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Development of herbal formulation of medicinal plants and determination of its antihyperuricemic potential *in vitro* and *in vivo* rat's model

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Abstract: Hyperuricemia is a common metabolic disorder and several herbal formulations are being used for its treatment. The study aimed to develop herbal formulation (Urinil B) and find its hypouricemic effects in vitro and in vivo. Urinil B was prepared by taking *Trachyspermum ammi*, *Piper nigrum* and *Berberis vulgaris* equally. In vitro Dissolution test and xanthine oxidase inhibition assay was performed for checking capsule absorbance and IC50 calculation respectively. For in vivo experimentation, the study comprised of 14 groups of rats (n=6). Results showed that significant xanthine oxidase inhibition was shown by herbal formulation with IC50 of 586±1.5µg/mL. Oral administration of Urinil B 250, 500 and 1000 mg/kg decreased serum and liver uric acid levels of hyperuricemic rats in dose and time dependent manner. 3 day and seven day administration of Urinil B reduced serum and liver uric acid level more significantly as compared to one day administration. However, allopurinol normalized serum and liver uric acid levels in all study groups. The present study indicated marked hypouricemic effects of Urinil B in hyperuricemia induced by potassium oxonate in rats. However, due to caveat of small sample size in this study, clear conclusion regarding hypouricemic potential of Urinil B can't be made.

Keywords: Urinil B, Hyperuricemia, herbal formulation, Rat model.

INTRODUCTION

Hyperuricemia is a metabolic disorder which is found in 5-30% of the population and appears to be increasing around the world. Hyperuricemia is characterized by increased level of Serum uric acid (sUA) (Zhao *et al.*, 2006). Over 6.8mg/dL, about saturation level of uric acid solubility at 7.4 pH and 37°C temperature (Punzi *et al.*, 2012). Uric acid is a final product of endogenous as well as dietary purines metabolism (Cantor *et al.*, 2017, Gaur *et al.*, 2018) uric acid is catalyze by hydroxylation of hypoxanthine and xanthine in the presence of Xanthine dehydrogenase (XDH) and xanthine oxidase (XOD). Both enzymes are the target for the treatment of hyperuricemia as well as gout (Zhao *et al.*, 2006). It may be caused by over production or under excretion or both of uric acid. It has been considered a causative precursor (Kiadaliri *et al.*, 2018) and believed a risk factor for the establishment of gout (Delbarba *et al.*, 2016) and also has been linked to hypertension, cardiovascular diseases, hyperlipidemia, diabetes, cancer, renal dysfunction and metabolic syndrome (Sánchez-Lozada, 2018, Seyed-Sadjadi *et al.*, 2017, Vargas-Santos and Neogi, 2017, Yiu *et al.*, 2017, Yu *et al.*, 2017).

Urate oxidase (uricase) an enzyme present in many mammalian species, play important role in the excretion of uric acid by breakdown of uric acid into allantoin, a more excrete able form of uric acid but in human the gene loss its function due to mutation (El Ridi and Tallima, 2017). Uric acid, chiefly in liver, is catalyze by the oxidation of hypoxanthine and xanthine in the presence of enzyme xanthine oxidase (XOD) (Wang *et al.*, 2010).

In human body, 90% of uric acid filtered through renal system is being reabsorbed in proximal tubules. This process of urate absorption depends on specific molecule. Recently Human Urate transpoter 1(hURAT1, SLC22A12) is confirmed that the transport of urate across cell membrane of proximal tubules, is being channeled back into the lumen of proximal tubules (Enomoto *et al.*, 2002). Like human, mouse reabsorbs uric acid in the proximal tubules, while uricase keeps the uric acid at low level in plasma. It is verified that mURAT1 a urate transporter present in mouse kidney. Therefore, Xanthine oxidase and mURAT1 are the important target for investigation of mechanism of hyperuricemia and development of drug for its treatment (Wang *et al.*, 2010).

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For the treatment of hyperuricemia and other urate related disorders, hypouricemic drugs are used (Mazzali *et al.*, 2001). These agents include XOD inhibitors, such as allopurinol is most often prescribed and uricosoric agents that inhibit the reabsorption of renal urate, is benzbromarone. Though they have some side effects include hypersensitivity and Stevens Johnson syndrome by allopurinol and hepatotoxicity by benzbromarone (Schlesinger, 2004, Wade *et al.*, 2017). So there is need of new agents or curative strategies for the physiologically regulation of uric acid and obstacle of hyperuricemia (Wang *et al.*, 2010).

