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EFFECT OF AMLA (*EMBLICA OFFICINALIS*) AND MILK THISTLE (*SILYBUM MARIANUM*) ON MANAGEMENT OF HYPERURICEMIA AND RISK OF GOUT IN HYPERURICEMIC RATS

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Abstract

Background: *Hyperuricemia is the increased level of uric acid in blood. The occurrence of hyperuricemia is increasing day by day, worldwide. Medicines used for hyperuricemia show multiple side effects by damaging liver and kidneys. Now a days, focus is increasing towards herbal medicines and nutraceuticals. Amla and milk thistle are nutrient dense foods with medicinal benefits. Such type of foods can be used to reduce hyperuricemia and gout naturally.*

Aim: *This study has been carried out to evaluate the medicinal effect of amla and milk thistle, individually and synergistically, in potassium oxonate-induced hyperuricemic rat model.*

Materials and Methods: *For phase-I, amla fruit and milk thistle seeds were purchased from local market of Rawalpindi, Pakistan. After processing, amla fruit and milk thistle seeds were grounded into fine powder. Proximate analysis, total polyphenols and ascorbic acid content were determined of both powders. For phase-II (in-vivo experiment), 36 albino male rats (250-300 g) acclimatized and then divided into 6 groups having 6 rats in each group. Group I, II and III were normal control, positive control and negative control groups. Group IV, V and VI were treatment groups. All the groups were given potassium oxonate (250 mg/kg/day) intra-peritoneally to induce hyperuricemia in them except Group I. Group III, IV, V and VI were administered allopurinol (5mg/kg), amla fruit powder (300mg/kg), milk thistle seed powder (300mg/kg) and combined dose of amla and milk thistle (150+150 mg/kg) orally. All the treatments were given after 1h of injecting potassium oxonate daily for 15 consecutive days. On day 15, animals were slaughtered and blood samples were preserved for testing. The serum levels of uric acid, creatinine, ALT and bilirubin were measured using commercially available kits according to recommendations of manufacturers. Statistical analysis was performed by applying analysis of variance (ANOVA) through SPSS and significance of differences was determined at the level of* $\rho < 0.05$.

Results: *Proximate analysis determined the high amount of moisture (10.26%), ash (4.28%), fiber (9.12%) and carbohydrates (68.74%) in amla and milk thistle*

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contains significantly high protein (21.78%) and fat (18.46%) content than amla. Between two powders, Total polyphenols (31.10 mg GAE/ g of extract) and Total flavonoids (21.06 mg QE/ g of extract) contents were high in milk thistle seed powder. But amla contains significantly high Vitamin C content (159.05 mg/100g) than milk thistle seed powder (29.16 mg/100g). At the end of study, rats of PO group-III showed significantly high levels of serum uric acid, creatinine, ALT except bilirubin. On the other hand, treatments given to rats of group III, IV, V and VI showed significant reduction in kidney and liver parameters.

Conclusion: *Oral administration of amla fruit and milk thistle seeds for 15 days has reduced serum uric acid levels along with creatinine, ALT and bilirubin. It is recommended that therapeutic potential of amla and milk thistle should be further tested and used for the treatment of hyperuricemia, gout and other diseases in varying doses individually and synergistically.*

1. INTRODUCTION

Hyperuricemia is a metabolic disorder caused by an imbalance in synthesis and excretion of uric acid, leading to multiple comorbid conditions like gout, diabetes, chronic renal disease, obesity, hypertension, and coronary artery disease (Wu, M et al., 2023) (An et al., 2023). Overall, prevalence of hyperuricemia is 25.8%, majorly effecting men more than women due to genetic factors and increased consumption of fat, sugar, and protein (Dehlin et al., 2020) (Wen et al., 2020) (Charan et al., 2022). Gout can develop if level of serum urate content is 6.8 mg/dL or more (Singh & Gaffo, 2020). Gout causes the buildup of uric acid crystals in joints specifically in the foot's metatarsophalangeal joint, knees, ankles, and arms, causing swelling and reddening (Engel et al., 2017) (Daoudi et al., 2020). It affects 2.68 out of every 1000 people annually, particularly in western countries (Charan et al., 2022). Gout patients also have a high prevalence of metabolic syndrome (Uaratanawong et al., 2011).

Hyperuricemia treatment involves reducing uric acid production or its elimination through kidneys (An et al., 2023). Medications like Allopurinol and febuxostat significantly lowers uric acid levels (Wen et al., 2020), but they also have side effects like nephrotoxicity, allergic reactions, liver damage, bone marrow suppression, hypersensitive reactions, (Li, S et al., 2019; Sarvaiya et al., 2015) recurrence risks and cardiovascular illnesses (Tao et al., 2022) (Chen et al., 2019). Research on herbal medicines is gaining attention due to their diverse biological activities, cost-effectiveness, and safety (Shivaraj et al., 2021). Plant-based compounds play a crucial role in preventing and treating hyperuricemia and other diseases (Serrano et al., 2020). Food components which Inhibit XO enzyme activity can reduce uric acid formation and alleviate inflammation (Sarvaiya et al., 2015).

Phyllanthus emblica also known as amla, its fruit is used in Ayurveda to treat inflammation, diarrhea, and jaundice and to extend life. It promotes vitality, power, and wellbeing while strengthening the immune system (Saini et al., 2022). Polyphenols and ascorbic acid are key ingredients in its health-promoting properties (Wu, M et al., 2022; Sonkar et al., 2020; Tewari et al., 2019; KC et al., 2020; Hussain et al., 2021). Amla is a potent anti-oxidant, contains minerals, polyphenols, tannins and bioactive compounds like gallic acid, ascorbic acid, pedunculagin, punigluconine, phyllaemblicin B, emblicanin A, emblicanin B, (Ikram et al., 2021) (Jagdale et al., 2021) and tannins in it prevent vitamin C oxidation by binding with it (Priya & Islam., 2019). Emblica officinalis has numerous beneficial properties, including anti-inflammatory, (Wang et al., 2019) antioxidant, (Chaphalkar et al., 2017; Sheoran et al., 2019) anti-jaundice, anti-dyslipidemic, (Quranayati et al., 2023) nephroprotective, (Huang et al., 2023) anti-apoptotic, (Chekdaengphanao et al., 2022) anticancer (Chekdaengphanao et al., 2022; Naik, & David., 2023) anti-aging, (Wu et al., 2022) anti-

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diabetic, (Naik and David, 2023) antibacterial and immunomodulatory (Jantan et al., 2019).

