



Research Paper

EFFECT OF BIHERBAL EXTRACTS OF *Bryophyllum calycinum* AND *Tribulus terrestris* ON HAEMATOLOGICAL CHANGES ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN FEMALE WISTAR RATS

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Abstract

The present research work was undertaken to study the therapeutic efficacy of biherbal extracts of *Bryophyllum calycinum* and *Tribulus terrestris* on ethylene glycol induced urolithiasis in female Wistar rats. The experimental Wistar rats were equally distributed in five different groups. Induction of urolithiasis was done by administration of ethylene glycol at the rate of 0.75% v/v in drinking water for 21 days. Confirmation of urolithiasis was done by evaluating serum biochemical parameters. After confirmation of urolithiasis, rats of group IV and V were administered alcoholic biherbal extracts (1:1) at 200 mg/kg and 400 mg/kg respectively for 28 days in Wistar rats. The blood samples from 90 Wistar rats were screened for detection of therapeutic efficacy of biherbal extracts of *Bryophyllum calycinum* and *Tribulus terrestris* on ethylene glycol induced urolithiasis in female Wistar rats. After 21 days of induction rats of all groups, except control group, showed significant variation in haematological profile. After 28 days of biherbal drug administration, rats from lithiatic control group (group II) showed significant lower haemoglobin value as compare to group I (Normal control) as this group was kept as a lithiatic control group while Group VII (BHE-II) showed significant increase in mean value of haemoglobin on 49th day as compared to group II. There is no significant difference in mean values of TEC, TLC, monocytes, lymphocytes, granulocytes and mean corpuscular volume values in different groups on 49th day. Mean value of MCHC of group II was significant ($P < 0.05$) lower as compare to group I (Normal control) while Group VI, VII and group XI showed significant increase in mean value of MCHC as compared to group II. Results of haematological profile reveals alcoholic extract of biherbal combination of *Bryophyllum calycinum* and *Tribulus terrestris* possess good therapeutic efficacy against urolithiasis.

Key words: Biherbal extract, urolithiasis, ethylene glycol, wistar rat, therapeutic efficacy.

INTRODUCTION

Urolithiasis or renal stone is the world's most common ailment with maximum chance of relapse. Stones are often more commonly found in males than females due to a longer urethra [1, 2]. It leads to decreased productivity and higher cost of maintenance. Stones that cause obstruction to the flow of urine, set up an environment of urine stasis and bacterial growth which leads to alteration in kidney functions tests and leads to increase Non Proteinous Nitrogenous (NPN) substances [3].

The treatment modalities like surgery and drug therapy are practiced in the management of kidney stones but have some limitations. Indigenous plants have been used as a potential source of medicine since ancient times. Although many plants have been evaluated for anti-urolithiatic effect, search for medical treatment for renal calculi is still going on [4, 5].

Monotherapy sometimes leads to failure in curing diseases having multiple aetiology and one of these problems exist in treatment of Urolithiasis. Traditional medical practitioners prescribe a combination of herbal products with synergistic action. There is no single satisfactory method available for complete cure of urolithiasis. For this reason biherbal drugs are gaining popularity among practitioners for treating urolithiasis. The fruits of *Tribulus terrestris* are recommended for the treatment of urinary disorders and erectile dysfunction [6] while anti-oxidant and nephroprotective effect of *B. calcynium* documented in various studies [7]. So, taking into view these points the present research work was carried out to evaluate the therapeutic efficacy of combination of biherbal alcoholic extract of *Tribulus terrestris* and *B. calcynium*.

MATERIAL & METHODS

The study was held on female Wistar rats procured from Zydus research center, Ahmedabad, Gujarat. Rats were adult in age having 250-300 g. weight. The experimental designing was done as described in Table no. 1. Fresh fruits of *Tribulus terrestris* and leaves of *Bryophyllum calcynium* were procured from Medicinal & Aromatic Plant Unit, Anand Agricultural University, Anand. The plant was washed thoroughly with clean water and dried in shade. The dried plant materials were powdered in electric grinder and subjected to extraction in Microwave extraction unit using alcohol following the method as described by [8].

Induction of urolithiasis was done by administration of ethylene glycol at the rate of 0.75% v/v in drinking water for 21 days. Confirmation of urolithiasis was done by evaluating serum biochemical parameters. Administration of alcoholic extract of *Bryophyllum calcynium* and *Tribulus terrestris* was mixed in 1:1 ratio and administered by oral route using sterile 1ml syringe with oral rat gavage needle. Dose was calculated according to body weight of animal and administered as per concentration strength of formulation. Urolithiasis induced Wistar rats were given biherbal treatment; in group IV and V were at the dose rate of 200 mg/kg and 400 mg/kg respectively, for 28 days. Blood samples were collected from all the animals by retro-orbital plexuses puncture under light ether anaesthesia with the help of capillary tube. Serum was harvested by centrifugation at 3000 rpm for 10 minutes at 10°C (Eppendorf 5804 R, Germany) and stored at - 40°C for biochemical analysis and analyzed within 12 hrs.

