WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 01, 1141-1154. Research Article ISSN 2277– 7105

EFFECT OF DUSHIVISHARI AGADA ON FLUID RETENTION IN DICLOFENAC INDUCED NEPHROTOXICITY- AN ANIMAL STUDY

Nitu Yashwant Wadkar[1]* and Mamata Narvekar[2]

[1]MD. Scholar Agadtantra Department of Agadtantra Evum Vidhi Vaidyak, Y.M.T. Ayurvedic Medical College, Kharghar, Navi Mumbai, Maharashtra, India.

[2]Professor, Department of Agadtantra Evum Vidhi Vaidyak, Y.M.T. Ayurvedic Medical College, Kharghar, Navi Mumbai, Maharashtra, India.

Article Received on
23 October 2020,

Revised on 13 Nov. 2020,
Accepted on 03 Dec. 2020

DOI: 10.20959/wjpr202101-19404

***Corresponding Author**
Dr. Nitu Yashwant Wadkar
MD. Scholar Agadtantra Department of Agadtantra Evum Vidhi Vaidyak, Y.M.T. Ayurvedic Medical College, Kharghar, Navi Mumbai, Maharashtra, India.

ABSTRACT

Diclofenac, a phenyl acetic acid derivative has good analgesic antipyretic and anti-inflammatory activity is additional diclofenac appears to reduce the intracellular concentration of free arachidonate in leukocytes perhaps by altering the release or update of fatty acid. However, in spite of such beneficial role of these drugs serval reports and investigation were issued dealing with their toxic and pathological side effects on various body organs including kidney. Toxic doses of diclofenac can cause nephrotoxicity related fluid retention in human and animals. Ill effects of modern drugs on human beings and the expense for its treatment have certainly initiated for the need of research into herbal drugs. The theory of Diclofenac effect on kidneys can be compared with the concept of dushi visha. Dushivishari agad is frequently used medicine in different condition like venomous snake bike and scorpion stings in the state of Kerala. Oxidative stress is one of the causes that is responsible for Diclofenac toxic action on nephrons. Most of the contents of Dushivishari agad have antioxidants property and they act on kidneys as well according to Ayurveda. All these facts demand for the research to find out safe, cost effective and efficient Ayurvedic formulation to treat the Diclofenac induced fluid retention and observe its effects on internal organs. The current study is aimed to develop a new standard herbal formulation with its in-vivo study to screen its effectiveness in albino wistar rats. The obtain results and conspicuous observation have been discuss with scientific reasoning. Effect of antidote Churna was previously done by researcher, its further step to observed its effects on fluid

retention of albino wistar rats.

KEYWORDS: Diclofenac, Nephrotoxicity, Dushivishari agada, Weight of Albino Wistar rats

INTRODUCTION

Any poison that is devoid of the natural ten properties of Visha, incapable of producing acute symptoms of poisoning can also be designated *DUSHIVISHA*. A poison, which is having fewer properties, which means less than ten classical properties that actually a poison should have, or either the poison, which is having lesser potency of all the ten properties, attains a latent or hidden stage in the body called Latent poison (*DushiVisha*).Low potency of all the ten qualities is said to be responsible for the delayed action and cumulative toxicity on the body.[12,18]

Diclofenac a NON STERIODAL ANTI- INFLAMMATORY DURG (NSAID) which is frequently prescribed in human and veterinary medicine as an inflammatory, antipyretic and analgesic. The adverse effects of Diclofenac on kidney are not surprising because when compared with other organs, kidney is uniquely susceptible to chemical toxicity, due to high blood flow in kidneys. The processes in kidney involve forming concentrated toxicants in the tubular fluid. As water and electrolytes are absorbed from the glomerular filtration, drugs in the tubular fluid may be concentrated; there by driving passive diffusion of toxicants into tubular cells as the consequence, a non-toxic concentration of the drug can reach to a toxic concentration in the kidneys. Kidney is also known to be sensitive to circulating vasoactive substance including NSAIDS.[8,14]

Ill effects of modern drugs on human beings and the expense for its treatment have certainly initiated for the need of research into herbal drugs. The theory of Diclofenac nephrotoxicity can be compared with the concept of dushi visha.

