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ACUTE AND SUBACUTE TOXICITY STUDIES OF POLYHERBAL FORMULATION: SHATGANDHA

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ABSTRACT

International regulations relating to human health require that all new pharmaceutical drugs are tested for their safety prior to their use in human being. Though the Herbal formulation thought to be safe, their toxicity study is necessary. Also the World Health Organization (WHO) has indicated assessment of the safety, efficacy and quality of herbal medicines. In this project Shatgandha, the herbal formulation manufactured by Indu Pharma is evaluated for toxicity acute and subacute toxicity as per OECD Guidelines 425. The present finding suggests that Shatgandha is non toxic at dose of 100 mg/kg & 200 mg/kg since no any marked changes in the Hematological, biochemical and histopathological parameters were observed, but at 400 mg/kg it has shown significant increase in AST, ALT, Creatinine & Urea as well as marked Pathological changes in Histopathology of liver. In this study dose of 400 mg/kg has shown toxicity in mice.

Key Words: Shatgandha, acute and sub acute toxicity.

1. INTRODUCTION

A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used. These studies are usually conducted in rodents. This test is particularly contentious as it is the only test in pharmaceutical development where lethality is a key endpoint. [1] [2] Increasingly, there is also controversy about the scientific value of the data obtained and its correlation with predicting acute toxic effects in humans, particularly when compared with

the suffering caused to the animals used. [3] Though the herbal formulation thought to be safe and their toxicity study is necessary. [4] Herbal formulation is thought to be more safe and efficacious way to treat disease but WHO indicate assessment of the safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization. [5] In this study the Proprietary herbal formulation "Shatgandha" is evaluated for acute & subacute toxicity study. Each tablet of Shatgandha consists of Guduchi Satwa 300 mg, [6] Ashwagandha Ghan 200 mg [7] & Excipient quantity sufficient.

2. MATERIAL & METHODS

2.1 Chemicals, Drugs & Diagnostic kits

Shatgandha a herbal formulation was obtained as gift sample.

Carboxy methyl cellulose was purchased from research labs, Pune, India.

Diagnostic kits were purchased from Erba Mannheim.

2.2 Experimental Design

a) Physicochemical Analysis [8]

The formulation was evaluated for physical and chemical properties.

b) Phytochemical Analysis [9]

The chemical test for Carbohydrates, proteins, amino acids, glycosides, saponins, flavonoids, Alkaloids & steroids were as per performed with appropriates reagent.

2.3 Standardization Parameter

As per Ayurvedic Pharmacopoeia the following parameter were evaluated for standardization of herbal formulation. [10]

a. Total Ash

b. Acid insoluble Ash

c. Water soluble Ash

d. Water soluble extractive value

e. Alcohol soluble extractive value

2.4 Animals

Swiss albino mice in the animal house facility of PDEA's SGRS College of Pharmacy were used. The animals were maintained under controlled temperature, humidity and light cycle as per prescribed by the CPCSEA. The experimental protocol was approved by the IAEC (SGRS/IAEC/07/2012-13)

2.5 Acute Toxicity Study

Acute toxicity study was carried out as per the OECD 425 guidelines. Swiss albino mice of either sex weighing 20-25 gm were administered with the dose of 2000 mg/kg orally of Shatgandha and observed closely for first 4 hours for behavioral and Neurological symptoms and then for 72 hours for mortality. [11]

2.6 Sub Acute Toxicity Studies

Swiss albino mice of either sex weighing 20-25 gm were divided in four groups. Group I: Normal control received vehicle, Group II: received 100 mg/kg, Group III: received 200 mg/kg and Group IV: received 400 mg/kg Shatgandha per orally respectively for 28 days. Body weight, food intake and water intake were monitored. [5] The blood was collected from treated animals by retro-orbital puncture method under anesthesia. The serum was separated and analyzed for hematological parameters such as heamoglobin, RBC, WBC, hematocrit and biochemical parameters such as Aspartate Amino Transferase (AST) [12],Alanine Aminotransferase (ALT) [12], Serum Creatinine [13], Blood Urea Nitrogen [14] The biochemical parameters were evaluated by using Autoanalyser CHEM-5 Plus V2 Erba Transasia with Erba Chem diagnostic kits. The Heart, Liver, Kidney & Spleen were isolated & evaluated for histopathological Changes.