There are two major categories of hypouricemic drugs, the agents that decrease the synthesis of uric acid. The agents that increase the excretion of uric acid (Dan *et al.*, 1994). The medicinal plants used in hyperuricemia or gout are based on the knowledge of traditional system of medicine (Guerrero and Guzman, 1998, Kong *et al.*, 2000). These uses of medicinal plants are lack of scientific evidence. There has been paying attention on identifying such phytochemicals that hold potential to inhibit Xanthine oxidase (Zhu *et al.*, 2004) or increase excretion or both, so that urate levels can be reduced. Flavonoids and phenolics compounds have significant ability to inhibit xanthine oxidase and have hypouricemic effect (Mehmood *et al.*, 2017, Zhao *et al.*, 2014). *Piper nigrum*, *Trachysermum ammi*, *Berberis vulgaris* are reported to have flavonoids and phenolics compounds (Damanhouri and Ahmad, 2014, Damodar *et al.*, 2011, Javed *et al.*, 2012, Koncic *et al.*, 2010) and commercially available Sat Ajwain. Due to above said phytochemicals, herbal formulation (Urinil-B) was developed which contains these three plants extracts to evaluate its antihyperuricemic action.

MATERIALS AND METHODS

Preparation of plant extract

Selected plants parts were collected from Bahawalpur. They were carefully cleaned and identified from Botany Department, The Islamia University of Bahawalpur, Pakistan. The plants parts were powdered following by proper cleaning. These three plants were macerated in hydro-ethanolic 30/70 V/V (Abbasi *et al.*, 2018) for 15 days with occasionally stirring in a day at room temperature. The filtrate was obtained following by filtration and residue was again macerated with fresh solvent two more times. After the collection of all filtrate of each plant extract was evaporated under reduce pressure and controlled temperature 30-40°C. While sat ajwain, a solid crystal was purchased from market.

Chemicals

Different chemicals and drugs utilized in the study were purchased from different source as: Potassium oxonate, xanthine oxidase from bovine milk (Sigma-Aldrich), Xanthine (Alfa-Aesar), allopurinol (Mega Pharmaceutical, Lahore), ethanol, methanol, HCl, NaCl, DMSO, KH_2PO_4, KOH pellets, $CaCO_3$, (Merk), Ketamine (Global Pharmaceuticals (Pvt) Ltd. Pakistan), Xylazine (Prix Pharmaceutica, Lahore).

In vitro experiment

Herbal Formulation (Urinil-B) Preparation

All three plant extracts in the formulation *Trachyspermum ammi* (Fruits extract & Sat Ajwain in ratio 3:1), *Piper nigrum* (Fruits) and *Berberis vulgaris* (Bark) were taken equally, mix with adsorbing material i.e. microcrystalline cellulose, calcium carbonate. Formulation and adsorbing material was mixed thoroughly with equal ratio to make fine powder and powder was sieved through sixteen number mesh and capsules were filled manually (Singh and Waldia, 2015).

Physical appearance of capsule

The capsules were observed by shell completeness, shell shape, color and ingredients inside of the capsules were observed to evaluate the physical appearance of the capsule (Tunsirikongkon *et al.*, 2013).

Weight variation of capsule

20 filled capsules were weighed and then drug was discarded and empty capsules were also weighed separately to evaluate the capsule weight uniformity. The difference of empty and filled capsule defined the drug in the capsule (Tunsirikongkon *et al.*, 2013).

Determination of λ_{max}

Drug extract was dissolved in phosphate buffer saline (PBS) (pH 7.4) and simulated gastric fluid (SGF) (pH 1.2) separately. These two solutions diluted suitably and scanned in UV-Vis region 200-800 nm via UV-Vis spectrophotometer (IRMECO-2020) to get $\lambda_{max.}$

Calibration Curve preparation

10 mg of formulation drug extract was weighed correctly and dissolved in each 200 ml SGF (1.2 pH) and 200 ml PBS (7.4 pH). These solutions of 50µg/mL concentration were considered as a stock solution which was used for the preparation of further serial dilutions of 5, 10, 15, 20, 25, 30, 35, 40, 45µg/mL. The absorbance was measured by UV-Vis spectrophotometer using individual solvent as blank at $\lambda_{max.}$ Calibration curve was found by plotting the values absorbance against concentration µg/mL (Singh and Waldia, 2015).