Dushivishari agad is frequently used medicine in different condition like venomous snake bike and scorpion stings in the state of Kerala. Oxidative stress is one of the causes that is responsible for Diclofenac toxic action on nephrons. Most of the contents of Dushivishari agad have antioxidants property and they act on kidneys as well according to Ayurveda.[8,11,13,14,15]

MATERIAL AND METHODS

Study design

1. Type of study design: Experimental study

This study was carried out in two phases.

a) Standard preparation of drug.

b) Pre-clinical toxic effect in animal model**.**

Crude material list

1. Diclofenac sodium

Preparation of dushivishari agada

i. Pippali - 1 part
ii. Dhyamaka – 1 part
iii. Jatamansi - 1part
iv. Lodhra – 1 part
v. Ela- 1 part
vi. Musta – 1 part

i. Tagar – 1 part
ii. Kustha – 1 part
iii. Yashtimadhu – 1 part
iv. Chandana – 1 part
v. Suvarchika – 1 part
vi. Gairika – 1 part

MATERIALS

Following raw drugs was used for research work with the standard references mentioned in Astang hridayam-

Table no. 1.

Sr. no	Dravya	*botanical name*	Family	Part used
1.	Pippali	*Piper longum, Linn*	Piperacease	Fruit
2.	Dhyamaka	*Cymbopogon martinii. Roxb*	Graminease	Stem
3.	Jatamamsi	*Nardostachys jatamansi. DC*	Valerianacease	Root
4.	Lodhra	*Symplocos racemosa. Roxb*	Symplocaceace	Stem bark
5.	Ela	*Elettaria cardamomum. Maton*	Zingiberacease	Seed
6.	Musta	*Cyperus rotundus*	Cyperacease	Tuber
7.	Tagar	*Valeriana wallichii*	Valerianaceacse	Root
8.	Kustha	*Saussurea lappa DC*	Asreraceae	Root
9.	Yastimadhu	*Glycyrrhiza glabra L.*	Leguminaceae	Root
10.	Chandana	*Pterocarpus santalismus L.*	Santalaceae	Stem
11	Suvarchika	*Pottasiicarbons*		
12.	Gairik	*Ferric oxide*		

Collection of drugs

The contents of *DUSHIVISHARI AGAD* was identified, collected and made in the form of churna.

Authentication of drugs was carried out with the help of Dravyaguna department and Ayurvedic pharmacopeia, India.

Methodology

a) Identification authentication and standardisation of drugs.
b) Preparation of Churnas of all the mentioned drugs separately.
c) Preparation of Dushivishari agada as per SOP.

Steps of prepration of dushivishari agada

1. Identification, collection and standardization of raw drugs i.e. Pippali, Dhyamaka, Jatamansi, Lodhra, Ela, Kustha, Tagar, Yastimadhu, Chandana, Musta, suvarchica, Songeru was done.
2. 50 gms of each mentioned drug 1 part were taken in a clean wide mouthed stainless-steel vessel. Total of 250 gms of the herbs.
3. The above drugs were grinded into fine coarse powder in an electronic mixer grinder
4. Made fine powder (85-120 mesh) of raw materials in equal amount each content to mixed with each other.
5. Standardization and authentication of final product done with pharmacognosy department.

Preclinical antidote effect of poison in animal model

- Study was done in Albino Wistar Rats as per O.E.C.D. guidelines. (Guideline no.420 and 421)
- IAEC permission was taken from respective institute prior to animal study.
- Permission of (C.P.C.S.E.A.) committee for the purpose of control and
- Supervision on animal of (I.A.E.C.) internal animal ethics committee was taken.
- Only those Albino Wistar rats were selected for experiment, which was showing
- Signs of toxicity of Diclofenac
- Test drug details:

Test Article: Dushivishari Agada Physical State: Solid

Quantity: 100 gm

Identification

By unique identification number marked by writing on cage tag and by corresponding colour body markings.

Rats were coded as follows- Code name-meaning.

H: Head B: Back T: Tail

HB: Head Back BT: Back Tail

Table no. 2: Dosage and route.

Dose Calculation	Human to animal dose conversion formula (Pagets and Barners formula) was used for both Diclofenac and Dushivishari Agada.
Dosage	a. Diclofenac was given in BD dose (13mg/kg) to create toxicity. b. Dushivishari Agada was given in BD dose (15mg/kg) in test drug 1 group. c. Dushivishari Agada was given in BD dose (30mg/kg) in test drug 2 group.
Route of administration	Oral route administration for Diclofenac and Dushivishari Agada was done.

W: White (No marking)

Table no. 3: Test System and Management.