2.7 Statistical Analysis

Data is represented as the mean ± SEM. Results were analyzed statistically by using Graphpad Prism 5 software by One Way ANOVA / Two-way ANOVA followed by post Dunnet's test. The minimum level of significance was set at $p<0.05$.

RESULTS

I. Physicochemical Analysis

Table No .1: Physicochemical Properties of Formulation

Parameter	Observation
Size & Shape	1.1cm ,Round Shape
Thickness	10.13mm
Color	Yellowish green colored
Odour	Characteristic
Taste	Bitter
Solublity	Insoluble in water

II. Phytochemical Analysis

The formulation showed the presence of Carbohydrate, Glycosides, Alkaloids Steroid, Saponin and phenolic compounds. Protein and Tannins were found to be absent in the formulation.

III. Standardization Parameter

Table No. 2: Evaluation of Shatgandha

Sr. no	Standardization Parameter	%w/w
1.	Total Ash	17.9
2.	Acid insoluble Ash	1.06
3.	Water soluble Ash	2.9
4.	Water soluble extractive value	13.18
5.	Alcohol soluble extractive value	1.38

IV.Acute toxicity

In acute toxicity study the mice administered with the dose of 2000 mg/kg orally did not show any behavioral and neurological symptoms. No any sign of mortality and morbidity was observed after 72 hours of administration of Shatgandha.

V. Subacute toxicity studies

As seen in the Fig. 1 in the sub acute toxicity study the groups treated with Shatgandha Showed significant increase in body weight as compared to control group.

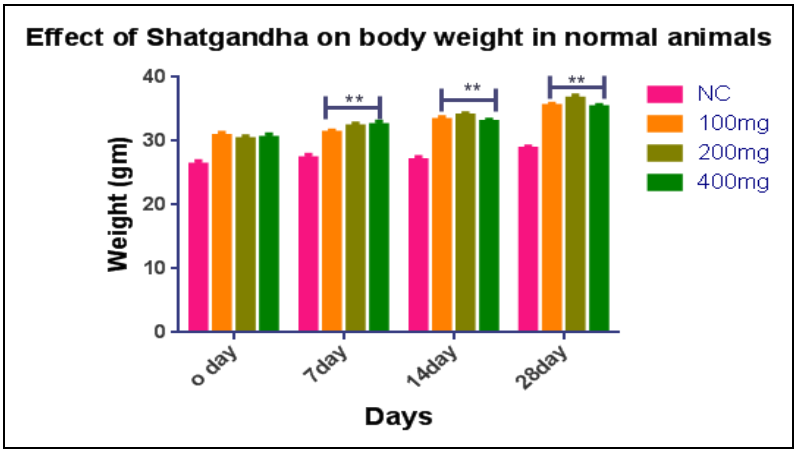


Fig. 01:Effect of Shatgandha on body weight in normal animals

Values are expressed as mean ± S.E.M., n=6; Statistical analysis by Two-way ANOVA followed by post test Dunnet's using Graphpad Prism 5 software; Normal control groups are compared with all treatment groups. * * $p<0.01$.

Table No. 3shows the hematological parameters such as Hemoglobin, RBC, WBC Count and differential leukocyte count, haematocrit and platelet count measured on 29 th day.

Table No. 3Haematological parameters in normal animals

Parameter	Normal control	Shatgandha 100mg/kg	Shatgandha 200mg/kg	Shatgandha 400mg/kg
Haemoglobin (gm %)	10.9±0.16	10.5±0.19	11.1±0.33	10.4±0.12
RBC (cells/cumm)	5.42±0.12	5.69±0.21	5.61±0.27	6.43±0.22
Total WBC (cells/cumm)	7300±108	7570±170	7010±154	7070±181
Neutrophil (% of cell)	55±0.57	56.5±0.76	58.5±0.76	58.5±0.76
Lymphocytes (% of cell)	26.83±0.60	28±0.57	23±0.57	21.33±0.55
Eosinophills (% of cell)	1.33±0.21	0.83±0.30	0.83±0.30	1±0.36
Monocytes (% of cell)	1±0.36	0.66±0.33	0.66±0.33	0.83±0.40
Basophils (% of cell)	00±0	00±0	00±0	00±0
HCT (%)	39±0.31	36.48±1.70	38.38±0.38	36.48±0.43
MCV (Cubic Micron)	83.15±1.56	79.88±0.42	79.78±0.65	80.03±0.73
MCH (Picogram)	26.56±0.92	26.41±1.23	26.41±1.50	28.40±0.83
MCHC (gm/dl)	33.31±0.38	34.5±0.32	34.21±0.32	34.51±0.40
Platelet count (cells/cumm)	251000±3270	279000±4800	271000±3250	260000±2440

