

Plant Isolated Compound Remove Uric Acid from Blood Immediately as Compare to Anti Uric Acid Drug

Rana Kausar* and Nazish Waris

Department of Biochemistry, Federal Urdu University, Karachi, Pakistan

Research Article

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*For Correspondence

Rana Kausar, Department of Biochemistry, Federal Urdu University, Karachi, Pakistan, Tel: +922199244141.

E-mail: ranakausar4@gmail.com

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ABSTRACT

The excess of uric acid in blood results in hyperuricemia which can also lead to other conditions, like kidney stones (uric acid nephrolithiasis) and kidney failure etc. Excess of uric acid is removed from the body by using different drugs which also produces harmful effects on other organs in many cases. But, in the current study, treatment was done by bottle gourd which not only helped to remove excess uric acid in blood, but also other organs remained unaffected. In the current study, uric acid was increased by direct oral dose of uric acid. Rats were divided into four groups: control, zyloric treated, bottle gourd orally treated and bottle gourd extract treated. After three weeks of treatment behavioural activity was monitored by locomotors activity in open field experiment, stimulatory activity in home cage, anxiolytic effects in light and dark apparatus and also in T maze, antidepressants by forced swimming activity, short term memory (STM) and long term memory (LTM) by water maize activity, were significantly increased in bottle gourd orally treated rats. After decapitation, laboratorial activity was determined by LFT (liver function test) including, ALT, GGT, creatinine and BUN to analyse liver and kidney effect. Results of bottle gourd orally treated rats showed a good effect on LFT as compare to extract and Zyloric treated rats. Behavioural and laboratorial variables were analysed by using SPSS v. 20 and p-value <0.05 was considered statistically significant. Thus, the aim of the study is to enhance the function of bottle gourd as an ant uric acid agent.

INTRODUCTION

Uric acid is the end product of purine metabolism engender during the breakdown of nucleic acids (DNA and RNA) and ATP, and can also be generated from proteins. Previous research shows that serum uric acid levels vary greatly in humans and can range from 2.5 mg/dl to 12 mg/dl or more. It results in hyperuricemia which can also lead to other conditions, like kidney stones (uric acid nephrolithiasis) and kidney failure [1]. More importantly, an elevated uric acid also predicts the development of obesity various metabolic syndrome such as diabetes, fatty liver, hypertension cardiovascular and renal diseases [2-7]. Currently, uric acid is under enormous investigation by the immunologists since it acts as a danger associated molecular pattern (DAMP) [8-10]. Monosodium urate (MSU) crystals, the etiological agent of gout in macrophages leading to inflammasome formation are recognized by the Nacht domain, leucine-rich repeat, and pyrin domain-containing protein 3 (NALP3). Once inflammasome is established, caspase-1 is activated and transform pro-interleukin-1 β (pro-IL-1 β) and pro-interleukin-18 (pro-IL-18) to active IL-1 β and IL-18 respectively. Then these potent proinflammatory cytokines are secreted [11]. The rigorous mechanism by which NALP3 is activated by MSU crystals in macrophages is not fully understood. According to a suggested mechanism, extracellular ATP binds to the purinergic receptor P2X7 leading to opening of this ATP-gated cation channel that induces potassium efflux and formation of a large pore by the hemi channel protein pannexin [12]. Intracellular potassium depletion is the trigger that stimulate NALP3, while inhibition of potassium efflux prevents NALP3 dependent caspase-1 stimulation in response to MSU crystals [13]. A dignified study showed that uric acid when released from dying cells induces inflammation. However, urate is not involved in the immune response to microbial stimuli or sterile irritant particles. Similarly, in a bleomycin-induced lung injury model, released from injured cells uric acid complements a major DAMP that through NALP3 activation forms the inflammasome and prompts IL-1 β secretion [14]. Under uric acid treatment caspase-1 activity and IL-1 β secretion were both markedly elevated. Because NALP3 is expressed in both human B-cells and T-cells [15,16]. The calabash, bottle gourd, or white-flowered gourd, also called lauki or doodhi, used as a vegetable [17,18]. Previous study shows that bottle guard is found to be the healer for mental disorder due to its highest content of choline than any other vegetables and the vitamins, minerals and any other amino acids that are present in it are used to

synthesize neurotransmitters [19]. Bottle guard have different functions such as good food to help digestion, help for weight loss, helps to treat urinary disorders, refreshes during summers, prevents heart disease, reduces stress etc. [20]. Various studies show that drugs which are used to treat uric acids can also damage other organs and produces side effects. In the current study, bottle gourd is used to treat uric acid which has no side effect than other.