In vitro dissolution test

Absorption rate, bioavailability and in vivo performance of dosage form can be predicted by dissolution test. Paddle apparatus USP type II (Pharma Test, Germany) was used for dissolution in the study. PBS (7.4 pH) and artificial gastric juice SGF (1.2 pH without enzymes) were used in the study as dissolution media. The dosage

form containing 250 mg drug extract was putted in the apparatus, volume 500 ml of dissolution medium, temperature 37±0.5°C and rotation was set at 50 rpm. The samples of 5 ml were taken at different intervals of time by replenishing with same amount of fresh medium. The samples were analyzed through UV-Vis spectrophotometer (UV/Vis Model U2020 IRMECO Germany).

In vitro xanthine oxidase inhibition activity

Xanthine oxidase (XOD) inhibition activity was followed as reported by (Zafar *et al.*, 2017) with some changes. XOD inhibition activity was assayed in phosphate buffer 50mM of pH 7.4. Test drugs including standard drug allopurinol was dissolved in dimethyl sulpho oxide (DMSO). Test drugs were dissolved at 5 mg/ml and allopurinol at 0.5mM concentrations, XOD (0.003 unit/well) and xanthine were dissolved in phosphate buffer 50mM of pH 7.4, 20µL buffer and 10µL of test drug was poured in the well in 96 well microplate and it was pre incubated at 30°C for 10 minutes. Reaction was started with the addition of substrate 20µL/well (0.1mM of xanthine), and then incubated at 30°C for 30 minutes. The inhibition of enzyme XOD was measured by uric acid formation at 295 nm spectrophotometerically by means of ELISA microplate reader (BioTek). Enzyme inhibition was calculated by following formula:

% Inhibition=100-(OD of test/OD of control)*100

In vivo experimental section

In vivo Experiment

For *in vivo* study, procedure and protocols for care of laboratory animals were strictly followed according to animal care committee, Department of Pharmacy, The Islamia University of Bahawalpur, Punjab, Pakistan. The study procedure and protocols were approved by Pharmacy Research Ethics Committee (PREC), Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Punjab, Pakistan (37-2015/PREC).

Animal model

Wistar albino male rats of 170-200 g weight were selected in the study. All animals were housed in the animal house of Pharmacology and Physiology research laboratory at Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Punjab, Pakistan. All the animals which were included in the study housed in polycarbonate cages 47×34×18 cm^3 (6 animals per cage) with standard conditions temperature 25±2°C and humidity 56±5% with 12:12 hour dark and light schedule, light start at 7am. All animals were allowed to adopt the environment for 1 week before the start of study. All animals were fed with standard chow and water *ad libitum.*

Hyperuricemia model in rats

Hyeruricemia in rats was induced by potassium oxonate, a uricase inhibitor as mentioned (Stavric *et al.*, 1975, Wang *et al.*, 2010, Zhao *et al.*, 2006). Potassium oxonate was injected intraperitoneally at dose 250 mg/kg before 1 h to the last drug administration to enhance hepatic and serum urate level (Zhao *et al.*, 2006).

Drug administration

Experiment was divided into three portions 1 day, 3 day and 7 day experiment. In each experiment animals were divided into 6 groups which contain 6 animals in each group. Sample size was calculated through "resource equation" method and was sufficient for statistical analysis (Charan and Kantharia, 2013). Animals were kept fasting (only food) for one hour prior to administration of drug. All drugs including allopurinol at different concentrations were dissolved or dispersed in distilled water. The amount or volumes of drug solution or suspension were given to animals depends upon the body weight. Six groups (normal control and hyperuricemic control) received distilled water orally for 1, 3, 7 day respectively. Standard control (3 groups) received allopurinol 10 mg/kg orally and 3×3 groups received formulation at concentration 250 mg/kg, 500 mg/kg and 1000 mg/kg for 1, 3 and 7 day respectively. All drugs were administered orally once daily via gavage at 9:00-10:00 am. Potassium oxonate was injected intraperitoneally at dose 250 mg/kg in all groups except normal control to induce hyperuricemia.

Collection of samples

Urine, blood and liver tissue were collected two hour after the administration of last drug. Urine was collected as mentioned by (Murugaiyah and Chan, 2009) with little changes. After final dose of drug administration animals were held in metabolic cages for 2 h while water ad libitum was given. Urine was collected and stored in tubes at -20°C till they were assayed. After urine collection, rats were anesthetized by ketamine HCl and xylazine (4:1) at dose 0.2mL/100 g intraperitoneally. After anesthesia blood samples were collected via cardiac puncture and allowed to clot for an hour. Serum was obtained by centrifugation (5000 rpm for 15min) of clotted blood and stored at -20°C till assayed. Simultaneously liver tissues were also excised and separated on ice plate carefully. Liver tissues were stored at -70°C till further process.