Figure 2, 3, 4 & 5indicates the biochemical parameters on 29th day only 400 mg/kg treated group showed significant increase in AST, ALT, Creatinine & BUN , while the dose of 100 mg/kg, 200 mg/kg did not show any significant increase in biochemical parameters.

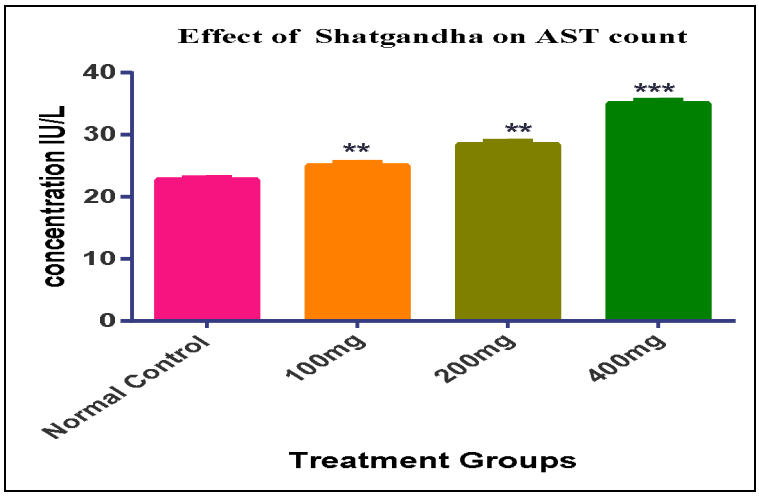


Fig. 02: Effect of Shatgandha on AST in normal animals

Values are expressed as mean ± S.E.M., n=6; Statistical analysis by Two-way ANOVA followed by post test Dunnet's using Graphpad Prism 5 software; Normal control groups are compared with all treatment groups. * * p<0.01, * * * p<0.001.

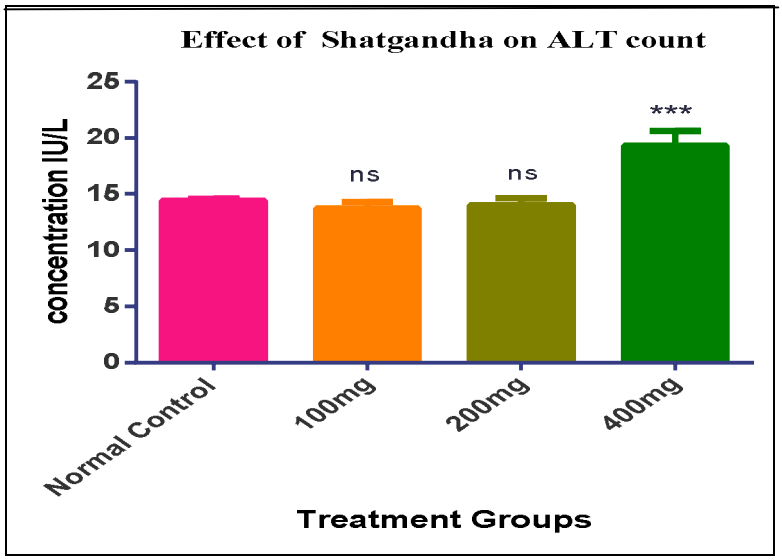


Fig. 03: Effect of Shatgandha on ALT in normal animals

Values are expressed as mean ± S.E.M., n=6; Statistical analysis by Two-way ANOVA followed by post test Dunnet's using Graphpad Prism 5 software; Normal control groups are compared with all treatment groups * * * p<0.001, ns=non significant.