Researches suggests that consuming amla can cause dose-dependent antigout effect and reduces arthritic pain, supporting the role of vitamin C for gout treatment (Sarvaiya et al., 2015). Researches showed that vitamin C supplementation given at dose 500mg/day can aid in gout prevention and treatment by lowering serum uric acid levels (Zhang et al., 2022) (Towiwat& Li, 2015) (Sun et al., 2018) (Choudhury et al., 2016).

Silybum marianum L. Gaertn, also known as milk thistle or mary thistle is a frequently found plant in the Asteraceae family (Meddeb et al., 2017). It is a well-known herb with a wide-ranging history of medicinal use and it is renowned for its powerful antioxidant, regenerative, anti-inflammatory (Javeed et al., 2022) and hepatoprotective effects, due to presence of specifically Silybin, as a proven medicine, with safe doses range from 420 to 600 mg per day (Harrabi et al., 2018) (Gillessen & Schmidt., 2020).

The main active compound of milk thistle is silymarin, present in fruits, leaves and seeds with the highest amount present in seeds (1–3%) (Harrabi et al., 2018) (Hobbs, 2008). In addition, 50–70% of silymarin consist of silybin, which is the most biologically active compound with powerful medicinal properties (Kalinowska et al., 2022) (Bijak, M., 2017). Milk thistle also contains diverse compounds and flavonolignans such as silybin (silibinin) A, silybin (silibinin) B, isosilybin (isosilibinin) A, silybin (isosilibinin) B, silychristin (silicristin), and silydianin which possess free radical scavenging and strong antioxidant properties (Javeed et al., 2022; Asmarian et al., 2017; Raclariu-Manolică & Socaciu, 2023; Bhattacharya, S., 2020).

Research showed that extracts of Silybum marianum, silibinin, silymarin effectively reduced serum urea, creatinine, and uric acid levels in kidney carcinogenesis in male Wistar rats (Yassin et al., 2021). Researches showed that silymarin can help reduce inflammation, lowers oxidative stress, hepatic fat deposition, insulin resistance and boost antioxidant enzymes levels (Marin et al., 2017) (Wadhwa et al., 2022) (Gillessen & Schmidt., 2020) (Valková et al., 2020). It also has anti-Parkinson's and Alzheimer's benefits (Ullah & Khan, 2018). It also has anti-inflammatory benefits for arthritis sufferers (Hussain et al., 2009), significantly reduce symptoms of Rheumatoid arthritis (RA) (Shavandi et al., 2017). Several researches suggested the strong anti-oxidant and anti-inflammatory potential of amla and milk thistle owing to the presence of abundant bioactive compounds in them. Studies also showed that the consumption of amla and milk thistle can aid in reducing serum uric acid levels along with the reduction in the risk of comorbids related to hyperuricemia like gout, cardiac diseases, hypertension, kidney dysfunctioning etc. Although, literature suggest that more well-designed trials are required to fully understand their effect. Therefore, the current study aims to study their bioactive compounds content, also to evaluate the individual and synergistic effect of amla fruit and milk thistle seeds on hyperuricemia and RFT and LFT parameters.

2. MATERIALS AND METHODS

1.1 Amla and Milk Thistle Seeds Processing

For this study, amla fruit and milk thistle seeds were purchased from local market of Rawalpindi. Amla was washed, cleaned, deseeded, cut into small pieces. Afterwards, amla and milk thistle seeds were shade dried and converted into fine powder with the help of a mechanical grinder. Powders were sieved and stored in air tight containers, separately.

1.2 Proximate Analysis

Proximate analysis of amla and milk thistle seed powder was done in the labs of National Agriculture Research Center (NARC), Islamabad. The moisture, fiber, protein, fat, carbs and ash content were measured in the sample. Each component is determined through different methods as given below;

Moisture analysis: Moisture analysis was done in the hot air oven through AOAC Method No. 925. 10 (2010). Samples were dried at 130 ˚C for 60 minutes. The equipment directly gives the moisture content in percentage, which is visible on LCD screen.

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Ash determination: Ash determination was done through AOAC Method No. 923. 03(2010), in the muffle furnace. Samples were placed in muffle furnace at 550 ° C for 16 hours and allowed to incinerate until light grey ash is obtained. The percentage of moisture was calculated in the following way;

Ash % = $\frac{W_{3-}W_1}{W_2} \times 100$

- W1= Weight of empty crucible
- W2= Sample weight
- W3= Weight of ash with crucible

Crude fat determination: Crude fat in the sample was determined by Soxhlet apparatus through AOAC Method No. 2003. 06(2010). Crude fat in the sample was extracted with the help of n-hexane. After extraction, solvent was evaporated and fat content was determined. The percentage of crude fat was calculated in the following way;

Crude fat %= $\frac{W_{3-}W_1}{W_2} \times 100$

- W1= Weight of empty beaker
- W2= Weight of beaker containing extracted fat
- W3= Weight of sample

Fiber determination: Total crude fiber in the sample was determined through a method based on cereal chem. 59(4): p.318 (1982). Sample was treated with 0.2549N H_2SO_4 and 40.742% NaOH solution. Residue was dried and ignited to obtain crude fiber. The percentage of crude fiber was calculated in the following way;

Crude fiber %= $\frac{A-B\times 100}{\text{Weight of sample}}$

Determination of protein: Determination of crude protein in the sample was done by kjeldahl method. Sample was digested with concentrated sulphuric acid, then the product ammonium sulfate was boiled with sodium hydroxide and converted into ammonium hydroxide. The titrant (dilute sulphuric acid) was calculated against the amount of ammonium hydroxide present in the distillate. Results were reported as percent of nitrogen or percent protein.

