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EFFECT OF *BILWADI AGADA* ON BODY WEIGHT IN PARACETAMOL-INDUCED HEPATOTOXICITY: AN ANIMAL STUDY

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ABSTRACT:
Paracetamol (Also Known as Acetaminophen, N-acetyl-p-aminophenol) is a commonly used over-the-counter medication with potent analgesic and antipyretic properties. It is effective in mild to moderate pain such as headache, dysmenorrhoea, post-operative pain, and toothache.
It is very safe when used in limited doses, but at the same time, if it is used for a prolonged period, it is capable of causing dose-related hepatocellular toxicity.
Toxic doses of Paracetamol can cause hepatotoxicity-related fluid retention (weight gain) or alteration in haematological profiles in humans and animals. The ill effects of modern drugs on human beings and the expense for their treatment have certainly highlighted the need for research into herbal drugs. The theory of Paracetamol effect on the liver can be compared with the concept of dushi visha. Bilwadi agad is a frequently used medicine in different conditions like venomous snake bites, insect bites and scorpion stings in the state of Kerala. Most of the contents of Bilwadi agad have antioxidant properties and they act on Liver and Kidneys as well, according to Ayurveda. All these facts demand for the research to find out a safe, cost-effective and efficient Ayurvedic formulation to treat the Paracetamol-induced haematological changes and observe its effects on internal organs. The current study aims to develop a new standard herbal formulation with its in-vivo study to screen its effectiveness in Swiss albino mice. The obtain results and conspicuous observation have been discuss with scientific reasoning. Effect of antidote Churna was previously studied by researcher, its further step to observe its effects on Haematological Profiles and Body Weight.

KEYWORDS: Paracetamol, Bilwadi Agada, Liver, hepatotoxicity, *Dushi visha*

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.
Paracetamol (Acetaminophen, N-acetyl-p-aminophenol) is one of the commonly utilized over-the-counter medications with potent analgesic and antipyretic action. It is effective in mild to moderate pain such as headache, dysmenorrhoea, post-operative pain, toothache.
It is very safe when used in limited doses but at the same time if it is used for prolonged period, it is capable of causing dose-related hepatocellular toxicity.
Liver is an important target site for the untoward effect of paracetamol. The liver is an essential organ of the human body that performs over 500 vital functions, and is responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, and vitamin storage, among other functions.
Ill effects of modern drugs on human beings and the expense for its treatment have certainly highlighted the need for research into herbal drugs. The theory of Paracetamol hepatotoxicity can be compared with the concept of dushi visha.
Bilwadi agad is frequently used as a medicine in different condition like venomous snake bites, insect bites and scorpion stings in the state of Kerala. Oxidative stress is one of the causes that is responsible for Paracetamol's toxic action on Liver. Most of the contents of Bilwadi agad have antioxidant properties, and they act on the liver as well, according to Ayurveda.

MATERIAL AND METHODS:

Study design:

1. Type of study design: Experimental study

This study was carried out in two phases.

A. Standard preparation of the drug.
B. Pre-clinical toxic effect in an animal model.

Crude material list:
1, Paracetamol
2, Silymarin

Preparation of Bilwadi Agada

i. Bilwa - 1 Part
ii. Karanja – 1 part
iii. Jatamansi - 1part
iv. Natam – 1 part
v. Surahwam- 1 part
vi. Haritaki – 1 part
vii. Bibhitaki – 1 part I
viii. Amalaki – 1 part
ix. Shunti – 1 part
x. Maricha – 1 part
xi. Haridra – 1 part
xii. Daruharidra – 1 part
xiii. Basta mutra - For Bhavana