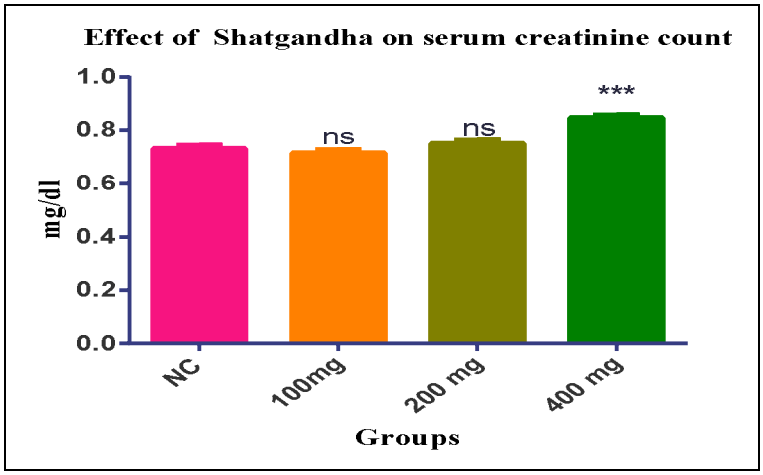


Fig. 04: Effect of Shatgandha on Creatinine in normal animals

Values are expressed as mean ± S.E.M., n=6; Statistical analysis by Two-way ANOVA followed by post test Dunnet's using Graphpad Prism 5 software; Normal control groups are compared with all treatment groups. * * * p<0.001, ns=non significant

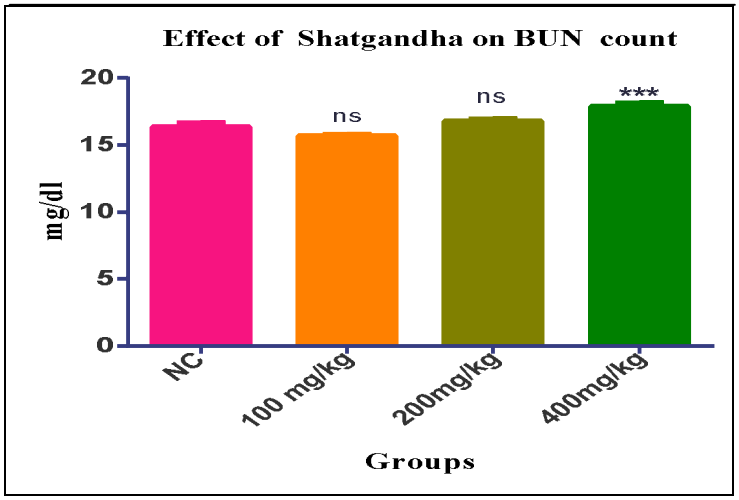
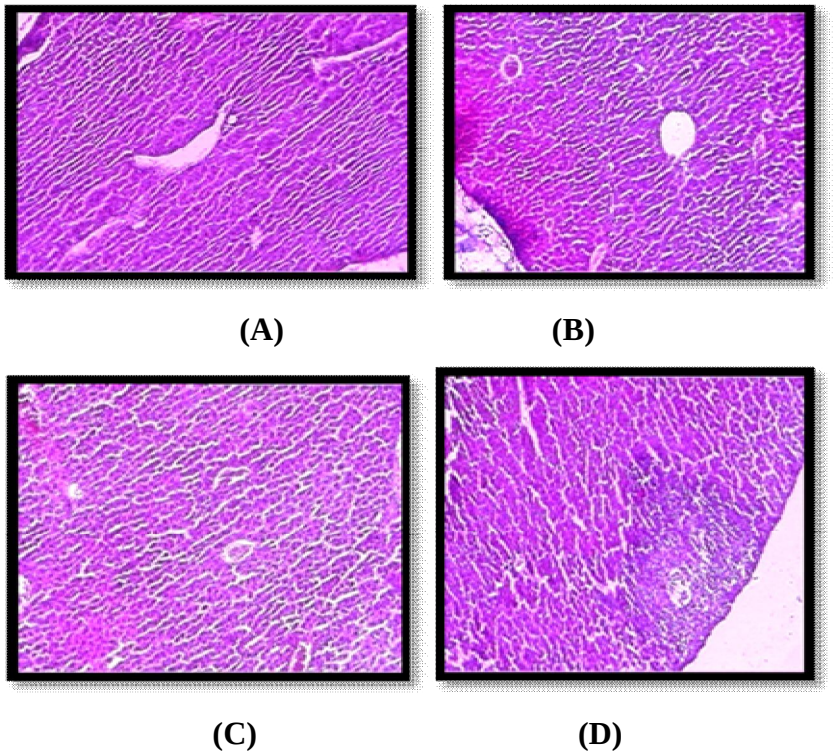


Fig. 05: Effect of Shatgandha on BUN in normal animals

Values are expressed as mean ± S.E.M., n=6; Statistical analysis by Two-way ANOVA followed by post test Dunnet's using Graphpad Prism 5 software; Normal control groups are compared with all treatment groups. * * * p<0.001, ns=non significant.

