity towards dopaminergic neurons hence the dopamine levels decreases gradually after the induction of MPTP. Along with this GSH, Catalase levels also decreases where as LPO level increases due to unsaturated fatty acids. All this in together leads to neurodegeneration . Manikya Bhasma and Pishti in the present study have increased the level of dopamine, GSH, Catalase and decreased the level of LPO ,hence gives the protection to the neurons .From this study it can be said that Manikya Pishti are efficient Neuroprotectives. The exact mechanism of action of Manikya Pishti in awarding Neuroprotection needs to be studied with further molecular and cytological assays.

APPLICATIONS OF THE PRESENT STUDY

ISSN: 2394-4404

Parkinsons disease is todays burning issue, Parkinson's disease (PD) is the most common form of a group of progressive neurodegenerative disorders characterized by the clinical features of parkinsonism, including bradykinesia (a paucity and slowness of movement), rest tremor, muscular rigidity, shuffling gait, and flexed posture. Although defined clinically as a movement disorder, it is now widely appreciated that PD can be accompanied by a variety of non-motor symptoms, including autonomic, sensory, sleep, cognitive, and psychiatric disturbances. Nearly all forms of parkinsonism result from a reduction of dopaminergic transmission within the basal ganglia. The discovery of dopamine in the brain, the demonstration of its depletion in PD, and the success of dopamine replacement therapy by its precursor, levodopa, are all major landmarks in the field of neurology. Treatment given in this disorder are levodopa and dopamine agonists like Bromocriptine which also have the lots of the side effects .Dopamine directly cant be given because it cannot cross the blood brain barriers hence the alternative are used.From the present study it can be said that Manikya pishti increases the dopamine levels and hence its use in Parkinsons Disease may prove beneficial.

The greatest risk factor for neurodegenerative diseases is aging. Mitochondrial DNA mutations as well as oxidative stress both contribute to aging. Many of these diseases are late-onset, meaning there is some factor that changes as a person ages for each disease. One constant factor is that in each disease, neurons gradually lose function as the disease progresses with age. In this case if neurodegeneration is due to oxidative stress than Manikya Pishti may prove beneficial in age related neurodegenerative disorders.

Also in Senile Dementia Manikya Pishti may proved beneficial by decreasing the oxidative stress in neuron cells because the main cause of senile dementia is increased oxidative stress in the neurons cells.

Manikya Pishti in the present study have increased the level of dopamine, GSH, Catalase and decreased the level of LPO, hence gives the protection to the neurons .From this study it can be said that Manikya Pishti is efficient Neuroprotective drug. The exact mechanism of action of Manikya Pishti in awarding Neuroprotection needs to be studied with further molecular and cytological assays.

V. CONCLUSION

Following conclusions can be drawn from the present study.

The experimental part proved that Manikya Pishti is neuroprotective drug and following points were concluded from the animal study.

Treatment with Manikya Pisti dose dependently reduced lipid peroxide concentrations compared with Toxicity control and Vehicle control. However, Vehicle control did not produce any statistically significant changes in the lipid peroxide concentrations when compared with Toxicity control.

Prominent oxidative stress in colonic mucosa was induced by MPTP in the saline control as shown by the decrease in brain GSH levels when compared with healthy control .This oxidative abnormality in brain tissue was ameliorated by pishti in dose dependent manner respectively, that is manifested as the significant increase in GSH levels when compared with toxicity & vehicle control ($P<0.05$).

Treatment with high doses of pishti and bhasma 4 mg/kg & 2 mg/kg significantly ameliorated the catalase levels as compared to toxicity and vehicle control. Nevertheless, vehicle control and low doses of pishti and bhasma did not produce any significant change in the catalase levels when compared with toxicity control.

Treatment with high doses of pishti 4 mg/kg & 2 mg/kg significantly improved the dopamine levels as compared to toxicity and vehicle control. Nevertheless, vehicle control and low dose of pishti did not produce any significant change in the dopamine levels when compared with toxicity control.

Histopathological reports too showed that Manikya Pishti proved to be effective in the Neurodegenerative disorders, hence it can be said that Manikya Pishti have the potency of neuroprotection.

Hence from these study it can be concluded that Manikya Pishti both are efficient neuroprotective formulations.m The results found in preclinical or experiment study are encouraging which will definitely form the base line for upcoming researchers .

The exact mechanism of action of Manikya Pishti in awarding Neuroprotection needs to be studied with further molecular and cytological assays.

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