MATERIALS

No	Name of the drug	Synonym	Botanical name	Part used	Quantity
1.	*Bilwa*	*Maha kapittha*	Aegle marmelos	*Moola*	1 part
2.	*Surasa*	*Tulasi*	Ocimum sanctum	*Pushpam*	1 part
3.	*Karanja*	*Naktamala*	Pongamia pinnata	*Phala*	1 part
4.	*Natam*	*Tagaram*	Valeriana wallichi	*moola*	1 part
5.	*Surahwam*	*Devadaru*	Cedrus deodara	*Kanda sara*	1 part
6.	*Haritaki*	*Abhaya*	Terminalia chebula	*Phala*	1 part
7.	*Bibhitaki*	*Aksha phala*	Terminalia bel-lerica	*Phala*	1 part
8.	*Amalaki*	*Dhatri*	Embica officinalis	*Phala*	1 part
9.	*Shunti*	*Vishwa bheshaja*	Zingiber officinale	*Kanda*	1 part
10.	*Maricha*	*Vellaja*	Piper nigrum	*Phala*	1 part
11.	*Pippali*	*Kana*	Piper longa	*Phala*	1 part
12.	*Haridra*	*Nisha*	Curcuma longum	*Kanda*	1 part
13.	*Daruharidra*	*Darunisha*	Berberis aristata	*Kanda*	1 part
14.	*Basta mutra*	*Goat urine*			Quantitysufficient

Following raw drugs was used for research work with the standard references mentioned in Bharat Bhaishajya Ratnakara-

Collection of drugs
The contents of Bilwadi Agada 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 Bilwadi agada as per SOP.

Steps of prepration of Bilwadi agada

1. Identification, collection and standardisation of raw drugs i.e. Bilwa, Karanja, Jatamansi, Natam, Surahwam, Haritaki, Bibhitaki, Amalaki, Shunti, Maricha, Haridra, Daruharidra, Basta mutra
was done.
2. 50 gms of each of the 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 mix with each other.
5. Standardisation and authentication of the final product were done with the pharmacognosy department.

Preclinical antidote effect of poison in animal model

Study was done in Swiss Albino Mice as per O.E.C.D. guidelines. (Guideline no.420 and 421)

IAEC permission was taken from the respective institute prior to the animal study.

Permission of (C.P.C.S.E.A.) committee for the purpose of control and Supervision on animals of (I.A.E.C.) internal animal ethics committee was taken.

Only those mice were selected for the experiment, which was showing signs of toxicity of Paracetamol

Group I - DC		**Group II- SC**		**Group III- VC**		**Group IV- T1**		**Group V- T2**	
Day 0	Day 45	Day 0	Day 45	Day 0	Day 45	Day 0	Day 45	Day 0	Day 45
25	23	25.5	27	24.6	28.8	26.9	25.6	28.3	26.4
24.8	22.5	25.2	27.5	25.3	27.6	26.4	24.8	27.9	26.9
24.5	22.8	25.4	27.9	24.3	28.5	27.3	25.9	28.5	27.9
25.4	23.8	24.5	27.1	25.3	29.5	27.7	26.7	28.8	27.2
24.9	22.9	24.7	26.9	25.1	28.2	26.7	25.9	28.5	27.9
25.5	23.8	25.8	27.6	24.9	27.6	27.3	25.9	25.9	27.9

Intra-Group Comparison	**Repeated measures ANOVA**	**Repeated measures ANOVA**	**Repeated measures ANOVA**	**Repeated measures ANOVA**	**Repeated measures ANOVA**
p value	< 0.0001, extremely significant	< 0.0001, extremely significant	0.0003, extremely significant	< 0.0001, extremely significant	< 0.0001, extremely significant
Day 0 vs Day 15	< 0.001, highly significant	< 0.001, highly significant	< 0.01, very significant	< 0.001, highly significant	< 0.001, highly significant
Day 0 vs Day 45	< 0.001, highly significant	< 0.001, highly significant	< 0.001, highly significant	< 0.01, very significant	> 0.05, not significant
Day 15 vs Day 45	< 0.001, highly significant	< 0.001, highly significant	> 0.05, not significant	< 0.001, highly significant	< 0.001, highly significant

Test drug details:

Test Article: Bilwadi Agada with honey.

Physical State: Solid Quantity: 100 gm

Identification:

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

Mice were coded as follows:

Code name-meaning. H: Head B: Back T: Tail

HB: Head Back BT: Back Tail

W: No markings

Dosage and route.

Dose Calculation	Human to animal dose conversion formula (Pagets and Barners formula) was used for both Paracetamol and Bilwadi Agada.
Dosage	a. Paracetamol was given in BD dose 250mg/kg/day to create toxicity. b. Bilwadi Agada was given in BD dose 3.25mg/kg/day orally with Honey on Test Drug 1 Group. c. Bilwadi Agada was given in BD dose 8.25mg/kg/day orally with Honey in Test Drug 2 Group.
Route of administration	Oral route administration for Paracetamol and Bilwadi Agada was done.