MATERIALS AND METHODS

Locally bred male albino Wister rats being about 150 grams to 200 grams on arrival purchased from animal house of research institute of Aga Khan university, Karachi, Pakistan were used throughout the experiment. The rats were placed individually in specially design cage with saw dust cover floor in a quiet room with three axes, two cubes of standard food and water at least 4 days before starting the experiment. So, the rats could adopt themselves to new environment. In this study, 12 rats were used divided into four groups of three rats. In which group 1 was used as control while, in other groups uric acid was increased by direct oral dose of uric acid. After this, group 1 control (C) was treated with saline, group 2, (T1) with Zyloric drug, group 3, (T2) with bottle gourd orally and group 4, (T3) with bottle gourd extract for 3 weeks. For the preparation of saline 4.5 gm NaCl is dissolved in 500 ml water and then, it was freezed to get chilled saline. Zyloric drug was given to rats as 30 mg/kg body weight, after dissolving in water. Bottle gourd extract was prepared in ethanol and precipitate were dissolved in saline water, which was given to rats as 80 mg/kg body weight. These animals were handled on a regular basis in non-life threatening situations like weighing, giving food treats etc.

Behavioral Technique

After three weeks of treatment activity was monitored for 10 minutes in the open field apparatus. The open field test (OFT) is a commonly used qualitative and quantitative measure of general locomotor activity and willingness to explore in rodents. Commonly, the open field is marked with a grid and square crossings. The open field apparatus consists of a square area (76 × 76 cm) with walls of 42 cm high. The floor divided by lines into 25 equal squares. Rearing and time spent moving are used to assess the activity of the rodent. The OFT is also often used to assess anxiety by including additional measures of defecation, time spent in the center of the field. Activity was monitored after treatment for ten minutes, in light and dark environment for anxiety. The apparatus used in light and dark experiment consisted of small square area (26 × 26 × 26 cm) with an access (12 × 12 cm) walls of one compartment was transparent and other dark. Ten minutes in home cage specially designed made up of Perspex (26 × 26 × 26 cm) with saw dust covered floor was used for this purpose. Ten minutes also in Plus T maze shaped apparatus used for screening and research anxiogenic and anxiolytic drugs, which have two open and closed arm each have open tool and elevated 40 cm to 70 cm from the floor. Number of entries and time spent in open arm are the anxiolytic drugs effect. For forced swimming activity, animals are subjected to two trials during which they are forced to swim in an acrylic glass cylinder filled with water. The first trial lasts 15 minutes. Then, after 6-hours, a second trial is performed that lasts 5 minutes. The time that the test animal spends in the second trial without making any movements beyond those required to keep its head above water is measured. This immobility time is decreased by various types of antidepressants and also by electroconvulsive shock. In water maize activity, rats were placed in to the water facing the wall of the tank and allowed 120 seconds to locate and climbed into the submerged platform. Rats were allowed to stay on the platform for 15 seconds. If the rats were failed to find the platform within the allowed time it was guided gently onto the platform. After training short term memory (STM) and long term memory (LTM) were determined by recording retention latency. Cut off time for each session was 2 mint. learning acquisitions (LA) can be tested immediately after training by recording initial latency. After monitoring these activities, the animals returned to their cages. After behavioral activity, rats were decapitated, by a special kit, the juglar veins were then punctured with a clean and sharp syringe and the blood collected in the gel containing tube and then tube placed in an incubator for 30 minutes and the serum collected after centrifugation on a bench centrifuge at 300 gav for 15 minutes. This procedure, if carefully followed, gave clear samples of serum with no hemolysis. Also blood collected in anticoagulant containing tube to gel plasma.

Preparation of Tissue Extracts

Rats were killed by dislocation of neck from the base of the skull the kidneys and liver rapidly taken out. The tissues were thoroughly washed in ice cold 0.9% saline for further study.

Statistical Analysis

Results were represented as mean, ± SD (n=4) significant difference by Tukey HSD and Duncan test p<0.05 level from C, T1, T2 and T3 following one-way ANOVA.