Carbohydrates analysis: Carbohydrates were determined by subtracting the percentage of ash, protein, fat, fiber and moisture in the sample from the total percentage.

Carohydrates content= [100 - (% moisture+ % ash + % crude protein + % crude fat + %crude fiber)].

1.3 Ascorbic Acid Determination

Amount of Ascorbic Acid (vitamin C) present in the sample was determined by the AOAC official method 967.21. Vitamin C present in the sample was extracted and titrated in the presence of HPO3-CH3COOH or HPO3-CH3COOH-H2SO4 solution to maintain proper acidity for reaction and to avoid autoxidation of ascorbic acid at high pH. The end point was considered when ascorbic acid reduced the rose-pink dye, 2,6-dichloroindophenol, into a colorless solution.

1.4 Total Polyphenols Determination

Estimation of total phenolic percentage was performed by the AOAC official method 2017.13. Phenols were extracted in water and then reacted with the Folin-C reagent (a complex mixture of heteropolyphosphotungstate-molybdate) in the presence of sodium carbonate to form a blue-colored complex. The intensity of the blue color was proportional to the amount of reactive phenolic compounds in the sample. The phenolic content was determined by measuring the absorbance of the sample solution at 765 nm and comparing it with a calibration curve using gallic acid as a standard. The method was able to quantify total polyphenolic content of about 5 (w/w) to 100% (w/w) in the extracts.

1.5 Study Type and Ethical Consent

It was an in-vivo experimental study. All the procedures done in this study were approved by Ethical Committee, National Institute of Health (NIH), Islamabad, Pakistan.

1.6 Chemicals

Potassium oxonate was purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Allopurinol was purchased from a medical store in Islamabad. All the other essential equipments and chemicals like choloform, cages, standard diet and pure water etc were provided by animal house of NIH, Islamabad. The testing kits for measuring serum uric acid (UA), serum creatinine (CRE), serum ALT and serum bilirubin

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were purchased from Chronolab Systems (Barcelona, Spain).

1.7 Test compound preparation

Potassium oxonate (PO) (250 mg/kg body weight, IP) used for induction of hyperuricemia and allopurinol (5 mg/kg body weight, PO) given to negative control group (GIII) was dissolved in 0.9% saline solution. Powders of amla fruit and milk thistle seeds (300mg/kg individually, combined dose; 150+150 mg/kg per day) were dissolved in 0.9% saline solution.

1.8 Induction of hyperuricemia and Treatment

Potassium oxonate (250mg/kg) was used to induce the hyperuricemia in rats. Rats will be treated with allopurinol, amla powder and milk thistle seeds powder 1h after potassium oxonate induction. Potassium oxonate will be given intra- peritoneally by dissolving in it 0.9% saline solution and treatment solutions will be administered to rats directly in stomach by using rat oral gavage needle with 2 ml BD syringe once daily for 15 consecutive days. Normal control group will be given 0.9% saline (2ml/kg/day) orally with same method.

1.9 Experimental Animals and design

Thirty-six adult male albino rats weighing between 250 and 300 grams were purchased and kept at Animal House of National Institute of Health (NIH), Pakistan. Prior to the trial, animals were acclimatized for 1 week. The animals were housed in standard polypropylene cages and given access to an unlimited supply of purified water and a standard normal diet (SND). The animals were housed in settings that were specifically pathogen-free (SPF) at 22 ± 2℃ (20–25℃), with a humidity of 60 ± 5%, and on a 12-hour light/dark cycle for the entire duration of the experiment. After all the rats were weighted, they were split up into 6 groups (n = 6 per group) with average weights that were equal for each group, as following

Group 1: Control group (CO): the rats were given 0.9% saline (2ml/kg/day) orally for consecutive 15 days.

Group 2: Potassium Oxonate group (PO): The rats were given 0.9% saline (2ml/kg/day) orally, along with intra-peritoneally injected potassium oxonate (250mg/kg/day) in 0.9% saline for consecutive 15 days. **(Positive control group)**

Group 3: Allo-PO group (APO): The rats were injected with potassium oxonate (250 mg/kg/day) dissolved in 0.9% saline directly into their abdominal cavity. After one hour, they were then given allopurinol (5 mg/kg/day) in 0.9% saline for 15 consecutive days. **(Negative control group)**

Group 4: Treatment Group 1 (T1): The rats were injected with potassium oxonate (250 mg/kg/day) in 0.9% saline directly into their abdomen. An hour later, they were given amla powder (300 mg/kg/day) mixed in a 0.9% saline solution for 15 consecutive days.

Group 5: Treatment Group 2 (T2): The rats received an injection of potassium oxonate (250 mg/kg/day) in 0.9% saline directly into their abdomen. One hour later, they were given milk thistle seed powder (300 mg/kg/day) mixed in 0.9% saline for 15 consecutive days.

Group 6: Treatment group 3 (T3): The rats were injected with potassium oxonate (250 mg/kg/day) in 0.9% saline directly into their abdomen. An hour later, they were given a mixture of amla and milk thistle seed powder (150 mg/kg/day each, in a 50:50 ratio) dissolved in 0.9% saline for 15 consecutive days.

1.10 Blood Sample Collection and Biochemical Analysis

On the day 15, rats were sacrificed after being fasted for 12h and anesthetized by chloroform. Using the heart puncture technique, blood samples were extracted and allowed to clot for 1h at room temperature. The collected blood samples were centrifuged for ten minutes at 3000 rpm at 10´C. For analysis, serum samples were kept at -20˚C until the biochemical assays were performed. Using commercially available detection kits, the levels of serum uric acid, serum creatinine, serum ALT, and serum bilirubin were measured in accordance with manufacturer recommendations.

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1.11 Statistical Analysis

The data was presented as mean values ± standard error of the mean (SEM). Statistical test calculations regarding analysis of variance (ANOVA) were performed by SPSS. The significance of differences was determined by analysis of variance (ANOVA). A level of $\beta < 0.05$ was considered to be statistically significant.