1. Species :	Rats
2. Strain :	Albino Wistar
3. Source :	BVC Veterinary college Parel
4. Sex :	Both male and female
5. Body weight range :	18.0 g to 20.0 g
6. Identification :	By unique identification number marked by writing on cage tag and by corresponding colour

	body markings.
7. No. of animals :	24
8. Acclimatization :	The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination. Husbandry
9. Environmental conditions :	Room temperature maintained between 22+3 oC, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation :	Three rats per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet :	Pelleted feed supplied by Supplier.
12. Water :	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

Fig. no. 1: Marking of rats for identification.

Fig. no. 2: Cage condition and observation.

Fig. no. 3: Administration of diclofenac via oral gavage.

Fig. no. 4: Administration of Dushivishari agada via oral gavagevia oral gavage.

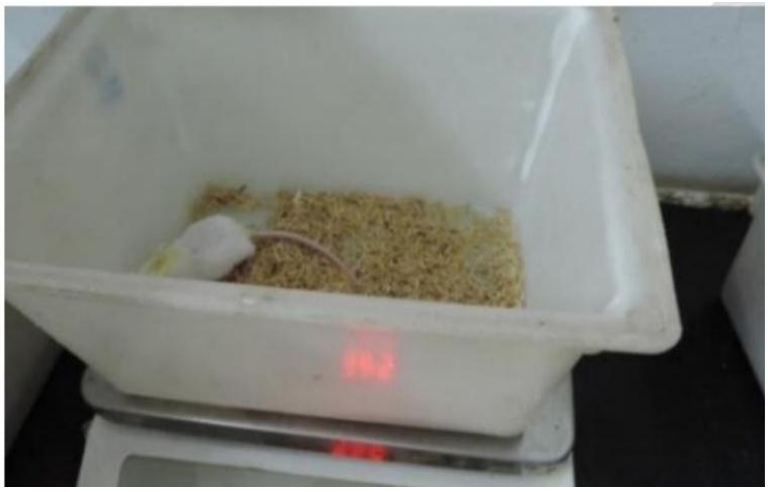

Table no. 4: Group of animals.

Sr. No.	Group name	Specification
1	NC	SHAM control
2	DC	Disease control
3	T1	Test Drug 1 (15mg/kg body weight)
4	T2	Test Drug 2 (30mg/kg body weight)

Inclusive criteria

Fig. no. 5: Measurement of weight of animal.

* Rats showing nephrotoxicity.
* 50% male and 50% female will be taken in each group.
* Weight of rats more than 120-180 gms.
* Rats of age ranging from 6 to 10 weeks

12. Exclusion criteria

* Pregnant Wister rats.

Observation of animals based on; Physical changes, Irritation, Behaviour changes was done.

Biochemical parameters which were observed for conformation of the nephrotoxicity were- BUN

* Creatinine
* Uric Acid
* Histopathology of kidneys
* Body weight (fluid retention)

Procedure

a) 24 Wistar rats was divided in 4 groups containing 6 in each group.
b) Every animal of the study group would be marked for identification.
c) Each animal would be weighted with digital weighing machine.
d) Nephrotoxicity induced by Diclofenac oral dose (13mg/ kg body weight) in2, 3, 4 groups respectively for 15 days.
e) Group 1 will be control group.
f) After 15 days GROUP 2 was untreated, whereas GROUP 3 was given *Dushivishari agad*, GROUP 4 was given increased dose of *Dushivishari agad.*
g) Blood samples collected on 0^{th}, 15^{th}, 21^{th} day for biochemical analysis of BUN creatinine, Serum uric acid and body weight was measured.
h) After drug administration animal was sacrificed for histopathology study.

Data analaysis

Following are the values obtained of the body weight of each animal per group.

Table no. 5: Weight of each animal.

Groups	Animal NO.	Body Wt. 0 Day grams	Body Wt. 21 Day Grams
Group A1 (Control Male)	Head	150	158
	Tail	165	168
	Back	182	195
Group A2 (Control Female)	Head	156	148
	Tail	162	168
	Back	180	189
Group B1 (Positive Control Male)	Head	185	178
	Tail	189	170
	Back	165	161

Group B2 (Positive Control Female)	Head	155	150
	Tail	165	161
	Back	190	181
Group C1 Test Gr 1 Male	Head	192	185
	Tail	165	170
	Back	155	160
Group C2 Test Gr 1 female	Head	185	188
	Tail	184	189
	Back	190	179
Group D1 Test gr 2 Male	Head	189	195
	Tail	198	224
	Back	201	230
Group D2 Test Gr 2 Female	Head	190	198
	Tail	185	190
	Back	191	198

Inter group comparison of values for BODY Wt. (n=6) at various intervals i.e Day 0 and Day 21 (*Table no 6 and 7)*

Table no. 6.

