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Original Article

Effect of *Raphanus raphanistrum* on chronic kidney disease induced by ethanol in animal model rats

Efeito do *Raphanus raphanistrum* na doença renal crônica induzida por etanol em ratos modelo animal

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Abstract

The aim of this study is to investigate the effect of *Raphanus raphanistrum* (radish) on chronic kidney disease damage by reactive oxygen species or free radicals in animal model rats. Total of 18 rats were used in this study, divided into 3 groups and each group consist of 6 rats. Group 1 control (C), group 2 model (M) and group 3 test (T). Model and test group were treated with alcohol to produce chronic kidney disease by reactive oxygen spices for 9 weeks a dose of 1 ml. After that test group was treated with *Raphanus raphanistrum* juice for 4 weeks 80mg/kg body weight to determine it effect. *Raphanus raphanistrum* juice effect on behavior of rats through increases the locomotor activity and anxiety. The serum creatinine and uric acid level were significantly improved in T group. The reactive oxygen enzyme test shows that Super Oxide Dismutase (SOD) and Glutathione Peroxidase (GPx) was increase in T group. The Glutathione S-Transferases (GST) and Catalase (CAT) enzyme level was nearly same in C and T groups. This study concludes that compound 1,1-diphenyl-2-picrylhydrazyl found in *Raphanus raphanistrum* juice and possess strong antioxidant activity on Chronic kidney disease induce by ethanol through reactive oxygen species. There is need of more researches to determine the use of natural compound to treat different disease.

Keywords: *Raphanus raphanistrum* (radish), reactive oxygen species, alcohol.

Resumo

O objetivo deste estudo é investigar o efeito de *Raphanus raphanistrum* (rabanete) nos danos da doença renal crônica por espécies reativas de oxigênio ou radicais livres em ratos modelo animal. Um total de 18 taxas foi utilizado neste estudo, divididas em 3 grupos e cada grupo consistia em 6 ratos. Grupo 1 controle (C), grupo 2 modelo (M) e grupo 3 teste (T). O modelo e o grupo de teste foram tratados com álcool para produzir doença renal crônica por meio de especiarias reativas de oxigênio por 9 semanas, uma dose de 1 ml. Após esse teste, o grupo foi tratado com suco de *Raphanus raphanistrum* por 4 semanas 80 mg / kg de peso corporal para determinar seu efeito. Efeito do suco de *Raphanus raphanistrum* no comportamento de ratos por meio do aumento da atividade locomotora e ansiedade. A creatinina sérica e o nível de ácido úrico melhoraram significativamente no grupo T. O teste da enzima oxigênio reativa mostra que a Super Óxido Dismutase (SOD) e a Glutationa Peroxidase (GPx) aumentaram no grupo T. O nível das enzimas Glutationa S-Transferases (GST) e Catalase (CAT) foi quase o mesmo nos grupos C e T. Este estudo conclui que o composto 1,1-difenil-2-picrilhidrazil encontrado no suco de *Raphanus raphanistrum* possui forte atividade antioxidante na doença renal crônica induzida pelo etanol por meio de espécies reativas de oxigênio. Há necessidade de mais pesquisas para determinar o uso de compostos naturais no tratamento de diferentes doenças.

Palavras-chave: *Raphanus raphanistrum* (rabanete), espécies reativas de oxigênio, álcool.

1. Introduction

The prevalence of chronic kidney disease has been spread rapidly in worldwide that increases mortality rate of approximately 850,000 annually (Mehmood et al., 2020). The consumption of alcohol is the main cause of chronic disorder (Varga et al., 2017). In animals the influence of alcohol on kidney morphology and performance are remains not clear. On the other hand evidence of damages and alteration in renal system on administration of ethanol has been mentioned (Lai et al., 2019).

Although, there is few options are available for the treatment of CKD. According to demand new therapeutic treatment are required to treat CKD (Mehmood et al.,

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2020). There are more than 600 herbal products,170 phyto constituents has been discovered that exhibits the nephron-protective effect. Whereas nephron-protective plants are pharmacologically analyzed which are used in traditional medicine found in small portion (Khan et al., 2021).

A property of Raphanus raphanistrum (radish) for root of hair initiation well recognized and improving through different factors which distress effectiveness in medicated property of *Agrobacterium rhizogenes* (Koelling and Karoly, 2007). Furthermore, the outcomes of ferric ion reduced antioxidant activity and 1,1-diphenyl-2-picrylhydrazyl exhibits antioxidant behavior of 2364 root MTCC extracts was better when associated with auxin induced roots of non-transformed Raphanus raphanistrum (Balasubramanian et al., 2018).