3. RESULTS

3.1 Phase-I

In phase-I of the research results, the proximate analysis of amla powder and milk thistle powder provided crucial insights into their basic nutritional composition, including moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate content. These parameters are essential for evaluating their potential as functional food ingredients or supplements. The analysis revealed that both powders possess unique nutritional profiles reflective of their botanical origins. Amla powder, known for its rich vitamin C content and antioxidant properties, demonstrated a relatively higher carbohydrate and moisture content, while milk thistle powder, commonly used for liver support, showed a notable protein and fiber presence. These findings underline the distinct compositional benefits of each powder, supporting their application in health-promoting dietary formulations.

Table 3.1 Proximate analysis of Amla powder and Milk thistle seed powder

Parameters	Amla powder (100 g)	Milk thistle seed powder (100 g)
Moisture (%)	10.26	7.44
Ash (%)	4.28	1.97
Protein (%)	5.59	21.78
Fat (%)	2.01	18.46
Fiber (%)	9.12	5.87
NFE (%)	68.74	44.48

3.1.1 Proximate analysis

The quantitative examination of food and food substances, such as moisture, crude protein, total fat, total carbohydrate, and crude ash, is done by means of proximate analysis (Suksaard & Ganogpichayagrai, 2020). Table 1 displays the approximate composition of amla fruit and milk thistle seed powder. Amla contains more carbohydrates (respectively, 68.74 g/100 g) as compared to milk thistle se eds (44.48 g/100 g). Whereas, the highest content of protein and fat was found in milk thistle seeds (21.78 g/100 g, 18.46 g/100 g) while amla had very less amount (5.59 g/100 g, 2.01 g/100 g). The fiber, ash and moisture content showed little variation in both samples with higher amount in amla (9.12 g/100 g, 4.28 g/100 g, 10.26 g/100 g) and a little lower in milk thistle (5.87 g/100 g, 1.97 g/100 g, 7.44 g/100 g).

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Table 3.2 Polyphenols, flavonoids and ascorbic acid results

Parameters	Amla powder (100 g)	Milk thistle seed powder (100 g)
Total polyphenols (mg GAE/ g of extract)	27.43	31.10
Total flavonoids (mg QE/ g of extract)	11.30	21.06
Ascorbic acid (mg/100g)	159.05	29.16

3.1.2 Total Polyphenols, flavonoid and ascorbic acid content

Slightly higher levels of polyphenols were found in milk thistle (31.10 mg CGA/100 g) as compared to amla (27.43 mg CGA/100 g). Total flavonoids obtained in milk thistle and amla were 21.06 mg QE/ g of extract and 11.30 mg QE/ g of extract. Ascorbic acid found in amla (159.05 g/100g) was lower than many similar studies on it whereas, milk thistle contains a good amount (29.16 mg/100g) of ascorbic acid, surprisingly.

3.2 Phase-II: Experimental Results

In phase-II experimental research results are presented. The effects of repeated oral administration of *P. emblica* fruit and *Silybum marianum* seed powders for 15 days in normal and potassium oxonate-induced hyperuricemic rats on different blood biochemical markers are shown in Table 3.3.

Table 3.3 Mean values of different treatments of amla, milk thistle and potassium oxonate.

Groups	Uric Acid (mg/dL)	Creatinine (mg/dL)	ALT (U/l)	Bilirubin (mg/dL)
I (CO)	4.01± 0.026	0.80±0.017	14±1.20	0.70±0.017
II (PO)	21.19±0.58	2.25±0.057	95±2.31	0.60±0.012
III (APO)	4.52±0.06	1.13±0.017	47±2.65	0.80±0.023
IV (T1)	17.15±0.08	1.25±0.023	59±2.60	0.70±0.026
V (T2)	10.24±0.026	0.58±0.023	67±2.88	0.50±0.012
VI (T3)	12.14±0.075	1.27±0.034	49±1.73	0.80±0.029

3.2.1 Effect of treatment on serum uric acid

Table 3.3 shows that mean values of uric acid in normal and potassium oxonate induced hyperuricemic rat groups. The level of uric acid significantly differed statistically at the level $p<0.05$. Serum uric acid levels were in range (4.01± 0.026 mg/dL) in normal control group (GI) as compared to PO induced hyperuricemic rats (Group II). However, injecting potassium oxonate (250 mg/kg/day) raised serum uric acid levels in Group II-VI, significantly in Group II (21.19±0.58 mg/dL). Whereas, Group III to VI which received allopurinol (5mg/kg/day), amla (300 mg/kg/day), milk thistle (300 mg/kg/day) and combined dose (150+150 mg/kg/day) as treatment demonstrated a significant reduction in blood uric acid levels during a 15- day period with mean values 4.52±0.06 mg/dL, 17.15±0.08 mg/dL, 10.24±0.026 mg/dL and 12.14±0.075 mg/dL, indicating that amla and milk thistle could