Species	Mice
Strain	Swiss Albino mice
Source	BVC Veterinary College, Parel
Sex	Both Male and Female.
Body weight range	25-40 gms
Identification	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
No. of animals	30
Acclimatization	The mice were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination. Husbandry
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
Accommodation	Six mice per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
Diet	Pelleted feed supplied by Supplier.
Water	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

Test System and Management:

Sr No	Group Name	Specification
1	DC	Disease Control
2	SC	Standard Control
3	VC	Vehicle Control
4	T1	Test Drug 1 (3.25mg/kg/day)
5	T2	Test Drug 2 (8.25mg/kg/day)

Inclusive criteria
Mice showing hepatotoxicity.
50% male and 50% female will be taken in each group.
Weight of mice more than 25-40 gms.
Mice of age ranging from 6 to 10 weeks

Exclusion criteria

Pregnant Mice.

Observation of animals based on:
Physical changes, Irritation, and behaviour changes was done. Biochemical parameters which were observed for confirmation of the Hepatotoxicity were CBC ESR, Bilirubin(Total), SGOT. SGPT, Histopathology of Liver and Body weight (fluid retention)

Procedure:
1) 30 Albino mice were divided into 5 groups containing 6 mice.
2) Every animal of the study group would be marked for identification.
3) Each animal would be weighed with a digital weighing machine.
4) Hepatotoxicity induced by Paracetamol oral dose (250mg/kg/day) in all groups, respectively for 15 days.
5) Group 2 will be the control group treated with Silymarin (50mg/kg Orally)
6) After 15 days, GROUP 1 was untreated, whereas GROUP 4 was given Bilwadi agad, and GROUP 5 was given an increased dose of Bilwadi agad.
7) Blood samples were collected on the 0th, 15th, and 45th day for Haematological analysis of CBC, ESR, Bilirubin(Total), SGOT. SGPT and body weight were measured.
8) After drug administration, animals were sacrificed for a histopathology study.

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

Body Weight	Group I - DC		Group II-SC		Group III-VC		Group IV-T1		Group V-T2	
Mean	25.02	23.13	25.18	27.33	24.92	28.37	27.05	25.80	27.98	27.37
SD	0.38	0.54	0.50	0.39	0.40	0.73	0.47	0.61	1.06	0.64

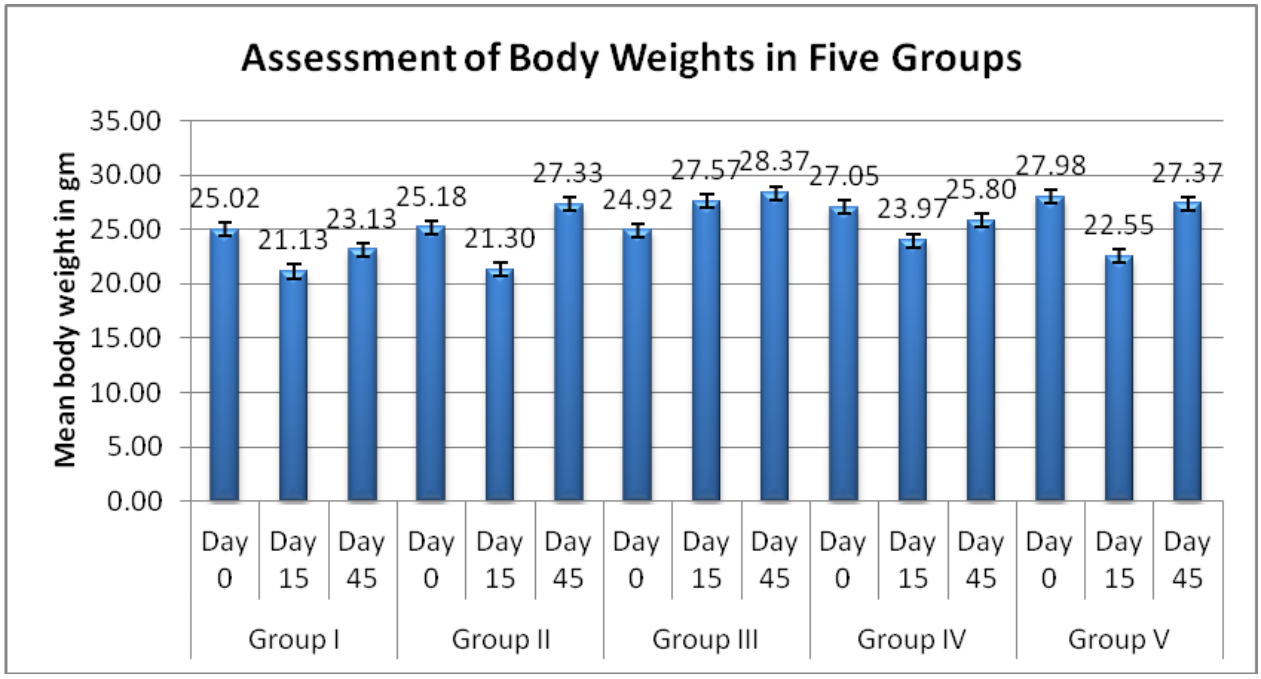
Intra-Group Comparison	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA
Day 0 vs Day 15	< 0.001, highly significant	< 0.001, highly significant	< 0.01, very significant	< 0.001, highly significant	< 0.001, highly significant
Day 0 vs Day 45	< 0.001, highly significant	< 0.001, highly significant	< 0.001, highly significant	< 0.01, very significant	> 0.05, not significant
Day 15 vs Day 45	< 0.001, highly significant	< 0.001, highly significant	> 0.05, not significant	< 0.001, highly significant	< 0.001, highly significant