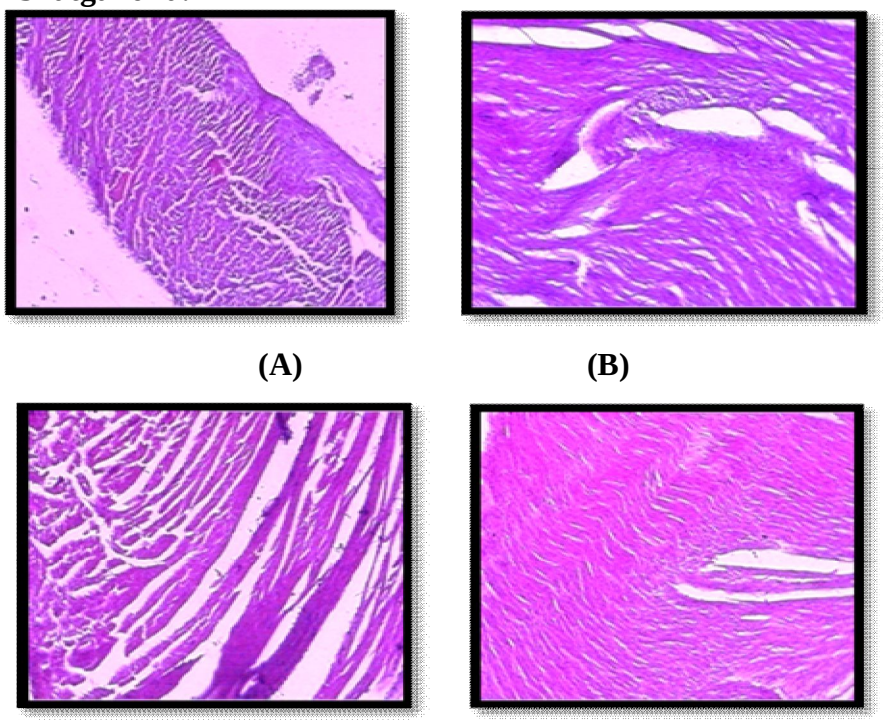
Figure 6, 7, 8 & 9 indicates the histopathological changes in the Liver, Heart, and Spleen & Kidney respectively. At dose 100 mg & 200 mg was found to be safe, but but at 400 mg has shown mark pathological changes in histology of the Liver, Heart, and Spleen & Kidney.



(A) (B)

(C) (D)

Fig. 06: Histopathological sections of Liver: A: Normal Control, B: Treated with 100mg/kg of Shatgandha. C: Treated with 200mg/kg of Shatgandha. & D: Treated with 400mg/kg of Shatgandha.



(A) (B)

(C) (D)

Fig. 07: Histopathological sections of Heart: A: Normal Control, A: Normal Control, B: Treated with 100mg/kg of Shatgandha. C: Treated with 200mg/kg of Shatgandha. and D: Treated with 400mg/kg of Shatgandha.