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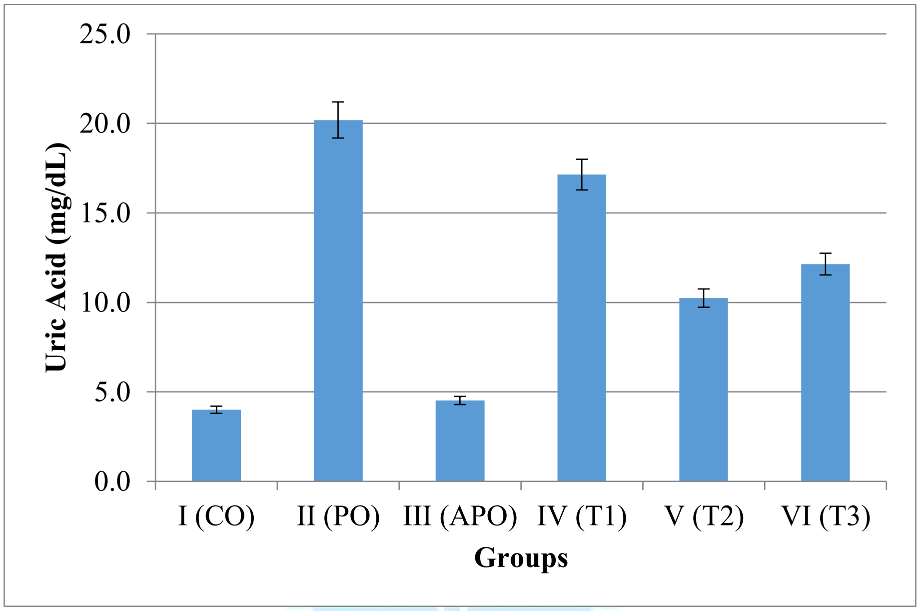
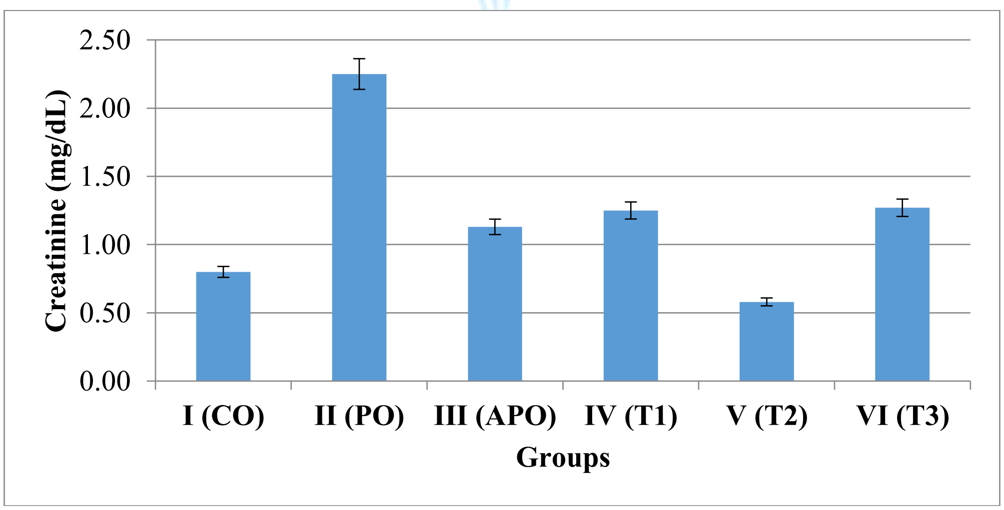
alleviate PO- induced hyperuricemia. Among all the treatments milk thistle consumption (Group V) caused significant lowering effect on serum uric acid levels after allopurinol in (Group III).



3.2.2 Effect of treatment on serum Creatinine levels

Table shows that mean values of all groups regarding creatinine level were significantly differed statistically at the level $p<0.05$. According to Chen et al. (2019), serum creatinine is another crucial marker of renal damage. As shown in table, PO treatment caused a significant elevation (2.25±0.057 mg/dL) in serum level of creatinine compared with the normal control group. Moreover, allopurinol, amla, milk thistle and combined dose also markedly decreased the levels of serum creatinine, indicating that milk thistle could effectively alleviate PO-induced hyperuricemia (0.58±0.023 mg/dL) as compared to all other treatments.



3.2.3 Effect of treatment on serum ALT

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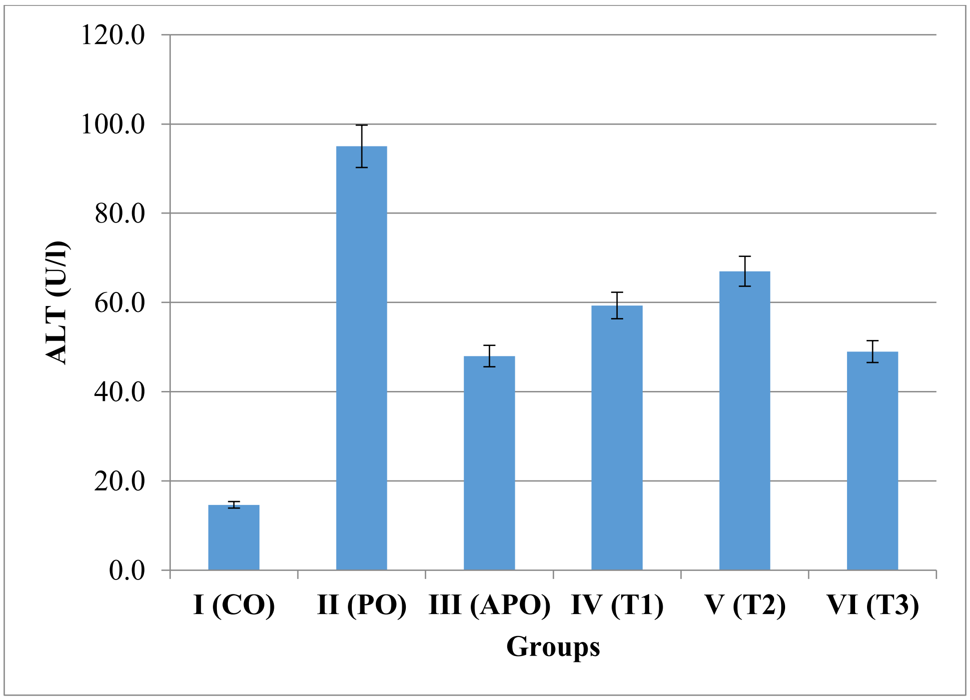
Table shows the mean values of serum ALT in all the groups are significantly altered ($p<0.05$). As demonstrated in Table 4.3, Group II (PO) showed a significant ($P<0.05$) increase in the levels of serum ALT (95±2.31 U/l) as compared to Group I (CO) (14±1.20 U/l), due to PO treatment for consecutive 15 days. Group III (APO) (47±2.65 U/l) administered allopurinol showed a significant decrease in the serum levels of ALT as compared with the Group II (PO) (47±2.65 U/l). Results showed the oral administration of amla and milk thistle, separately and combined in Group IV (59±2.60 U/l), Group-V (67±2.88 U/l) and VI (49±1.73 U/l) significantly decreased the levels of serum ALT as compared to the Group II (PO). According to the results, amla is more effective in reducing the serum ALT in potassium oxonate induced hyperuricemia rats as compared to milk thistle. Results also showed positive synergistic effects of amla and milk thistle on serum ALT in this research.