Groups		Mean	Std. Deviation	Median	Chi square value	p value of Kruskal-Wallis Test
day 0	Control grp	165.83	12.844	163.5	10.782	0.013*
	Positive control grp	174.83	14.972	175		
	Test drug1 grp	178.50	14.977	184.5		
	Test drug 2 grp	192.33	5.989	190.5		

Table no. 7.

Groups		Mean	Std. Deviation	Median	Chi square value	p value of Kruskal-Wallis Test
day 21	Control grp	171.00	17.978	168	13.316	0.004**
	Positive control grp	166.83	11.720	165.5		
	Test drug1 grp	178.50	11.467	182		
	Test drug 2 grp	205.83	16.762	198		

There was a statistically highly significant difference seen for the values between the groups ($p<0.01$) at day 21 with higher values in group 4.

There was a statistically significant difference seen for the values between the groups ($p<0.05$) at day 0 with higher values in group 4.

Pairwise comparison of values for body wt. at various intervals i.e Day 0 and Day 21 as in Table 8 and 9.

Table no. 8.

	Group	vs group	Mann-Whitney U value	Z value	p value
Day 0	1	2	10.000	-1.290	0.197#
	1	3	7.500	-1.684	0.092#
	1	4	0.000	-2.882	0.004**
	2	3	15.500	-0.405	0.685#
	2	4	4.500	-2.177	0.029*
	3	4	7.000	-1.768	0.077#

Table no. 9.

	Group	vs group	Mann-Whitney U value	Z value	p value
day 21	1	2	17.000	-0.161	0.872#
	1	3	13.500	-0.723	0.470#
	1	4	1.500	-2.656	0.008**
	2	3	8.500	-1.527	0.127#
	2	4	0.000	-2.892	0.004**
	3	4	0.000	-2.887	0.004**

There was a statistically highly significant difference seen for the values between the groups ($p<0.01$)

Between groups 1 vs 4 on day 0

Between groups 1 vs 4, 2 vs 4 and 3 vs 4 on day 21

There was a statistically significant difference seen for the values between the groups ($p<0.05$) between groups 2 vs 4 on day 0

When compaired days, time and group wise there was significant/ hihly significant change in body weight of animal.

DISCUSSION AND RESULTS

The current study was aimed to find out effectiveness of antidote against Diclofenac induced nephrotoxicity on kidney and body weight of albino wistar rats. The observations and results obtained during the study has been discussed with scientific reasoning.

The experiment protocol was approved by Animal ethical committee in accordance with guidelines formulated by the committee for the purpose of control and supervision of experiments of animals.

As the anatomical and physiological systems resembles human systems both being mammal, hence the use of albino wistar rat species were selected.

Multiple animals per group were used for statistical reasons in the calculation of result. Considering guidelines for animal model 6 animals were included per group.

Experiments Was performed and effects Diclofenac in kidneys was observed. There were changes in weight of rats. Inter group comparison showed changes in weight of different.

Considering statistical data it was observed that there is no significant changes in weight of rats due to effects of antidote Dushivishari agada on Diclofenac induced nephrotoxicity.[3,24,25]

According to contemporary view, *Piper longum* works as analgesic, anti-pyretic, anti-oxidant, etc., *Cyperus rotundus, Linn* acts on Shotha and Deepana, Pachana and also in vishajanya vikara and have anti-inflammatory, anti-bacterial, antimutagenic property. Chandana also have free radical Scavening properties.

Saussurea Lappa Roots have anti- oxidant properties. As per Ayurveda textuals all the contents acts in dushivishjanya vicar.[3,8,11,12,15]

According to histopathological report Antidote churna showed significant changes in renal manifestation in albino wistar rats but not on weight (fluid retention).