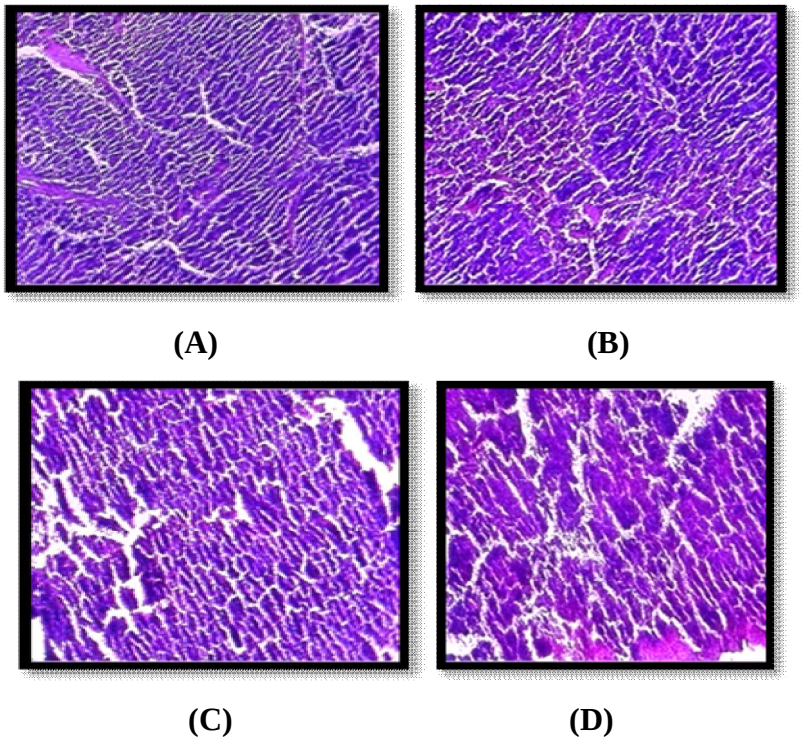


Fig. 08 : Histopathological sections of Spleen: A: Normal Control, B: Treated with 100mg/kg of test drug, C: Treated with 200mg/kg of test drug and D: Treated with 400mg/kg of test drug.

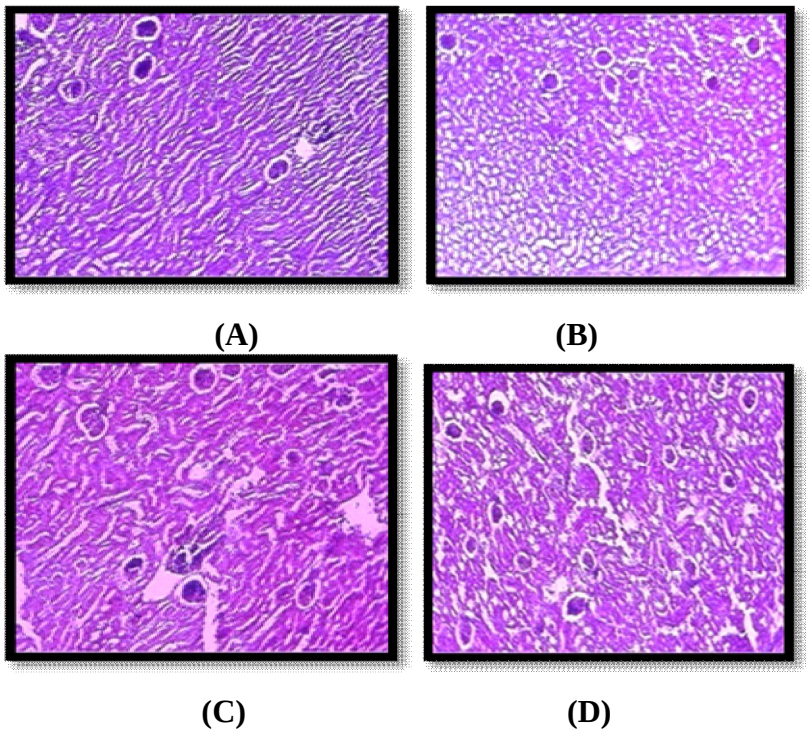


Fig. 09: Histopathological sections of Kidney: A: Normal Control, B: Treated with 100mg/kg of test drug, C: Treated with 200mg/kg of test drug and D: Treated with 400mg/kg of test drug.