3.2.4 Effect of treatment on serum bilirubin

Table shows that mean values of various treatment/groups regarding bilirubin were significantly differed statistically at the level $p<0.05$. The effects of potassium oxonate and the interventions administered to groups III–VII demonstrated that there is not a significant difference in any group's serum bilirubin levels. Group II (PO) and Group I (CO) did not significantly vary in their levels of serum bilirubin with value (0.60±0.012 mg/dL) and (0.70±0.017 mg/dL), irrespective of injecting potassium oxonate in group II. However, Group III (APO group) showed slightly higher serum bilirubin levels (0.80±0.023 mg/dL) than Group II (PO). Whereas, Groups IV, V, and VI treated with amla, milk thistle, and their combination dose for 15 days, didn't show any significant difference in serum bilirubin values of potassium oxonate induced hyperuricemic rats. Although, milk thistle treated group had lowest levels (0.50±0.012 mg/dL).

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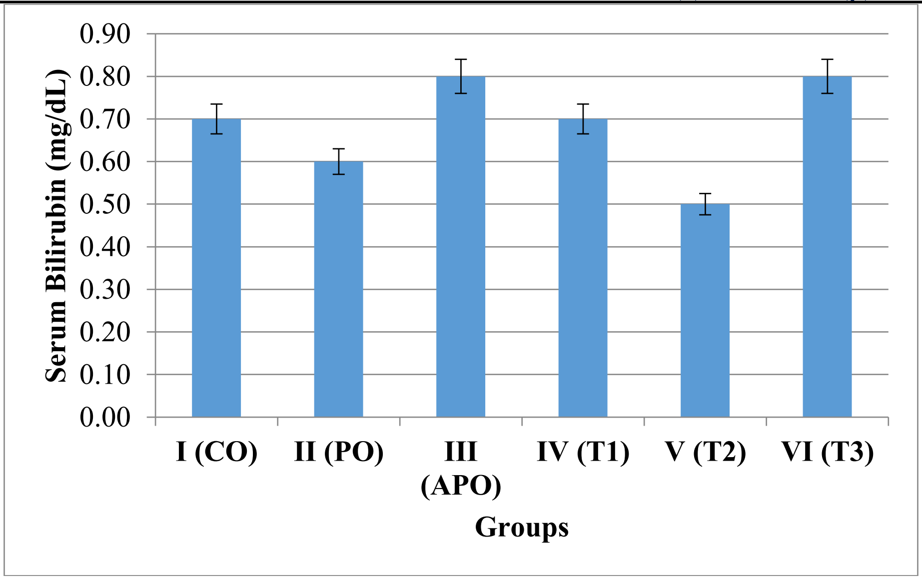

4. DISCUSSION

4.1 Phase-I

Proximate analysis was done to determine the moisture, crude protein, total fat, total carbohydrate, and crude ash in amla fruit and milk thistle seeds. After Barbados cherries, amla fruit has the second-highest vitamin C content of any fruit (Malpighia glabra L.). Together with phytochemicals including polyphenols, tannins, emblicol, linoleic acid, corilagin, phyllemblin, and rutin, it is also a great source of minerals and amino acids. The fruit is usually recognized for its capability to treat various conditions including diabetes, inflammation, atherosclerosis, acidity, asthma, skin issues, and obesity. It has also been famous for its antibacterial, blood sugar-lowering, and lipid-reducing properties. Therefore, amla is used in quite a lot of traditional medical systems such as Chinese, Tibetan, and Ayurvedic medicine (Tewari et al., 2019). Milk thistle (Silybum marianum L.), which has been valued for its medicinal properties for a long time. This plant, part of the Asteraceae family, contains a compound called silymarin. Silymarin is known for its effectiveness in treating liver conditions and other health issues, which is why milk thistle is often referred to as a liver tonic (Javeed et al., 2022).

The quantity of water and volatile compounds lost during drying is known as the moisture content (Suksaard & Ganogpichayagrai, 2020). The moisture content determined in amla and milk thistle was 10.26% and 7.44% in 100g. Kumar et al., (2022) reported a 2% to 7% moisture content in spray dried amla powder. According to Parveen and Khatkar (2015), various amla fruit types have a moisture content ranging from 81.26 to 84.65%. Whereas, several studies reported range of moisture content in different varieties of milk thistle seeds from 4.48% to 6.90% (Aziz et al., 2020; Khan et al., 2007; Javeed et al., 2022)

Crude protein in our sample was revealed to be 5.59% per 100 grams of sample, which is lower than the amount reported in another study as 7.02±0.02% per 100 grams (Kumar et al., 2022). According to several researches, crude protein content in amla ranged from 2.05 to 4.51% (Alkandari et al., 2019; Parveen and Khatkar, 2015; Tewari et al., 2019). Crude protein measured in our sample of milk thistle seeds was 21.78% per 100 grams powder, nearly similar to the findings of Elhassaneen et al. (2023). He determined the crude protein in milk thistle seeds as 22.17%. Protein content confirmed by Abd-El-hady & Arafa (2019) in milk thistle seeds was 23.43%. Javeed et al. (2022) also observed crude protein content of 23% in milk thistle seeds.