Therefore, it can be stated that there is least effect of Dushivishari agada churna to prevent fluid retention caused by Diclofenac considering changes in weight of rate.[3,22,24,27]

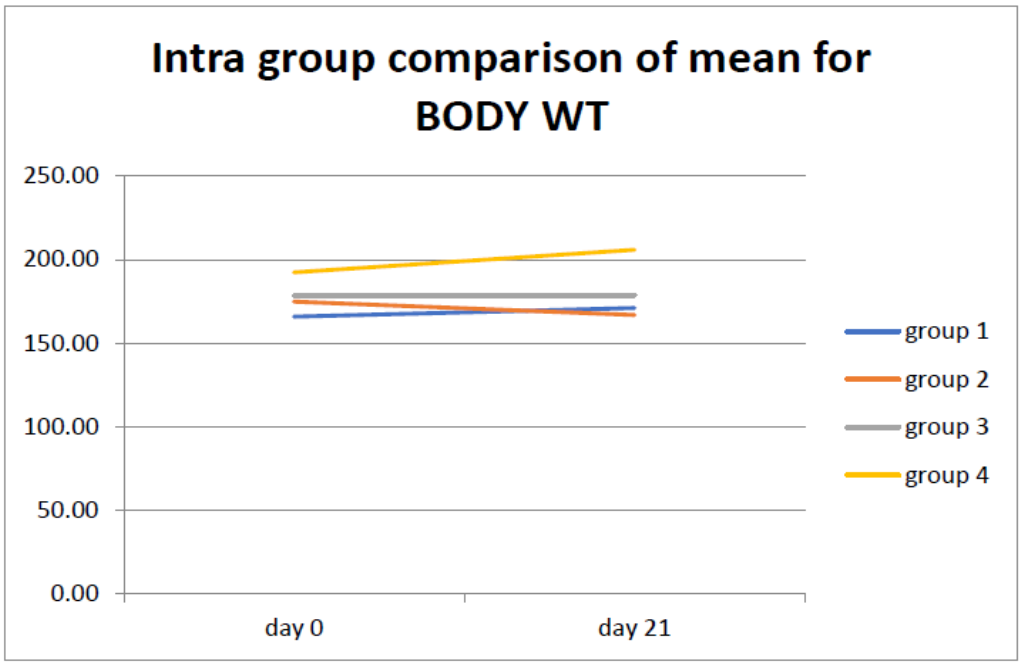

Fig. no. 6: Graphical representation.

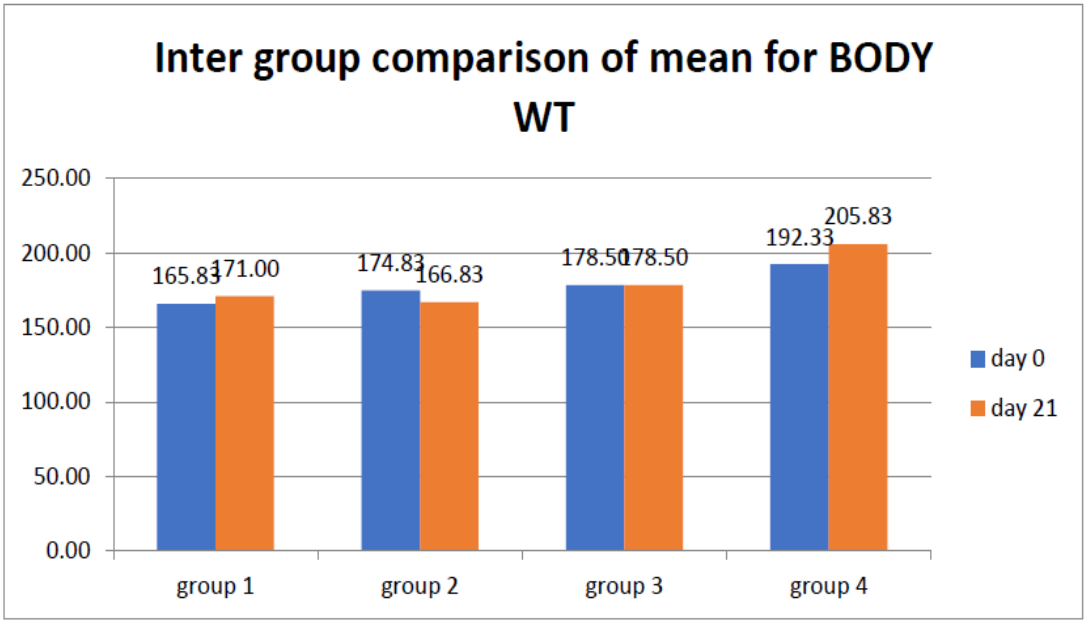

Fig. no. 7: Graphical representation.

CONCLUSION

In scientific study, observations obtained, results and logical discussions are the key points towards the definite conclusion.