RESULTS

Behavioral Results

Statistical analysis by one-way ANOVA (df3,11) (f=238.750) (p<0.0001) show that mean value of T2 was increased (**Figure 1a**), crossed more boxes as compare to C, T1 and T3. **Figure 1b** (df3,11) (f=32.000) (p<0.0001) show that mean value of T1 head movement was increased as compare to T2 and T3 **Figure 1c** treated rats, statistical analysis by one-way ANOVA

(df3,11) (f=2590.750) (p<0.0001) show that mean value of T2 was decreased as compare to T1 and T3. **Figure 1d** (df3,11) (f=247650.667) (p<0.0001) and **Figure 1e** (df3,11) (f=100.812) (p<0.0001) show that STM and LTM of T2 was significantly increased as compare to C, T1 and T3 because, T1 took less time to reach its platform. **Figure 1f** One-way ANOVA (df3,11) (f=1252.280) (p<0.0001) and **Figure 1g** (df3,11) (f=1.272) (p<0.348) show that mean value of T2 spent more time in light box and no. of entries was increased as compare to T1 and T3.

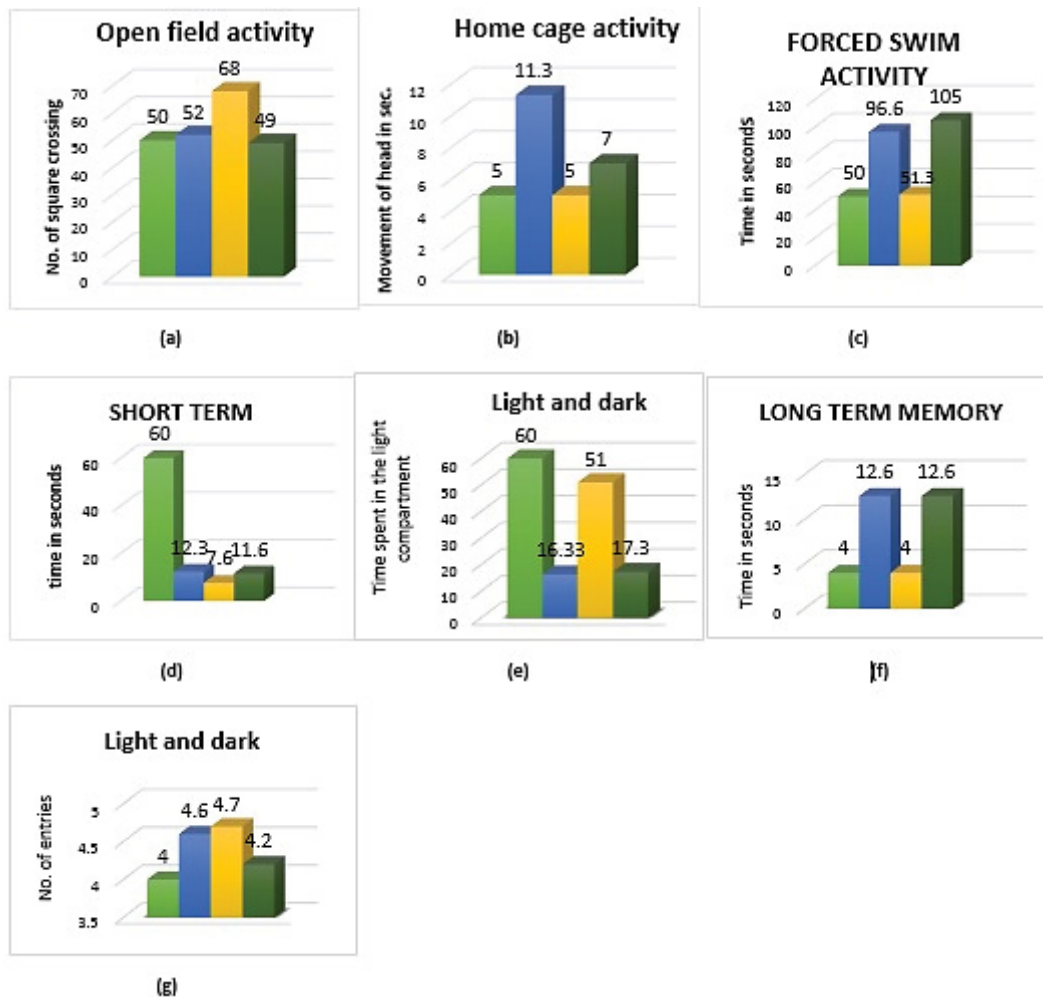


Figure 1. Behavioural Technique. In graphs colours represent control (C), drug treated T1, bottle gourd orally T2 and bottle gourd extraction T3 respectively.