Determination of uric acid

The liver tissues were homogenized with the help of homogenizer (WiseTis-HG-15D) in 10 volume of ice cold potassium phosphate buffer (7.4 pH), then centrifuged at 12,000 rpm at 4°C for 15 min. Supernatant was separated carefully, and was intended for the determination of uric acid (Wang *et al.*, 2010). Hepatic, serum and urine urate was determined by diagnostic Uric Acid FS TBHBA kit (DiaSys Germany). Urine and serum urate was determined in mg/dL, whereas hepatic urate was determined in mg/g of wet tissue.

Determination of creatinine
Creatinine of urine and serum (mg/dL) were measured by using Creatinine FS assay kit DiaSys Diagnostic System, Germany).

STATISTICAL ANALYSIS

Results were analyzed by SPSS version 20.0 software (I.B.M. SPSS. statistics.v20_32bit_oxava.com). Data was expressed as mean ± standard error of mean (S.E.M). For in vivo activity results, Student's *t*-test for independent means was applied for calculating statistical significance of the differences between the groups. $P \leq 0.05$ (Two tailed values) were considered significant. One way ANOVA was used for checking the significance for enzyme inhibition assay. $P \leq 0.05$ were considered significant.

RESULTS

Physical appearance of Capsule
There was no change in the physical appearance of the capsule and the ingredients present inside the capsule after shell were being emptying.

Weight variation of capsule
As shown in table 1.

λ_{max} and Calibration Curve
Diluted solution of formulation drug extract was scanned to determine λmax. The maximal absorbance was found at 0.34 µm. This wavelength was used for the preparation of calibration curve and analysis of dissolution samples. As in table 2 and fig. 1.

In vitro Dissolution test
Dissolution was performed in two different dissolution media i.e. pH 1.2 to simulate gastric condition and in PBS (pH 7.4) to simulate physiological pH. Results of dissolution in term of cumulative amount (mg) and percentage of drug release at pH 1.2 is given in table 3 and plotted in fig. 2. Results of dissolution in term of cumulative amount (mg) and percentage of drug release in PBS (pH 7.4) is given here under in table 4 and plotted in fig. 3.

In vitro xanthine oxidase inhibition activity
In this study herbal formulation (Urinil-B) was evaluated for xanthine oxidase inhibition activity. The results of xanthine oxidase inhibition activities of formulation as compared to standard allopurinol are given in table 5. Results showed that significant xanthine oxidase inhibition was shown by herbal formulation with IC_{50} of 586±1.5µg/mL.

The administration of Urinil-B, a herbal formulation formulate from poly herbal plants extracts was found significant reduction in the serum uric acid levels in time and dose dependent manner as in table 6. The dose 250 mg/kg of Urinil-B was effectively reduce the serum uric acid level in 1, 3 and 7 day treatment compared to hyperuricemic control group, though less effective as compared to allopurinol standard group, and was found as effective as allopurinol in hyperuricemia treatment. Three day treatment was found more effective than one day treatment, same way 7 day treatment was found more significant than one and three day treatment when 250 mg/kg drug was administered.