In the present study sincere attempts has been made to draw definite conclusion regarding efficiency of Dushivishari agada on weight of albino wistar rats due to Diclofenac induced

nephrotoxicity.

Observation, Statistical data and histopathological reports showed that there is no significant effect of Dushivishari agada on weight of albino wistar rats due to Diclofenac induced toxicity.

ACKOWLEDGMENT

The author wishes to express their profound gratitude to and appreciation to Dr. Mamata Narvekar and Agadtantra department of YMT Medical college and hospital. Dr. Sushant Sole for their support.

REFERENCES

1. Dr Sushil Kumar. Ayurvedic management of cumulative toxicity (*Dushi visha):* A review. Ayurvedic medical college and research centre, Mathura, 2017; 5.
2. Parvesh Kumar, Munish Kumar. Concept of dushi visha –Significance in current perspective. Dept. of Agadtantra, Uttaranchal Ayurveda college, 2016.
3. Sreejith GS, Ittoop J Ancheril. Chemical analaysis of *Dushivishari agad* An Ayurvedic herbo-mineral formulation to combat residual toxicity. SDM college of Ayurveda and hospital, Hassan, Karnataka, India, 2015
4. M Ramazan M ashraf shah. Evaluation of Diclofenac toxicity at different concentration in relation to time using broiler Chicken model, 2015.
5. D DRC. Antioxidant activities of *pippali* proteins, 2014; 2.
6. Zeynab kh. Hepato-Renal and Heamtological effects of Diclofenac in rats. Global journal of pharmacology, 2013.
7. Seluck DBM. Serological and Biochemical evaluation of Diclofenac Induced Nephrotoxicity in Rats, 2013.
8. Kim Kmcsiag. Antioxidant Activity effect of Saussurea Lappa Roots, 2012.
9. Olleges A. Defects in mouse Nephrogenesis Induced by Non selective Cyclooxgynase-2 inhibitors, 2010.
10. Horel WH. Non-steroidal anti-inflammatory drugs and kidney. July, 2010.
11. Beatrice Sasmkppmav. Free radical scavenging activity of Pterocarpus Santalinus in vitro. Indian Jorunel of science and technology, 2008.
12. Eng NL. Action of Diclofenac and Meloxicam Nephrotoxic cell Death, 2008.
13. Ardestani Rya. Free radical Scavenging activity of Cypersys rotundus, 2007.
14. Yasmeen T. Adverse effect of Diclofenac sodium on Renal paranchyme of adult Albino

rats. Sindh medical college, Karachi. July, 2007.

15. B MAKSMS. Comparison of Antioxidant Activity of Extract from Root of Glycyrrhia Glabra L. with artificial agents Nov-Dec, 2003.
16. Linton AL. Adverse effect of NSAIDS on Renal function. Aug, 1994.
17. Bruna Fernanda Murbach Teles, Camila Perira braga; Effect of Inhaling Cymbopogon martinii Essential Oil and Geraniol on Serum Biochemistry Parameters and Oxidative Stress in Rats, 2014.
18. Dr. Neha Kulkarni, Dr. Jayant Phadke; Concepteual study of Dushivisha with special reference to Bruhatryee, 2017.
19. Adam L, Linton,MBFRCP; Adverse effects of NSAIDS on Renal function, 1984.
20. Sun Young Kim and Aree Moon; Drug-Induced Nephrotoxicty and its Biomarkers, 2012.
21. Walter.H.Horli; Non-steroidal Anti-Inflammatory Drug and kidney, 2010.
22. Uday.V.Pawade, Abhay. H. Patkar; Role of Vishaghnayogas (Anti- Toxic Formulations)in current scenerio- A Review, 2020.
23. Kapil Lawrence,Reena Lawrence, Dharmendra Parihar, Reshmi; Antioxidant activity of *Palmarosa essential oil* (*Cymbopogon martini*) grown in north Indian plains, 2012.
24. *Evaluation of Nephro-Hepatoprotective activity of Dushivishari Agada in Mercuric chloride induced Acute Nephro-Hepatotoxicity in Wistar rats*. Diss. KLE University, 2015.
25. BG Ashish, P Sharon, S Garima ;Dushi Visha and Oxidative Stress-A Correlation, 2015.
26. E.JHickey, R.RRajeV, EReid S.MGross ›S.DRay[a] ; Diclofenac induced in vivo nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death, 2001.
27. B Bhargav, R Acharya; CONCEPT OF VISHA AND ITS PHARMACOLOGICAL BASIS IN AYURVEDA, 2012.