In published studies the ethanolic extract of Raphanus raphanistrum has proven diuretic effect, rifampicin concentration has and toxicity has declined. Moreover, the ethanolic extract reduced the concentration of rifampicin slightly more than water extract. In Raphanus raphanistrum strong antioxidant activity has found which tends to reduced nephrotoxicity as a potent nephroprotective agent (Khalid et al., 2018).

Therefore we aim to investigate the nephroprotective activity effect of Raphanus raphanistrum juice on chronic kidney disease induced by reactive oxygen species or free radicals in animal model rat.

2. Material and Methods

2.1. Quality of animals

Locally bred female Albino Wister rats weighing about 198 to 230gm on arrival purchased from animal house of International Center for Chemical and Biological Sciences University of Karachi Pakistan were used throughout the experiment. Animals were brought and studied in the Biochemistry department of Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan. These rats were individually kept in specially designed cage with saw dust cover floor in a quiet room, with free access to cubes of standard rat's food and water at least 3 to 4 days before starting the experiment. So, the rats could adapt themselves to the new environment. Total of 18 rats were used in this study, divided into 3 groups, each group contain six rats. Group 1 called control given saline. Group 2 called model, group 3 used as test. Chronic kidney disease was developed in model and test groups by 1ml alcohol administration for nine weeks as per body weight (Baqa et al., 2019). After inducing CKD model remained untreated till end of the experimentWhile, test group of rats were treated with radish juice (Baqa et al., 2019).

2.2. Mean weight of animals before treatment

RAT-1 group control (C) =198 gm
RAT-2 group model (M) = 190 gm
RAT-3 group *Raphanus raphanistrum juice* treated (T) =200 gm

2.3. Preparation of radish juice

Purchase fresh *Raphanus raphanistrum* (radish) from market and juice were obtain by the help of juicer blander, which was given to T rats as 80mg/kg body weight for 4 weeks.

2.4. Handling

The oral intake of drugs to experimental rats need extensive management and it is suggested that earlier to experimental operation, such animals would be handled on a routine basis in non-life-threatening conditions like giving food treats, petting and weighing. This makes the animals react positively to handler and learn to identify person. The animals should be handled gently but firmly avoiding loud noises or sudden movements.

To remove the rats from the cage, it is picked up by the tail close to the base and placed on the flat surface of a laboratory bench. While still holding to the tail with the right thumb and forefinger, the scruff of the animal is reached for with your left thumb and forefinger, positioning them firmly on either side of the animal's head at the level of the mandible. Simultaneously the rest fingers and palm of the left hand are used to firmly press the thorax or trunk down against the flat surface of the bench. Thereafter the scruff (the loose skin over the neck) is gathered between the thumb and the forefinger and used to lift up the animal by the scruff. The tail may be held either firmly against the trunk with the fifth finger of the left hand of left hanging free. When held firmly this way, the rat is restrained and the esophagus is as straight as possible.

2.5. Experimental protocol

Behavioral activities were monitored during treatment on weekly basis, in light and dark environment. Light and dark activity is specific for anxiety the apparatus used in light and dark experiment consisted of small square area (26x26x26 cm) with an access (12x12 cm) walls of one compartment was transparent and other dark. Experiment was performed under normal day light, the experimental rats, were placed on the dark side of the apparatus than observed that how many time takes to rat move in the light portion within 5 minutes. For the next five minutes the activity was monitored in the open field, and the open field apparatus consists of a square area (76×76cm) with walls of 42 cm high. The base is separated by lines in 25 squares. An animal taken out from the specialized cage and placed it in the center. Square of the open field apparatus, rats move from center square, crossing with all four paws, corner sitting, grooming. These all activities scored for five minutes. Ten minutes in home cage specially designed made up of Perspex (26×26×26cm) with saw dust covered floor was used for this purpose. This activity was monitored as the number of cage crossing and 0.4 scales of increasing intensities of grooming and gnawing. Home cage activity of experimental rats was scored alternatively in a balanced design home cage apparatus to avoid order effect. After observing the behavioral activities, the rats were returned to their cages.