According to and Suksaard and Ganogpichayagrai (2020), the amount of total fat or the ether extract comprises of all types of fats present in food such as fatty acids, oil-soluble colors, fat-soluble vitamins and steroids. In the study we performed,

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total fat content of milk thistle was discovered to be 18.46% and amla contained only 2.01% per 100g. Several other researches revealed even higher fat content in milk thistle seeds like 26.05% (Khan et al., 2007), 26.14% (Abd-El-hady & Arafa., 2019), 26.72% (Elhassaneen et al., 2023) and 19.74% to 23.19% (Aziz et al., 2020). For amla, several other researchers discovered the same fat content (range from 1.98% to 2.60%) as revealed by our study (Kumar et al., 2022; Alkandari et al. 2019).

In our study, milk thistle analysis showed crude ash in our sample was 1.97% per 100 grams of powder. One more study by Aziz et al. (2020) stated that the ash content present in various milk thistle seeds varied from 1.25% to 2.37%. Our findings are quite similar to the results of a study by Khan et al. (2007) and Javeed et al. (2022), they determined the total ash content of 1.93% in milk thistle seeds. Elhassaneen et al. (2023) determined the ash content in S. marianum seeds to be 2.83%. In our study, the ash content of the powder of dried amla fruit was discovered to be 4.28% per 100 grams. Research by Alkandari et al. (2019) revealed 1.99 to 2.36% per 100 g of ash content in amla, dried by different methods. Some other studies detected 2.08 to 2.97% and 2.24 to 3.08% of ash content in amla (Tewari et al., 2019; Parveen and Khatkar., 2015)

Our study revealed the crude fiber in milk thistle seeds as 5.87% per 100g. Our findings are quite similar with the results of Javeed et al. (2022) and Khan et al., (2007), who reported total crude fiber of 5.48% in the seeds. Several other studies reported even higher levels of crude fiber in milk thistle seeds such as 29.95% (El-haak et al., 2015), 25.74% (Abd-El-hady & Arafa, 2019), 4.39% to 7.4% (Aziz et al., 2020) and 7.17% (Elhassaneen et al., 2023). Total carbohydrate content found in our milk thistle seeds was 44.48% per 100 grams. The carbohydrate content in milk thistle seeds varies from 34.13% to 45.42% (Aziz et al., 2020). Other studies found content of 19.77% (Abd-El-hady and Arafa., 2019), 38.16% (El-haak et al., 2015), 67.21% (Elhassaneen et al., 2023), and 87.2% (Javeed et al., 2022), with varying results from different sources.

Total carbohydrate and Crude fiber detected in amla was 68.74% and 9.12% per 100g. Tewari et al. (2019) stated that the carbohydrate content in amla fruits varied from a maximum amount of 81.93% to a minimum of 77.18%. Kumar et al., (2022) revealed higher carbohydrate content (92.58±0.03%) and low Crude fiber content (1.99±0.04%) in amla as compared to our findings. The crude fiber content of amla fruit varies between 8.23 to 9.98% and 7.18 to 22.35% (Alkandari et al., 2019) (Parveen and Khatkar., 2015).

Polyphenols, found in amla, are natural antioxidants that inhibit oxidative damage caused by harmful free radicals (Javeed et al., 2022) (Zahiruddin et al., 2021). A recent study found that all polyphenols in amla have strong antioxidant properties, decreasing oxidative stress markers in 2K1C rats (Rahman et al., 2020). Tewari et al. (2019) found polyphenol levels in amla fruit varied from 24.61% to 31.12%, Other studies reported the highest content at 42.35±1.70% and 90±0.01 mg/g GAE (Zahiruddin et al., 2021) (Kumar et al., 2020). A study found total phenolics in milk thistle seeds at 35.65 mg GAE per gram, similar to our findings. Aziz et al. (2020) reported varying phenolic contents (15.08 ± 0.47 to 25.13 ± 1.50 mg GAE per gram) while Elhassaneen et al. (2023) reported a higher total polyphenolic content 127.65 ± 6.59 mg GAE per gram.

S. marianum contains flavonoids, which are known for treating liver and gall bladder problems (Javeed et al., 2022). Research by Abd-El-Hady & Arafa., (2019) reported 23.78 mg of flavonoids per gram in milk thistle seeds, close to our study. Milk thistle seeds were discovered with 65.1 ± 2.71 mg/g and 8.1 ± 0.24-15.19 ± 0.75 mg QE/g of flavonoids (Aziz et al., 2020) (Elhassaneen et al., 2023). Zahiruddin et al. (2021) studied different plants and discovered that amla had the highest flavonoid content of 20.15±1.22% as compared to other plants they studied. Flavonoids, rich in antioxidants, are known for their potential health benefits, including anti-inflammatory, anti-cancer, and antioxidant effects (Zahiruddin et al., 2021). They also play a role in defense against tumors, ulcers, inflammation, and bacterial platelet

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aggregation. Consumption of dietary flavonoids, such as myricetin, quercetin, and isoflavones, has been linked to a lower death rate from coronary heart disease (Abd-El-Hady & Arafa., 2019).
The amount of ascorbic acid in amla and milk thistle was found to be 159.05 mg/100g and 29.16 mg/100g. The vitamin C content of milk thistle seeds is nearly equal to the vitamin C content of lemons, which is 31 mg/100g of fresh weight (Doseděl et al., 2021). Studies show amla fruit's strong antioxidant effect due to 600-700 mg of vitamin C per 100 grams. However, drying processes can lower ascorbic acid levels, as noted by Kumar et al. (2022) in his study (450±0.05 mg/100g). several studies identified different ranges of vit C in amla such as 1.93 to 3.29 g/100g, 206.8 mg to 932.1 mg/100g, 498.81 to 585.00 mg/100g (Parveen and Khatkar., 2015; Alkandari et al., 2019; Tewari et al., 2019). Vitamin C plays a crucial role in collagen synthesis, hormone synthesis, carnitine synthesis, gene transcription, translation regulation, tyrosine removal, defense against ROS, and iron reduction (Doseděl et al., 2021). Studies have shown a link between vitamin C intake and lower uric acid levels (Peng et al., 2018), particularly in patients with hyperuricemia, type II diabetes, and other medical conditions (Abd El-Aal et al., 2018; Liu et al., 2021).