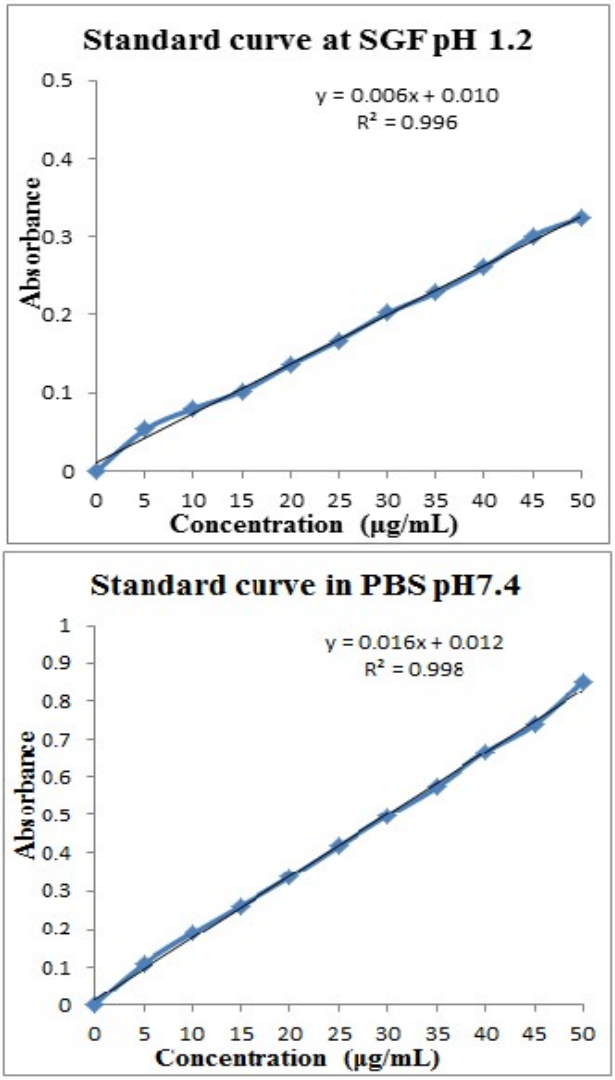

Fig. 1: Standard Curve SGF (pH 1.2) and PBS (pH 7.4)
Time and dose dependent effects of Formulation (Urinil-B) on serum and liver uric acid levels in hyperuricemic rat

Administration of Urinil-B at 500mg/kg dose was observed significant in time dependent manner compared to hyperuricemic group. One day treatment of 500mg/kg dose was effectively reduced the serum uric acid compared to hyperuricemic control. Seven day treatment was found more effective compared to one and three day treatment, while moderate reduction of serum urate was observed in three day treatment, though it was less significant compared to allopurinol standard control.

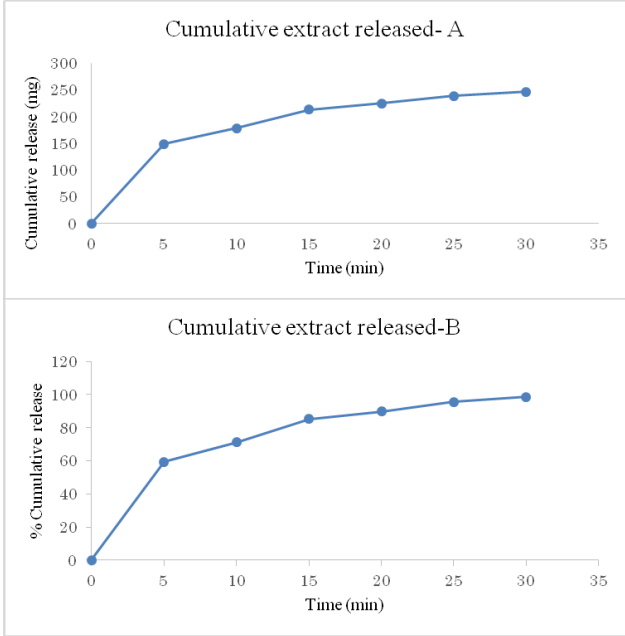

Fig. 2: Cumulative extract released at pH 1.2, A represents extract released in amount (mg) while B shows data in terms of percentage.

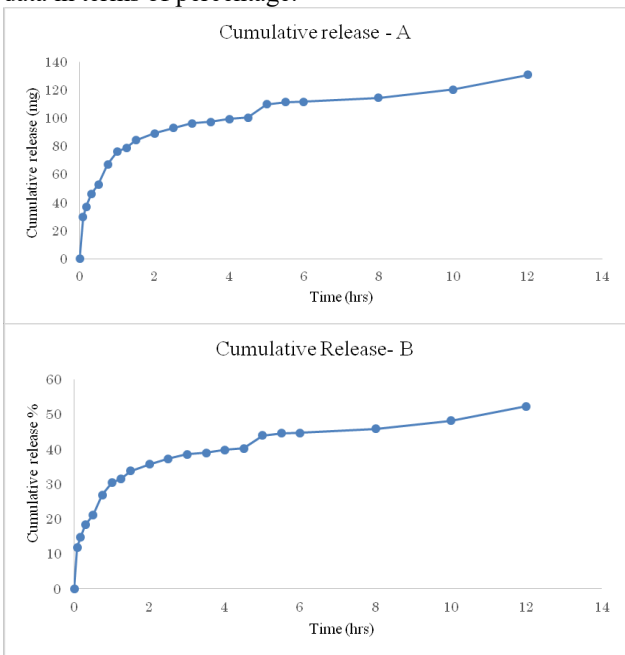

Fig. 3: Cumulative extract released in PBS (pH 7.4), A represents extract released in amount (mg) while B shows data in terms of percentage.