2.6. Mean weight of animals before decapitation

RAT-1 group control (C) =178 gm
RAT-2 group model (M) = 191 gm
RAT-3 group *Raphanus raphanistrum juice* treated (T) =196gm

2.7. Blood collection after decapitation

Rats were decapitated after 3 months. The blood was collected from wound of neck in the Serum-separating tubes for biochemical test.

2.8. Biochemical test

All biochemical test was performed at Dow University of Health and Sciences. These tests were performed in serum. Test includes SOD, CAT, GPx, GST, Creatinine and uric acid. All materials and quality controls are provided by Roche, Pakistan. The principle of this system is photometry.

2.9. Statistical analysis

All Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) Version 20. Continuous variables were presented as mean ± SD. LSD-test (Least Squire Difference Test) or ANOVA, Tukey HSD and Duncan test Mann–Whitney U test, and Chi square test were used as applicable. Results were considered to be statistically significant at P-value <0.05.

3. Results

Effect of *Raphanus raphanistrum juice and alcoholic intake on* behavioral activities such as anxiety, stimulatory and locomotors behavior shown in Figure 1a-1c. In Figure 2a-2b the creatinine and uric acid levels were determined to monitor the level of chronic kidney disease. In Figure 3 Reactive oxygen enzymes level are access in C, M and T groups.

3.1. Home cage activity

In Figure 1a Week 1 before the starting of treatment the stimulatory activity of rats in C,M and T was (41.67±7.06, 25.33±9.35 and 14.83±9.99) the rats show exploratory behavior in the new environment in habituation period after week 1 we start treatment. After 2 months of treatment the stimulatory activity of rats was decrease in groups of rats C was 25.17±9.64, M was 21.17±7.22 and T was 24.5±9.81 respectively as compare to control group. In last week of treatment activity of M was 11.5±3.33 significantly decrease compare to control whereas Test group 49.5±15.77 stimulatory activity was increase as compare to control.

3.2. Open field activity

In Figure 1b Week 1 before the staring of treatment the locomotors activity of rats in C,M and T was (77.17±9.62,