4.2 Phase-II

In this study, individual and synergistic effect of amla and milk thistle on hyperuricemia along with other kidney and liver parameters was investigated in Potassium Oxonate (PO) induced hyperuricemic rats for 15 days. Intra-peritoneal injections of PO (250 mg/kg/body weight) have led to increased serum concentration of creatinine, uric acid, serum ALT and bilirubin. The production of uric acid by the liver and its elimination by the kidneys makes it a significant marker of hyperuricemia. Kidney injury may result from underexcretion of UA (Chen et al., 2019). A study was conducted on gout in rats using Phyllanthus emblica fruits. They found that both alcoholic and aqueous extracts significantly reduced blood creatinine, uric acid, BUN, and XO enzyme levels (Sarvaiya et al., 2015). Another study showed that the amla extracts can lower uric acid levels, suppress inflammation, protect ankle cartilage and treat gout with its anti-oxidant and anti-inflammatory properties (Tao et al., 2022). Several bioactive compounds in amla inhibits the formation of inflammatory cytokines, reducing pain and inflammation (Saini et al., 2022). Research by Mo et al. (2007) showed flavonoids in amla and milk thistle possess hypouricemic effect by blocking xanthine oxidase activity. An in silico and in vitro study on ethanolic extract of amla showed strong antioxidant and enzyme-inhibiting effects, which suggest its potential to treat gout (Hamsarekha et al., 2017). Chatuphalatika's (CTPT), a Thai herbal mixture with amla, has been found to effectively inhibited hepatic XOD activity and reduce plasma uric acid levels in hyperuricemic mice by 40% (Sato et al., 2018). Amla and ginger leaf extracts protect kidneys and liver from lead and arsenic damage by repairing antioxidant enzyme levels and decreasing reactive oxygen species (Fazal et al., 2021).
A study found that amla fruit extract can prevent liver damage from copper, recover liver parameters, and reduce oxidative stress. (Zulfiqar et al., 2023). Studies have shown that amla can restore normal plasma concentrations of creatinine and uric acid in 2K1C rats (Rahman et al., 2020), decrease serum creatinine levels, and restore hematological and biochemical markers like ALT, AST, urea, and creatinine in rats (Arora et al., 2024; Rajkumar et al., 2021). A study revealed that aqueous extract of Emblica officinalis at doses of 200 mg/kg and 400 mg/kg did not change the oxidative stress indices and biochemical markers of liver and kidney function, such as ALT, AST, GGT, ALP, albumin, and bilirubin (Mishra et al., 2020). Another study done by Mishra et al., (2022) showed that aqueous extract of Emblica officinalis (200 mg/kg) had reduced liver and kidney functioning biochemical indicators but it didn’t change serum bilirubin levels. According to a clinical trial, after two weeks of supplementation of Emblica Officinalis's, ALT and bilirubin levels decreased, but there was no significant difference in the levels of creatinine, uric acid, urea-nitrogen, total protein and albumin (Kapoor et al., 2020).

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Research on silymarin supplements and seeds found that they can lower blood sugar, cholesterol, triglycerides, LDL, ALT, AST, ALP, urea, and uric acid levels (Ali et al., 2015). Silymarin extract also decreased liver xanthine oxidase activity (Alizadeh et al., 2018). Several other studies confirmed the nephroprotective and hepatoprotective effects of milk thistle due to the lowering of kidney and liver parameters such as urea, creatinine, ALT, AST etc. (Malkani et al., 2020) (Mengesha et al., 2021) (Ibrahim et al., 2022). Milk thistle, when administered to rats exposed to carbon tetrachloride, improved lipid profiles, kidney function, and liver function (El Hassanen et al., 2021). Silymarin also decreased creatinine and uric acid levels in rats exposed to carbon tetrachloride-induced kidney damage (Saed et al., 2023; El Rabey et al., 2023). Aziz et al., (2020) suggest effectiveness of milk thistle in treating liver diseases due to its high silymarin content. Cengiz et al. (2016) stated that silymarin have nephroprotective properties, it extensively lowers the oxidative stress markers, decreases the serum creatinine and urea levels. Another study on male Wistar rats found that silymarin, silymarin, and Silybum marianum extracts reduced urea, creatinine, and uric acid levels (Yassin et al., 2021) (Elhassaneen & Mahran, 2024). According to research done by Talbi et al., (2020), milk thistle seeds solution lowered the elevated levels of ALT, AST, ALP, total cholesterol, triglycerides, LDL, urea, and creatinine. Results from another research showed a decrease in the levels of ALT, AST, ALP and total bilirubin by the intake of silymarin in diabetic rats (Kheiripour et al., 2019). The increasing knowledge and awareness about the safety, affordability and insignificant side effects of natural herbs and superfoods has boost their demand globally. Based on the studies mentioned above as well as further studies, foods like amla and milk thistle are natural source of nutrients. Phenolic chemicals present in them such as flavonoids, have been shown in several studies to have antioxidative, anticancer, antibacterial, cardio-protective, anti-inflammatory, and immune-stimulating properties. Amla and milk thistle are proved to fight against a wide range of illnesses such as diabetes, cancer, heart disease, arterial hardening, inflammation, digestive problems, vision issues, arthritis, and rheumatism, liver and kidney diseases, owing to their strong antioxidant properties (Varnosfaderani et al., 2018; Elhassaneen et al., 2023).

5. CONCLUSIONS

Based on the study's findings, it can be concluded that Phyllanthus Emblica (amla) fruit and Silybum Marianum (milk thistle) seed powders, taken orally, either alone or in combination, can be a powerful superfood that lowers blood uric acid and other liver and kidney parameters. Both of them have sufficient quantities of vitamin C, flavonoids, and polyphenols, which are naturally occurring antioxidants and can be very helpful in the treatment of a wide range of illnesses, including hyperuricemia, nephropathy, hepatotoxicity, cardiopathy, and many others. Further research is required to examine the advantages of Phyllanthus Emblica (amla) fruit and Silybum Marianum (milk thistle) seeds in relation to varying dosages in humans.

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