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Therapeutic effect of *Moringa oleifera* pods extract and Raspberry ketone against Thioacetamide toxicity in male rats

Soha M. Hamdy[1], Amany M Shaaban[1], Zakaria A El-khayaht[2], Abdel Razik Farrag [3] and Marwa El-Sayed[1]

1Chemistry Department, Faculty of Science, El Fayoum University, El- Fayoum, Egypt
2Biochemistry Department, National research center, Cairo, Egypt
3Pathology Department, Medical division research, National research center, Cairo, Egypt

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ABSTRACT

Background and Aim: Phytochemicals of antioxidant efficiency have either defensive or disease protective properties are widely used worldwide. **Aim:** This study was performed to assess the therapeutic impact of hydroethanolic extract of Moringa oleifera (M.O) pods and Rasperry ketone (R.K) against the complications accompanied with thioacetamide (TAA) toxicity in male rats. **Materials and methods:** 88 healthy male rats were separated equally into eleven groups: control group (1); M.O control group (2); R.K control group (3); M.O+R.K control group (4); TAA group (5); M.O protected group (6) RK protected group (7); M.O +R.K protected group (8); Treated M.O (9); Treated RK (10) and Treated M.O+R.K group (11). **Results:** The results obtained indicated significant elevation of serum GPT, GOT, AFP, creatinine and urea in TAA model in comparison to all control groups. TAA model also induced significant increase of NO, PON-1 and MDA in both hepatic and renal tissues. However all these parameters are improved in both protected and treated groups as compared to TAA model. The severe damages in both kidney and liver tissues developed by TAA administration resolved by administration of M.O alone or better with both M.O and RK as natural antioxidants. **Conclusion:** The current research proposed that subjunction of Moringa pods presented more therapeutic effect than Rasperry ketone against the toxicity of TAA in hepatic and renal tissues.

INTRODUCTION

Thioacetamide (TAA) acts as a sulfur source in the production of organic compounds for example elastic chemicals, healing and treatable agents, mineralogy, insecticides, and drug manufacturing [1]. TAA also used as antifungal agent, a drug

Corresponding author: Amany M. Shabaan, E-mail address: amm11@fayoum.edu.eg

component, a raw medicine, a textile dye, and a finishing auxiliary [2]. Predominantly, it serves as hepatotoxic and hepato-carcinogenic agent as it causes centrilobular cell death complemented with improved liver enzymes and bilirubin. TAA is widely used as hepatotoxin rats model this result in development of cirrhosis, fibrosis in addition to necrosis of liver [3]. To provoke these influences, TAA results in oxidative bio activation into its *S*-oxide (TASO) finally leading to its chemically reactive $TASO_2$ form [4]. These metabolites leads to oxidative stress then circulated among some organs as liver, kidney, and bone marrow. Liver is a major organ attacked by reactive oxygen species (ROS) [5].

Antioxidants are usually natural substances that can reduce the effect of the free radicals, convert them from unsafe to safe and lowering the cell injury. Liver cirrhosis and kidney failure can be developed if the inflammation doesn't be stopped [6]. Medicinal phytochemicals have extremely vital position as they used in treatment of numerous diseases, involving hepatic disorders with no toxicity [7].

Moringa oleifera (M.O) is a common vegetable plant in various countries like Egypt, has many therapeutic compounds with beneficial healthcare features, including anti-cancer [8] and anti-oxidant properties [9]. It is popularly known as drumstick, because it is long and thin, with triangular pods of seeds. It was recently found that M. oleifera seeds were good antioxidants, and can decrease oxidative damage accompanying by cancer [10]. Raspberry Ketone (RK) is an aromatic compound present in red raspberry fruits (*Rubus idaeus*) which is the principal substance responsible for the sweet odor of raspberries and is utilized in a perfume and cosmetics, beverages and food stuffs [11]. RK have a double influence of liver protection and body fat drop, and the process included liver inflammation decline, and enhanced antioxidant capability [12].

The purpose of the current research is to assess the effectiveness of both hydroethanolic extract of *Moringa oleifera pods* and Raspberry ketone opposed to thioacetamide (TAA) toxicity in male rat.

Subjects and methods

Chemicals and reagents

Thioacetamide [TAA] with purity 99%, and Raspberry ketone (4-(4 Hydroxyphenyl)-2- butanone) 99% pure were obtained from Sigma chemical company (St Louis, MO, USA). The extract of Moringa oleifera pods was obtained from the Egyptian Scientific Society of Moringa (ESSM), National Research Centre, Egypt.

Preparation of plant extract

Dry milled pods of Moringa oleifera were put in the Soxhlet thimble with 80% ethyl alcohol in conical flask, additional refluxed for 18 hours at 80° for 48 hrs. Gathered solvent were cooled at 25° C and emptied in a glass plate. The extract was concentrated under vacuum at 40° C to produce a semisolid mass, dried in hot air oven below 50° for 48 hours and stored in desiccators. Suspension of the extract was prepared in distilled water for use.

Animals

88 health mature male Wistar rats weighting 120-130 g were purchased from the Animal House Colony of the National Research Centre, Egypt. All animals were held in typical circumstances of light, moisture, and warmth with chow and tap water available. This study was conducted according to the guiding principles permitted by the Ethical Committe of Medical Research, National Research Centre, Egypt.