Inter group comparison for BODY Wt. (n=6) of values at various intervals i.e Day 0 and Day 45

	Day 15	Day 45
Group II vs Group I	> 0.05, not significant	< 0.01, very significant
Group II vs Group III	< 0.01, very significant	< 0.01, very significant
Group II vs Group IV	< 0.01, very significant	< 0.01, very significant
Group II vs Group V	< 0.05, significant	< 0.01, very significant

There was a highly statistically significant difference seen for the values between the groups ($p<0.01$) at day 45, with higher values in group 5.
There was a highly statistically significant difference seen for the values between the groups ($p<0.01$) Between groups 1 vs 4 and 5 on day 0.
Between groups 2 vs 1, 2 vs 3, 2 vs 4 and 2 vs 5 on day 45.

There was a statistically significant difference seen for the values between the groups ($p<0.05$) between groups 2 vs 4 on day 15
When comparing days, time and group-wise, there was significant/highly significant change in the body weight of the animal.



DISCUSSION AND RESULTS

The current study aimed to find out the effectiveness of the antidote against Paracetamol-induced hepatotoxicity on the liver and body weight of albino mice. The observations and results obtained during the study have been discussed with scientific reasoning.

The experiment protocol was approved by Animal Ethics 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 resemble human systems, both being mammals, hence the use of albino mice 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 were performed, and the effects of Paracetamol in Liver were observed. There were changes in the weight of mice. Inter-group comparison showed changes in weight of different. Considering statistical data it was observed that there is no significant changes in the weight of rats due to the effects of the antidote Bilwadi agada on Paracetamol-induced Hepatoxicity.

According to contemporary view, Piper longum works as analgesic, anti-pyretic, antioxidant, etc., All other drugs acts on Shotha and Deepana, Pachana and also in vishajanya vikara and have anti-inflammatory, anti-bacterial, antimutagenic properties.

According to the histopathological report Antidote churna showed significant changes in Hepatic manifestation in albino mice but not in weight (fluid retention). Therefore, it can be stated that there is least effect of Bilwadi agada churna to prevent fluid retention caused by Paracetamol, considering changes in weight.

CONCLUSION

In a scientific study, observations obtained, results and logical discussions are the key points towards the definite conclusion. In the present study, sincere attempts have been made to draw definite conclusions regarding the efficiency of Bilwadi agada on the weight of albino mice due to Paracetamol induced hepatotoxcicity.

Observation, Statistical data and histopathological reports showed that there is no significant effect of Bilwadi agada on the weight of albino mice due to Paracetamol-induced toxicity.

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