RESULTS

After biherbal treatment of 28 days in urolithiatic rats Mean±SE values of haematological parameters like Haemoglobin (Hb), Total Erythrocyte Count (TEC), Pack Cell Volume (PCV), Total Leucocyte Count (TLC), granulocytes, lymphocytes,

monocytes , Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) are described in Table no. 2 and 3. Haemoglobin and MCHC value were significantly differed in various groups of rats. No significant difference could seen in other parameters.

Table no. 1 Different groups of Wistar rats

Sr. No.	Group No.	Substance	Dose (mg/kg)	No. of Animals	
1	I	Normal control	---	6	
2	II	Lithiatic Control (Ethylene glycol)	Ethylene glycol 0.75%v/v p.o. alongwith drinking water	6	
3	III	Vehicle Control [±]	Same volume	6	
4	IV	Biherbal extract – I {1:1} (Aqueous plant extract)	Dose-I 200mg/kg	8	
5	V		Dose-II 400mg/kg	8	
6	VI	Biherbal extract – II {1:1} (Methanolic plant extract)	Dose-I 200mg/kg	8	
7	VII		Dose-II 400mg/kg	8	
8	VIII	Biherbal extract– III {1:1} (Chloroform plant extract)	Dose-I 200mg/kg	8	
9	IX		Dose-II 400mg/kg	8	
10	X	Extract control-I (Aqueous plant extract)	Dose-I 200mg/kg	4	8
			Dose-II 400mg/kg	4	
11	XI	Extract control-II (Methanolic plant extract)	Dose-I 200mg/kg	4	8
			Dose-II 400mg/kg	4	
12	XII	Extract control-III (Chloroform plant extract)	Dose-I 200mg/kg	4	8
			Dose-II 400mg/kg	4	
Total				90	

Biherbal Extract I : Aqueous extract of *Bryophyllum calycinum* and *Tribulus terrestris*
 Biherbal Extract II : Methanolic extract of *Bryophyllum calycinum* and *Tribulus terrestris*
 Biherbal Extract III : Choloroform extract of *Bryophyllum calycinum* and *Tribulus terrestris*

Table no. 2 Effect of biherbal treatments on Hb, TEC, PCV, MCV and MCHC in different groups of Wistar rats

Group	Description of group	Hb Mean±SE (g/dl) (After biherbal treatment on 49 th day)	TEC Mean±SE(10 ¹² /L) (After biherbal treatment on 49 th day)	PCV Mean±SE(%) (After biherbal treatment on 49 th day)	MCV Mean±SE(fL) (After biherbal treatment on 49 th day)	MCHC Mean ± SE(g/dl) (After biherbal treatment on 49 th day)
I	Normal control	16.22 ^a ±0.45	7.94±0.21	44.75±0.44	58.01±0.26	39.81 ^a ±0.19
II	Lithiatic control	8.60 ^{ab} ±0.30	7.84±0.17	45.22±0.20	57.82±0.27	31.40 ^a ±0.35
III	Vehicle control	15.55 ^{de} ±0.18	7.85±0.13	44.86±0.24	58.00±0.25	40.01 ^c ±0.27
IV	BHE – I (Aq.) 200 mg/kg	8.36 ^{ab} ±0.33	7.59±0.13	45.17±0.30	57.96±0.28	35.51 ^b ±0.91
V	BHE – I (Aq.) 400 mg/kg	8.30 ^{ab} ±0.35	7.62±0.10	44.51±0.25	57.94±0.25	32.86 ^{ab} ±2.49
VI	BHE – II (Meth.) 200 mg/kg	9.83 ^b ±0.31	7.42±0.13	45.05±0.42	58.01±0.31	39.91 ^c ±0.10
VII	BHE – II (Meth.) 400 mg/kg	13.90 ^c ±0.70	8.01±0.19	44.95±0.26	58.23±0.23	38.61 ^c ±0.77
VIII	BHE – III (Chlor.) 200 mg/kg	8.20 ^a ±0.32	7.92±0.09	45.39±0.23	57.66±0.17	34.86 ^b ±1.50
IX	BHE – III (Chlor.) 400 mg/kg	8.86 ^{ab} ±0.16	7.60±0.20	45.26±0.73	57.87±0.24	40.01 ^c ±0.15
X	Extract control-I (Aq.)	13.33 ^c ±0.66	7.93±0.07	45.27±0.79	58.01±0.23	39.88 ^c ±0.43
XI	Extract control-II (Meth.)	14.38 ^{cd} ±0.69	7.57±0.25	44.05±1.11	58.19±0.20	39.93 ^c ±0.16
XII	Extract control-III (Chlor.)	13.86 ^c ±0.84	7.94±0.29	45.58±0.30	57.70±0.23	39.46 ^c ±0.41