Administration of Urinil-B at 1000mg/kg to hyperuricemic rats, there was significant reduction in the serum urate levels was observed in time dependent manner in table 6. One day treatment of Urinil-B at 1000mg/kg was effectively reduce the serum urate level compared to hyperuricemic control, while less effective compared to standard control allopurinol and more than normal control.

There was significant reduction of serum urate level was observed in the pretreated group for 3 and 7 day than 1 day treatment. The rats which were pretreated for 7 days at dose 1000 mg/kg was reduced the serum uric acid less than the normal control.

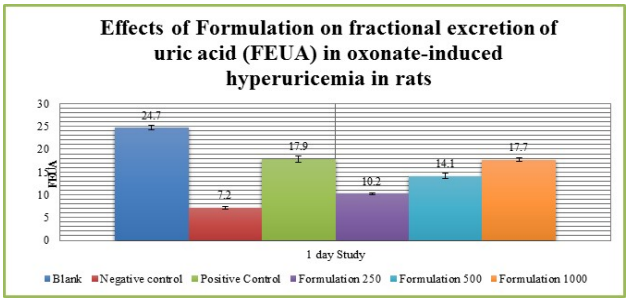

Fig. 4: FEUA after one-day treatment of *Formulation* (Urinil-B). For statistical significance, Student's t-test was used between control and study groups.

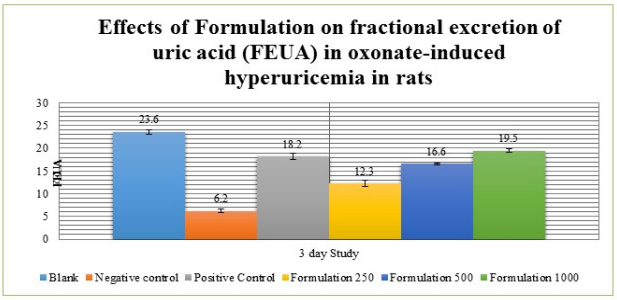

Fig. 5: FEUA after three-day treatment of *Formulation* (Urinil-B). For statistical significance, Student's t-test was used between control and study groups.

In same result of serum uric acid, Urinil-B *Formulation* was observed to be effective in the reduction of liver urate in time and dose dependent manner table 7. It was found that the administration of 250mg/kg effectively reduce the liver urate compared to hyperuricemic control, though it was still above the level of normal control in 1 day and 3 day treatment, while in 7 day treatment the reduction of liver urate was found more than that the normal control group and show significant result compared to standard control. 500mg/kg of Urinil-B was effectively reduces the liver urate level compared to hyperuricemic control in time dependent manner, while liver urate level was above compare to normal control in 1 day and 3 day treatment. The rats 7 day pretreated with 500 mg/kg were observed more effective compared to standard control. Administration of Urinil-B at 1000mg/kg to hyperuricemic group was observed significant reduction in liver uric acid in 1, 3 and 7 day treatment compared to hyperuricemic control, although it was same effective in 1 day, less effective 3 day and more effective in 7 day treatment compared to standard control. It was observed that poly herbal Urinil-B *Formulation* show significant result in reduction in liver and serum urate levels in time and dose dependent sequence.

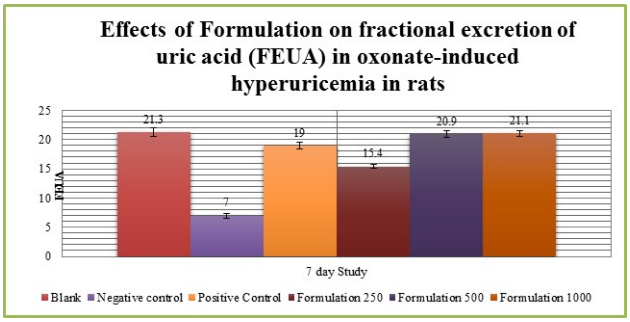

Fig. 6: FEUA after seven-day treatment of *Formulation* (Urinil-B). For statistical significance, Student's t-test was used between control and study groups.