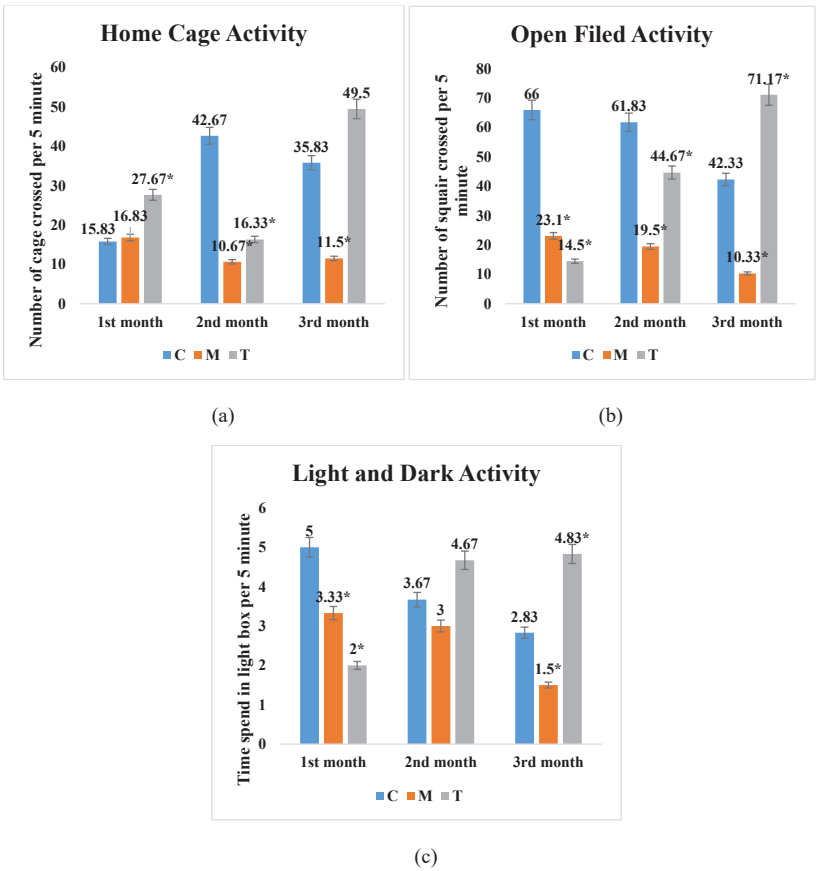
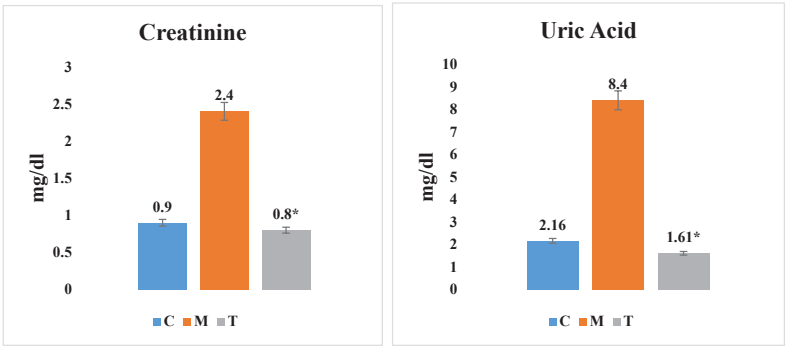
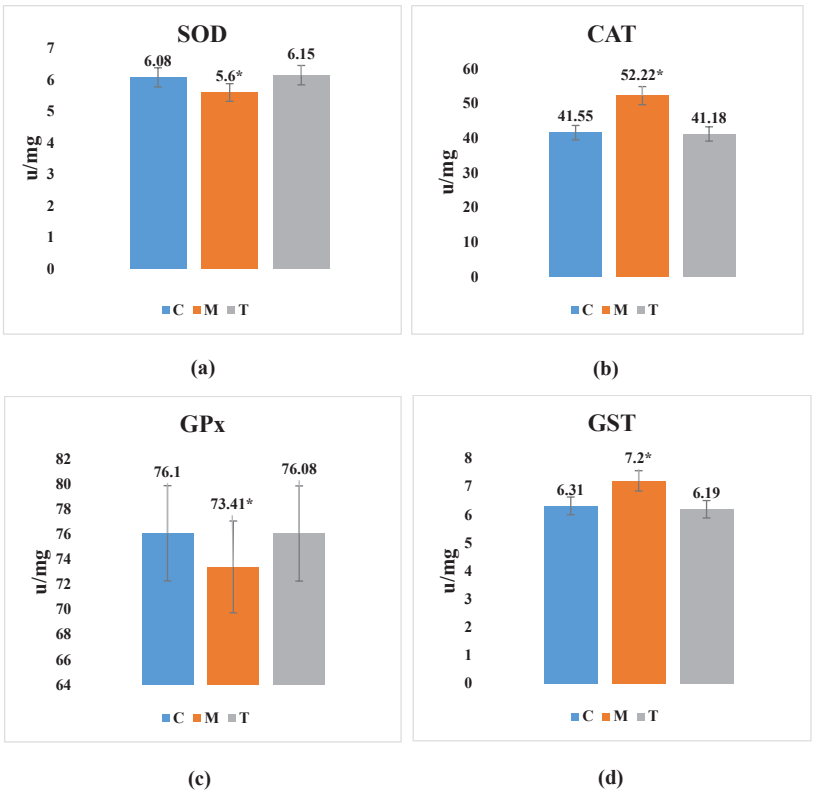


Figure 1. 1a- 1c. Comparison of behavioral results in Control (C), Model (M), Test (T) group of rats.
*Compared to c P-value < 0.05 considered to be statistically significant.



(a) (b)

Figure 2. 2a -2b. Comparison of creatinine and uric acid in Control (C), Model (M), Test (T) group of rats. *Compared to c P-value < 0.05 considered to be statistically significant.



(a) (b)

(c) (d)

Figure 3. 3a-3d. Comparison of biochemical results in Control (C), Model (M), Test (T) group of rats. Catalase (CAT) Super oxide dismutase (SOD) Glutathione S-transferase (GST) Glutathione peroxidase (GPx). *Compared to c P-value < 0.05 considered to be statistically significant.

46.17±26.18 and 34.5±10.25) the rats show exploratory behavior in the new environment in habituation period after week 1 we start treatment. After 2 months of treatment the locomotors activity of rats was decrease in groups of rats C was 55±17.78, M was 50.83±20.29 and T was 30.83±10.03 respectively as compare to control group T group show significantly decrease locomotors activity. In last week of treatment activity of M was 10.33±4.55 significantly decrease compare to control whereas Test group 71.17±4.88 locomotors activity was increase as compare to control.