Liver Function Test

Statistical analysis by one-way ANOVA (df3,11) (f=5190.750) (p<0.0001) show that mean value of alanine aminotransferase (ALT) was decreased in T2 (**Figure 2a**) as compare to T1 and T3. **Figure 2b** (df3,11) (f=3123.000) (p<0.0001) show that mean value of gamma GT was in limit in control and T2 but increased in T1 and T3. **Figure 2c** treated rats, statistical analysis by one-way ANOVA (df3,11) (f=17.029) (p<0.001) show that creatinine level was decreased in T2 while increased in T1 and also normal in control. **Figure 1d** (df3,11) (f=7697.408) (p<0.0001) show that blood urea nitrogen (BUN) was decreased in T2 and also in C while increased in T1 and T3.

DISCUSSION

Previously, it was studied that by using anti uric acid drugs such as Zyloric can produce no. of complications with the treatment of uric acid. Also many other drugs such as thiazide diuretics can also increase uric acid levels in the blood by interfering with renal clearance. As the liver and kidney play an important role for the excretion of uric acid so, the kidney is the main organ than others which was affected by anti-uric acid agent. Many diseases related to metabolic syndrome, type-2 diabetes, cardiovascular disease, Lesch-nyhan syndrome and gout are the main risk factors due to hyperuricemia for humans. In the current study, results show that by comparing data from treated groups with data from the control group using statistical evaluations as shown in **Figure 1a**, reveal that the open field apparatus was constructed with play wood. cerebellum is largely involved in coordination, rats whose cerebellum does not work well are generally clumsy and steady. In this study, the rat which is treated with drug show normal crossing, the drug which is treated with bottle gourd orally show good crossing whereas the rat which is treated with bottle gourd extraction show less crossing squares as compared to control. Home cage activity specific for stimulatory activity

like living in their home freely (**Figure 1b**). The present studies show that after Zyloric treatment stimulatory activities of rats significantly increased as compare to control groups, whereas the group of rats which are treated with bottle gourd in orally form show normal stimulatory activity, whereas the group of rats which are treated with bottle guard extraction show high stimulatory activity as compared to group of control animals (**Figure 1c**). Forced swimming activity is specific to monitor depression in rats. After treatment with Zyloric results show that depression is significantly increased in Zyloric treated rats as compared to control. The group of rats which are treated with bottle gourd orally shows normal value, whereas the group of rats which are treated with bottle gourd extraction show increased amount of depression as compare to control. Memory in rats is recognized by water maize activity. Short term memory allows us to do all the little things that make us productive (**Figure 1d**). Zyloric in low doses produces excitatory effects and that dopaminergic mechanisms may be involved in the observed effects of Zyloric. That's why Zyloric increases the short term memory of rats whereas, the group of rats which are treated with bottle gourd orally also show good short term memory, Whereas the group of rats which are treated with bottle gourd extraction also show good short term memory. long term memory is also recognized by water maize activity, is the final stage of the dual memory model proposed by Akinson and Schiffrin in which data can be stored for long periods of time (**Figure 1e**). Long term memory in Zyloric treated rats significantly decreases as compare to control group rats. whereas the group of rats which are treated with bottle gourd orally show good memory. The group of rats which are treated with bottle gourd extraction show bad long term memory as compare to control group rats. Light and dark activity is specific for anxiety. By using the light and dark box we can determine whether the drug is anxiolytic or anxiogenic. Prolonged administration can produce severe effects including cerebral, dermatological, myopathy, neuropathic and psychiatric disorders, irreversible retinopathy and maculopathy. The group of rats which are treated with Zyloric show anxiety more as compared to control whereas the group of rats which are treated with bottle gourd orally show less anxiety whereas the group of rats which are treated with bottle gourd extraction show anxiety as compared to control groups.