Table 1: Weight variation of capsule

No.	Weight (mg)	Deviation %
1	0.602	±0.95082
2	0.599	±0.44774
3	0.5952	±0.189492
4	0.6042	±1.31974
5	0.5989	±0.43097
6	0.5961	±0.038569
7	0.589	±1.229185
8	0.588	±1.396878
9	0.5935	±0.474569
10	0.6021	±0.96759
11	0.6015	±0.86697
12	0.598	±0.28005
13	0.5999	±0.59866
14	0.5859	±1.749032
15	0.5998	±0.58189
16	0.5986	±0.38066
17	0.589	±1.229185
18	0.5895	±1.145339
19	0.6019	±0.93405
20	0.5945	±0.306877
	Average weight 0.59633	

% of deviation was calculated by the formula:
Deviation (%)= (Average weight-weight of capsule)/ Average weight ×100

Effects of Urinil-B Formulation on FEUA in potassium oxonate induced hyperuricemia in rats

The effect of Urinil-B *Formulation* on fractional excretion of uric acid (FEUA) for 1 day treatment at 250mg/kg was observed significant compared to hyperuricemic group, but still below level of normal control. Although 250mg/kg was found less significant compared to 500mg/kg, 1000 mg/kg and allopurinol standard control. 1 day treatment of Urinil-B at 500mg/kg and 1000mg/kg were found significant compared to hyperuricemic control group, while 500mg/kg was observed less effective compared to standard control and 1000mg/kg was establish almost equal to standard control as in fig. 4.

The effect of Urinil-B *Formulation* on fractional excretion of uric acid (FEUA) for 3 day treatment at 250mg/kg was observed effective compared to hyperuricemic control, but still below than normal control. 250mg/kg was less effective as compared to 500mg/kg, 1000mg/kg and standard control. Three-day treatment with Urinil-B at 500mg/kg and 1000mg/kg were found to be effective compared to hyperuricemic control, while 500 mg/kg was observed less effective when compared to standard control and 1000mg/kg was observed more significant compared to standard control as in fig. 5.

Table 2: Absorbance of different concentration at SGF pH 1.2 and PBS pH 7.4

Concentrations (µg/mL)	Absorbance	
	SGF pH1.2	PBS pH 7.4
0	0	0
5	0.053	0.107
10	0.08	0.188
15	0.102	0.261
20	0.136	0.366
25	0.167	0.418
30	0.202	0.498
35	0.228	0.573
40	0.261	0.666
45	0.301	0.738
50	0.324	0.852

Table 3: CDR (mg) and CDR (%) at pH 1.2

Time (min)	CDR (mg)	CDR (%)
0	0	0
5	148.6507	59.460
10	178.1645	71.265
15	213.2631	85.305
20	224.5872	89.834
25	239.0969	95.638
30	246.4781	98.591

Table 4: CDR (mg) and CDR (%) at pH 7.4

Time (hrs)	CDR (mg)	CDR (%)
0	0	0
0.083	29.6875	11.875
0.167	37.0609375	14.824375
0.3	45.84796875	18.3391875
0.5	52.925	21.17
0.75	67.1965625	26.878625
1	76.01359375	30.4054375
1.25	78.901875	31.56075
1.5	84.29296875	33.7171875
2	89.2184375	35.687375
2.5	93.0571875	37.222875
3	96.2746875	38.509875
3.5	97.3078125	38.923125
4	99.435625	39.77425
4.5	100.4717188	40.1886875
5	109.79	43.916
5.5	111.305	44.522
6	111.5714063	44.6285625
8	114.4942188	45.7976875
10	120.3885938	48.1554375
12	130.66375	52.2655

Administration of Urinil-B for 7 day at 250 mg/kg was observed significant compared to hyperuricemic control, but still below the normal control. Although 250mg/kg

was observed less significant compared to 500mg/kg, 1000mg/kg and standard control. 7 day administration of Urinil-B at 500mg/kg and 1000mg/kg were found more effective compared to hyperuricemic control and standard control. FEUA level of 500mg/kg was more compared to standard control as in fig. 6, while result was still below the level of normal control, but 1000mg/kg was observed more effective compared to standard control and FEUA level almost same as normal control. Overall poly herbal *Formulation* Urinil-B was observed effective in time and dose-dependent manner.

DISCUSSION

The present study reveals that the herbal formulation (Urinil-B) possess xanthine oxidase inhibition in vitro and was able to significantly reduce serum and liver uric acid levels in rats. The individual plant components of the Urinil-B are well known for their antihyperuricemic effects (Damanhouri and Ahmad, 2014, Damodar *et al.*, 2011, Javed *et al.*, 2012, Koncic *et al.*, 2010).