3.3. Light and dark activity

In Figure 1c Week 1 before the starting of treatment the anxiety of rats in C,M and T was (6±1.41, 5±1.67 and 5±1.41) the rats show exploratory behavior in the new environment in habituation period after week 1 we start treatment. After 2 months of treatment the anxiety of rats was decrease in groups of rats C was 3.67±1.21, M was 2.83±1.6 and T was 4±1.41 respectively as compare to control group. In last week of treatment activity of M was 1.5±0.84 significantly decrease compare to control

whereas Test group 4.83±0.75 anxiety was increase as compare to control.

In Figure 2a Comparing the result of serum creatinine levels in all three groups it shows that creatinine is normal in control group and also in normal ranges in *Raphanus raphanistrum* juice (T) group that is significant result but in ethanol treated (M) group the value is increase that means kidney is not work properly.

In Figure 2b Administration of ethanol increase uric acid level in rats of M group but *Raphanus raphanistrum* juice treated rats have normal uric acid concentration in their blood that is significant as compare to M and C group. Subjects with kidney disease excretion of uric acid was decreasing and its concentration in blood was rising.

3.4. Effect of Raphanus raphanistrum juice on biochemical activity

In Figure 3a the level of Super Oxide Dismutase (SOD) level is compare in all three groups compare to control SOD is increase in T group and significantly decrease in M group. In Figure 3b CAT level is significantly increase in M group on the other hand the CAT concentration in T and C group is near the same. In Figure 3c the Glutathione Peroxidase (GPx) level was decrease in M group and significantly improve in T group. In Figure 3d GST level increase in group (M) and significantly decrease in T group of rats.

4. Discussion

Reactive Oxygen Species or free radicals is a by-product of alcohol metabolism which were recognized as cellular damage, except antioxidant system of body clean the free radicals. Oxidative stress happens when body is not able to detoxify free radicals as rapidly as they produce, essential in generating alcohol-related tissue damage.

Chronic kidney disease (CKD) was international community health problem with bad consequences and high price. There is increasing indication that some of the harmful results of CKD can be avoid or prevent by initial therapy of enzyme angiotensin II-receptor blockers and angiotensin II converting inhibitors (Li and Wang, 2005).

Creatinine level in plasma was come from catabolism of muscle and its elimination was occurring through kidney and creatinine was not reabsorbed. When distribution occur in renal function creatinine was accumulated in blood (Voncik et al., 2015).

In present study demonstrated about administration of ethanol increase uric acid level in rats of model group but Raphanus raphanistrum juice treated rats have normal uric acid concentration in their blood. Subjects with kidney disease excretion of uric acid was decreasing and its concentration in blood was rising. It is reported in previous study that hyperuricemia cause hypertension (Johnson et al., 2005).

Studies from past identified natural compound to treat kidney damage through different methods using antioxidants such as ginger (Shanmugam et al., 2010), ascorbic acid (Mailankot et al., 2009), grape leaf extract (Pari and Suresh, 2008), Hemidesmus indicus R.Br. root (Saravanan and Nalini, 2007) chrysin (Tahir and Sultana, 2011) or methanolic extract of Cnidoscolus aconitifolius (Adaramoye and Aluko, 2011). Conventionally used chines herb *Angelica sinensis*, Tripterygium wilfordii Hook F extracts and triptolide to treat Chronic Kidney Disease.

Recommended that SOD start its function through the stimulation received when level of lipid peroxidase enzyme and ROS was high (Rodrigo and Rivera, 2002), we observe that SOD level was significantly decrease in model rats and no changes occur in test group. Our result indicate that CAT enzyme was increase in model and normal level in treated group. High concentration of CAT enzyme was required for the degradation of hydrogen peroxide and in ethanol metabolism (Dinu et al., 2006).

Antioxidant defense mechanism of rat's kidney exhibit high level of GST to counter the ethanol toxicity and GPx concentration was decrease compare to the treated group show normal level of both GPx and GST. Previous study shown decrease GPx because of inactivation of ROS enzyme in long duration of ethanol intake (Adaramoye and Aluko, 2011). Some study reported in the exhaustion of GPx enzyme with ethanol intake and some study conclude that GPx activity was increase in ethanol injection in the kidney of rats (Chauhan et al., 2011).

5. Conclusion

This study concludes that compound 1,1-diphenyl-2-picrylhydrazyl found in *Raphanus raphanistrum* juice and possess strong antioxidant activity on Chronic kidney disease induce by ethanol through reactive oxygen species. There is need of more researches to determine the use of natural compound to treat different disease.

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