Table no. 3 Effect of biherbal treatments on TLC, Granulocytes, Lymphocytes and Monocytes in different groups of Wistar rats

Group	Description of group	TLC Mean±SE (10 ⁹ /L) (After biherbal treatment on 49 th day)	Granulocytes Mean±SE (%) (After biherbal treatment on 49 th day)	Lymphocytes Mean±SE (%) (After biherbal treatment on 49 th day)	Monocytes Mean±SE (%) (After biherbal treatment on 49 th day)
I	Normal control	11.51±0.28	11.41±0.12	85.03±0.58	2.22±0.24
II	Lithiatic control	11.86±0.32	11.39±0.11	83.95±0.37	2.47±0.15
III	Vehicle control	11.89±0.21	11.45±0.08	84.60±0.37	2.85±0.24
IV	BHE – I (Aq.) 200 mg/kg	11.65±0.25	11.69±0.11	84.65±0.31	2.69±0.39
V	BHE – I (Aq.) 400 mg/kg	11.93±0.21	11.38±0.05	84.66±0.25	2.40±0.32
VI	BHE – II (Meth.) 200 mg/kg	12.00±0.05	11.51±0.12	84.73±0.40	2.11±0.53
VII	BHE – II (Meth.) 400 mg/kg	12.00±0.17	11.39±0.11	80.35±4.36	2.63±0.05
VIII	BHE – III (Chlor.) 200 mg/kg	11.70±0.08	11.54±0.06	84.01±0.28	2.49±0.23
IX	BHE – III (Chlor.) 400 mg/kg	12.25±0.20	11.53±0.09	84.35±0.26	2.54±0.31
X	Extract control-I (Aq.)	12.05±0.24	11.24±0.10	84.41±0.23	2.56±0.05
XI	Extract control-II (Meth.)	11.40±0.10	11.05±0.07	84.41±0.31	2.67±0.13
XII	Extract control-III (Chlor.)	11.55±0.16	11.38±0.14	83.46±0.82	2.56±0.23

DISCUSSION

Results revealed that mean value of haemoglobin of group II (Lithiatic control) was significant ($P < 0.05$) lower as compare to group I (Normal control) as this group was kept as a lithiatic control group. Group VII (BHE-II) showed significant ($P < 0.05$) increase in mean value of haemoglobin on 49th day as compared to group II. It may be due to treatment with higher dose of methanolic biherbal extract at the rate of 400 mg/kg body weight. Group VI and IX showed non-significant ($P < 0.05$) increased in mean value of haemoglobin on 49th day as compared to group II. It may be due treatment of biherbal methanolic and chloroform extract. Group X, XI and XII were in range of group I (Normal control). It indicates that there is no adverse effect of all extracts of *Bryophyllum calycinum* and *Tribulus terrestris* on the mean value of haemoglobin. Results indicate that haemoglobin level decrease due to urolithiasis induction. Treatment with biherbal extracts shows significant increase in haemoglobin concentration. It may be due to some of the phytochemical constituents of biherbal extract may have stimulatory effect on the bone marrow to produce more haemoglobin when orally administered biherbal plant extracts [9]. The phytochemical active components may have effect on kidney. So it might be helping in recovery of kidney damage. Kidney is also responsible for erythropoietin synthases erythropoietin is also responsible for Hb synthases so haemoglobin level increase.

There is no significant difference in mean values of TEC in different groups on 49th day. It may be due to urolithiasis condition does not affect the erythropoiesis mechanism [10]. PCV have direct relationship with RBCs. In all the groups on 49th day no significant changes was observed in RBCs which reflected in PCV. So, PCV value showed non-significant difference in different group.

After biherbal treatment of 28 days in urolithiatic rats no significant difference in mean values of TLC, monocytes, lymphocytes, granulocytes and MCV values which implies that urolithiasis condition does not affect these haematological parameter [11].

Mean value of MCHC of group II was significant ($P < 0.05$) lower as compare to group I (Normal control). In lithiatic control group it has been observed that feed consumption in lithiatic control group is reduced so it may be one of reason and other reason that ethylene glycol have effect on kidney and kidney is damaged. Group VI, VII and group XI

showed significant ($P < 0.05$) increase in mean value of MCHC as compared to group II. It may be due to treatment with biherbal methanolic extract and biherbal chloroform extract respectively. Group IV and V showed significant ($P < 0.05$) increase in mean value of MCHC as compared to group II but not in normal reference range. It may be due to treatment of biherbal aqueous extract. Group X, XI and XII were in range of group I (Normal control). It indicates that there is no adverse effect of all extracts of *Bryophyllum calycinum* and *Tribulus terrestris* on the mean value of MCHC [9].

CONCLUSIONS

Results indicate that urolithiasis had no effect on erythropoiesis mechanism. Effect of methanolic extract of biherbal plant *Bryophyllum calycinum* and *Tribulus terrestris* is more as compare to aqueous and chloroform extracts for the treatment of urolithiasis.

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