In this study, formulation inhibits xanthine oxidase sufficiently. Previously reported study suggested that flavonoids and phenolics are the main component in plant materials for xanthine oxidase inhibition (Ahmad *et al.*, 2018), all the plants included in Urinil-B have flavonoids and phenolic compounds (Damanhouri and Ahmad, 2014, Damodar *et al.*, 2011, Javed *et al.*, 2012, Končić *et al.*, 2010). So, it might be suggested that Urinil-B inhibit xanthine oxidase due to presence of these active metabolites in its individual components.

In vivo experimentation showed a drastic increase of blood urate levels in negative control groups as compared to other groups after potassium oxonate administration. It showed that hyperuricemia model of rats was effectively established.

Table 5: Effects of herbal formulation on inhibition of xanthine oxidase (*in vitro*)

	% inhibition	IC_{50}
Formulation (5mg/mL)	84.6±0.3***	586±1.5 (µg/mL)
Allopurinol(0.5mmol)	92±0.5	24.3±1.8 µmol
*** p ≤0.001 ANOVA		

In the current study, it was evaluated that oral administration of herbal formulation Urinil-B was able to significantly reduce serum and liver uric acid levels of hyperuricemic rats in a dose-dependent manner. However, the onset of antihyperuricemic effects of herbal formulation Urinil-B appeared to be slower than that of allopurinol. Antihperuricemic drugs reduced hyperuricemia by inhibiting xanthine oxidase and action on renal or extra-renal urate transporters. In this study, orally administered allopurinol significantly decreased potassium oxonate induced hyperuricemia in rats. It has action on a urate transporter and Cl/urate transporter (Bordier *et al.*, 2004) and inhibit xanthine oxidase (Wang *et al.*, 2017). Results matched to the several studies showing the decrease of hyperuricemia with allopurinol (Shan *et al.*, 2015, Shi *et al.*, 2016, Zhao *et al.*, 2006).

Current study showed the marked effectiveness of Urinil-B against xanthine oxidase and it might be suggested that formulation showed its hypouricemic potential by affecting on a urate transporter and Cl/urate transporter. However, effect of Urinil-B on urate transporter and Cl/urate transporter is not investigated yet.

The formulation might be effective in humans also as several research studies showed that urate transporter 1 (gene provoking hereditary renal hypouricemia) in rat kidney transported UA similar to human urate transporter 1(Hosoyamada *et al.*, 2004, Lipkowitz *et al.*, 2004). However, the study results should be replicated in large sample sizes and should analyzed in clinical trials also.

Table 6: Time and dose dependent effects of Formulation (Urinil-B) on serum uric acid levels in hyperuricemic rat

Treatment	Dosage (mg/kg)	Serum uric acid Level (mg/dl)		
		1 day study	3 day Study	7 day Study
Normal animal	---	2.6±0.1	2.7±0.09	2.9±0.07
Negative control(Vehicle)	---	5.33±0.1###	6.1±0.1###	7.5±0.7###
Formulation	250	4.2±0.1*###	3.9±0.3**##	3.1±0.3**
	500	3.9±0.3*##	3.4±0.4**	2.8±0.5**
	1000	3.4±0.3**#	3.0±0.2**	2.7±0.1**
Allopurinol	10	2.8±0.2**	2.54±0.1**	2.51±0.2**

Table 7: Time and dose dependent effects of *Formulation* (Urinil-B) on liver uric acid levels in hyperuricemic rat

Treatment	Dosage (mg/kg)	Liver uric acid Level (mg/g wet tissue)		
		1 day study	3 day Study	7 day Study
Normal animal	---	0.23±0.009	0.23±0.01	0.24±0.009
Negative control	---	0.5±0.005###	0.6±0.006###	0.7±0.005###
Formu-lation	250	0.35±0.02**##	0.29±0.01**#	0.22±0.02**
	500	0.29±0.02**#	0.25±0.01**	0.22±0.02**
	1000	0.26±0.02**	0.26±0.02**	0.19±0.02**
Allopurinol	10	0.26±0.01**	0.23±0.001**	0.23±0.01**

Data represent mean ± S.E.M. of 6 animals. For statistical significance, Student's t-test was used between control and study groups.
* P < 0.01 (compared to hyperuricemia control group).
** P < 0.001 (compared to hyperuricemia control group).
# P < 0.05 (compared to normal control group).
P < 0.01 (compared to normal control group).
P < 0.001 (compared to normal control group).

CONCLUSION

The current study indicated antihyperuricemic potential (*in vitro* and *in vivo*) of Urinil-B in potassium oxonate induced hyperuricemia model of rats in dose dependent

manner. However, caveat of small sample size involved in making a firm conclusion regarding its hypouricemic potential.

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