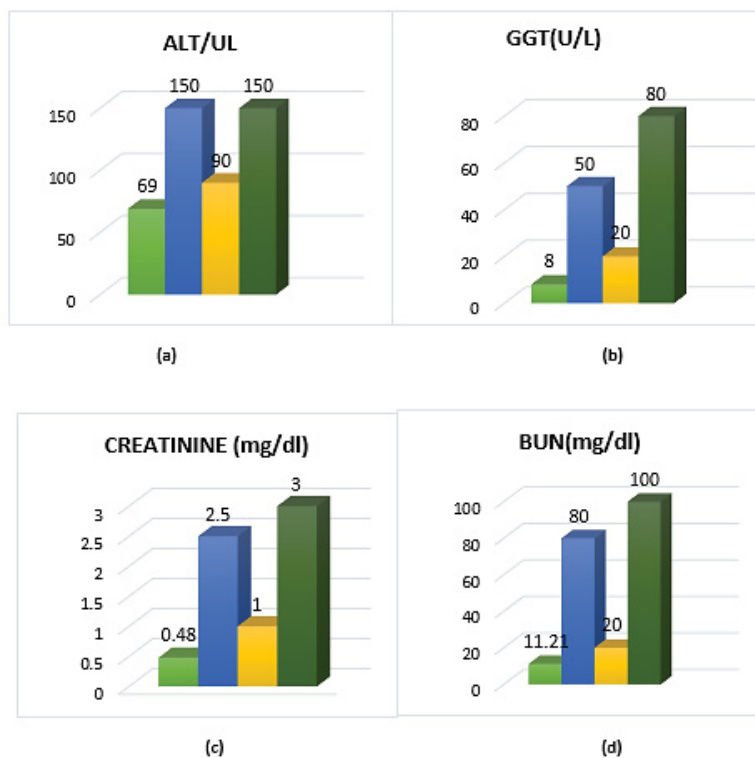


Figure 2. Liver Function Test. In graphs colours represent control (C), drug treated T1, bottle gourd orally T2 and bottle gourd extraction T3 respectively.

Alanine transaminase (ALT) also known as serum glutamic pyruvic transaminase (SGPT) (**Figure 2a**). ALT is an enzyme found mostly in the cells of the liver and kidney mainly used for the detection of liver damage. The group of rats which are treated with Zyloric and bottle gourd extraction show increased ALT as compared to control and bottle gourd orally treated rats show less ALT. Gamma glutamyl transferase (GGT) is an enzyme found in cell membranes of many tissues mainly in the liver, kidney, and pancreas. The gamma glutamyl trans peptidase (GGT) plays a significant role in helping the liver to metabolize drugs and other toxins. The GGT test is also currently the most sensitive enzymatic indicator of liver damage and other disease. Elevated serum GGT has also been found to correlate with cardiovascular diseases. GGT was also found to be in control in bottle gourd orally treated rats as similar in control group while, increased in Zyloric and bottle gourd extraction treated rats, shows the abnormal function of liver. The kidneys filter out most of the creatinine and dispose of it in the urine. Abnormally, high levels of creatinine thus warn of possible malfunction or failure of the kidneys. In the current study, bottle gourd orally treated rats show a normal function of kidney while group 1 and group 3 rats were marked with abnormal function of kidney. Blood urea nitrogen (BUN) level is another indicator of kidney function. Urea nitrogen is a waste product that's created in the liver when the body breaks down proteins. Having too much urea nitrogen in the blood or having high BUN levels can be a sign of kidney or liver problems. Here,

results also indicate the good effect of bottle gourd orally on kidney and rats were found to be normal but bottle gourd extraction and Zyloric drug treated rats were found to be with abnormal kidney function.

CONCLUSION

Bottle gourd is rich in dietary fiber both soluble and insoluble. Previous studies show that, it helps in curing constipation, flatulence and even piles. It is also easy to digest. The vitamins, minerals and dietary fibers in lauki or bottle gourd, keep the body well-nourished especially, after drinking its juice in the morning on an empty stomach. In the current study, it was concluded that if bottle gourd is orally given to the subject having hyperuricemia can not only good for treating uric acid but also it has good effect on other organ of the body. In this study, hence, it is concluded that group of rats 3 showed best result and proof that bottle gourd orally is very helpful in lowering of uric acid as compare to Zyloric and bottle gourd extraction. In behavioral activities the rats which were treated with bottle guard orally showed best results as compare to drug treated group and bottle gourd extraction.

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