DISCUSSION

In acute toxicity studies the polyherbal formulation Shatgandha did not exhibit any lethality or toxic symptoms at the dose of 2000 mg/kg. According to OECD guidelines 425 the polyherbal formulation was administered in increasing order i.e, 175 mg/kg, 550 mg/kg, 1000 mg/kg, 1750 mg/kg and 2000 mg/kg and it did not exhibit any mortality or toxic symptoms hence the formulation was found to be safe. As the dose of 2000 mg/kg was well tolerated by the animals treated further studies were conducted at dose of 100 mg, 200 mg and 400 mg of body weight. As seen in the Figure 1 in the sub acute toxicity study the groups treated with Shatgandha showed significant increases in body weight as compared to control group. This increase in body weight is due to presence of Ashwagandha in the formulation. Ashwagandha & Guduchi have been reported there anabolic effect therefore increasing body weight is inaccordance with the previous result. [15] On 29th day no any significant difference in haematological and biochemical parameters was observed between the Polyherbal Shatgandha formulation and the normal control group. There was no any significant increase in the haematological parameters such as Haemoglobin, RBC, WBC Count & Blood cell indices. In differential leucocytes count Monocytes count was found to be slightly decreased in groups treated with 100 mg/kg, 200 mg/kg, & 400 mg/kg as compared to normal control group. The groups treated with100 mg/kg & 200 mg/kg showed slightly increase in platelet count. But this change in blood cell count is within Physiological limits. This suggests that the test drug is non toxic to the circulating blood cells and does not showed effect on haematopoeisis.

As seen in the Figure No. 2, 3, 4 & 5 on 29th day only 400 mg/kg treated group showed significant increase in AST, ALT, Creatinine & BUN. Dose of 100 mg/kg & 200 mg/kg did not showed any significant increase in the liver parameters like AST & ALT but in case of 400 mg/kg treated group showed minor toxicity to the liver which was further confirmed by histopathological studies while as the normal levels of blood urea and serum Creatinine & normal histological structure of Kidney indicate that the test drug did not affect the renal function.

CONCLUSION

The present finding suggests that Shatgandha is non toxic at dose of 100 mg/kg & 200 mg/kg since no any marked changes in the hematological, biochemical and histopathological parameters was observed, but at 400 mg/kg has shown statistically significant increase in

Biochemical parameters and marked histopathological changes indicating hepatotoxicity, cardiac toxicity, nephrotoxicity & spleen toxicity in Mice.

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REFERENCES

1. ICH M3: Guidance on non-clinical safety studies for the conduct of human clinical trials for Pharmaceuticals in Health Care Management Int J Pharm Bio Sci 1997; 3(4): 612 – 28.
2. Robinson S, Challenging requirement for acute toxicity studies, National center for the Replacement, Refinement & Reduction of Animal: A workshop report 2007; 2-5
3. CDER Guidance for Industry: Single dose acute toxicity testing for pharmaceuticals; Position paper on non-clinical safety studies to Support clinical trials with a single microdose. 1996 CPMP/SWP/2599/02/Rev 1; 2004
4. Nippo Y, ICH Japan, Guidelines for new drug registration with harmonized tripartite guidelines, Challenging requirement for acute toxicity studies 1999
5. Bose A, Review on latest developments in the standardization of Ayurvedic drugs IJPRBS, 2012: Volume1 (3):96-119
6. Pandey M, Surendra K, Tinospora Cordifolia: A Climbing Shrub in health care management, Int J Pharm Bio Sci 2012; 3(4):612 - 28
7. Gupta G.L, Rana A.C, Withania somnifera (Ashwagandha): A Review, Pharmacognosy 2007; Vol 1: 129-32.
8. Indian Pharmacopoeia, Govt of India ministry of health and family welfare published by Indian Pharmacopoeia commission,Ghaziabad, vol-I & II ,2010:PP-14, 752-54
9. Khandelwal K.R, Practical of Phamarmacognosy, nirali prakashan, 21st edition, 2011: PP-25.1-25.9
10. Ayurvedic Pharmacopoeias of India, determination of foreign matter 1996; 1 : 189-97.
11. http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg 425.pdf.
12. King J, The transferase-alanine and Aspartate transaminase, in practical clinical enzymology, Van D Ed, Nostrand Company Limited, 1965; pp.191.

13. Slot C, Plasma Creatinine determination: a new and specific Jaffe reaction method,Scand. J. Clin. Invest. 1965; 17:381.
14. Natelson S, Scott ML, Beffa C, A rapid method for the estimation of urea in biological fluid by means of the reaction between diacetyl monoxime and urea, Am. J.Chem. Pathol, 1951; 21:275.
15.Aphale A.A**,** Chhibba A.D, Subacute toxicity study of the combination of ginseng (panax ginseng) and ashwagandha (withania somnifera) in rats,Indian J Physiol Pharmacol1998; 42 (2): 299-302.