Study plan

Acclimations for one week, then animals were distributed into eleven groups (8rats/group): Group1 (N. control); animals fed on standard diet. Group2 (M.O control); animals administered oral with M.O extract (400mg/kg) daily for 8 weeks. Group3

(RK control); animals administered oral with RK (4mg/kg) daily for 8 weeks. Group 4 (M.O+RK control); animals administered oral with M.O extract (400mg/kg) + RK (4mg/kg) daily for 8 weeks. Group 5 (TAA positive control); animals administered with TAA (200mg/kg) 3times/week for 8 weeks. Group 6 (M.O protected); animals administered oral with M.O extract (400mg/kg) daily for 14 days before received TAA (200mg/kg) 3times/week and continue administered oral with M.O extract (400mg/kg) daily for 8 weeks. Group 7 (RK protected); animals administered oral with RK (4mg/kg) daily for 14 days before received TAA(200mg/kg) 3 times/week and continue administered oral with RK (4mg/kg) daily for 8 weeks. Group 8 (M.O +R.K protected); animals administered with M.O extract (400mg/kg) + RK (4mg/kg) daily for 14 days before received TAA (200mg/kg) 3 times/week and continue administered with M.O extract (400mg/kg) + RK (4mg/kg) daily for 8 weeks. Group 9 (Treated M.O); animals administered with TAA (200mg/kg) 3times/week for 8 weeks then treated with M.O extract (400mg/kg) daily for 8 weeks. Group 10 (Treated RK); animals administered with TAA (200mg/kg) 3times/week for 8 weeks then treated with RK (4mg/kg) daily for 8 weeks. Group 11(Treated M.O+R.K); animals administered with TAA (200mg/kg) 3 times/week for 8 weeks then treated with M.O extract (400mg/kg) + RK (4mg/kg) daily for 8 weeks.

When study finished, rats were fasted 12 hrs then anesthetized with ether, and blood was obtained through retro-orbital venous plexus. Blood samples collected and centrifuged at 3000 rpm for quarter hour in order to get serum and kept at –20 ^{o}C. Serum obtained was utilized for evaluation of SGPT, SGOT as hepatic markers and AFP as tumor marker, urea and creatinine as renal markers. The liver and kidneys were immediately removed, rinsed with cool physiological saline solution and homogenized, centrifuged and the resulting supernatants were stored at –20°C for measuring malonadialdyehyde, paraoxinase, and Nitric oxide by spectrophotometer. Moreover, sections of the liver and kidneys of each group were immediately removed and fixed in 10 % neutral formaldehyde for histological study.

Biochemical study

Hepatic enzymes

SGPT and SGOT enzymes were assessed in accordance with the assays of Gella *et al*., [13].

Tumor biomarker assay

Alpha-fetoprotein (AFP) level was detected in serum (ng/ml) by the method described by Acosta [14], its kits obtained from Diagnostic Products Company (Los Angeles, CA, USA).

Kidney function

Urea and creatinine were detected in serum by the methods of Fabiny & Ertingshausen [15] and Tabacco *et al*., [16] respectively.

Oxidative stress markers

MDA concentrations were measured in both hepatic and renal tissues by the technique of Ohkawa *et al*., [17], Paraoxinase-1 (PON1) was performed in both hepatic and renal tissues by the technique of Higashino *et al*., [18], and Nitric Oxide (NO) was performed in both hepatic and renal tissues by the assay of Montgomery e*t al*., [19].

Histopathological analysis

The hepatic and renal tissues were removed and put in 10% buffered formaldehyde, inserted in paraffin wax afterward mounted into blocks and placed at 4˚ C. The paraffin blocks were partitioned on the microtome at 6 μm thicknesses. The slides were submerged in xylol to remove parafin then immersed in alcohol with concentrations (90–50%) and stained with Haematoxylin and Eosin [20].

Statistical test

It was achieved by microsoft excel and SPSS software (version 20). Results were examined by one way analysis of variance (ANOVA) then Post Hoc analysis to determine significant differences among means [21]. The results were stated as mean ± standard deviation (SD). Differences were considered significant at $p \leq 0.05$.

Results

There were non-significant elevations in SGPT and SGOT activities in the (M.O, R.K, M.O+R.K) control and treated (M.O and M.O+R.K) groups when compared to normal control. High significant elevation in SGPT and SGOT activities were detected in TAA, protected M.O and protected R.K groups as compared to normal control. Meanwhile as the protected and treated groups compared with TAA group, a high significant reduction was observed. By comparing SGPT and SGOT activities in the treated and protected M.O groups with their control (M.O), a non-significant increase was observed in treated group and significant increase observed in protected one. However by comparing SGPT and SGOT activities in the treated and protected R.K groups with R.K control, a significant elevation (p-values≤ 0.05) was observed in both. While comparing the treated and protected M.O+R.K groups with M.O+R.K control, non-significant increase was detected (Table 1).

A non-significant decline was detected in serum AFP concentrations in the control (M.O, R.K, M.O+R.K) groups, and highly significant elevation in the rest of groups in comparison to normal control. However as the protected and treated groups compared with TAA group, a high significant decrease was observed. Finally, by comparing the treated and protected groups with their controls, a significant increase was observed (Table 1).

There was a high significant elevation in serum urea and creatinine concentrations in the TAA positive control, protected and treated groups (M.O, R.K and M.O+R.K) compared to normal control. Meanwhile, a markedly depletion in serum urea and creatinine concentration was observed as comparing the protected and treated groups with TAA positive control model. Conversely, there was a highly significant elevation in serum urea and creatinine in the treated and protected groups in comparison with their controls (Table 2).

Table (3) has shown highly significant increase in renal tissue MDA and NO levels in the TAA positive control, protected (M.O, R.K and M.O+R.K) groups as compared to normal control. Whereas, by comparing the renal tissue MDA and NO levels in the protected and treated groups with TAA positive control group, a markedly reduction were detected. Again, by comparing the renal tissue MDA and NO levels in the treated and protected groups with their controls, significant increases were detected.

A non-significant decline in renal PON activity in the control (M.O, R.K, M.O+R.K) groups, protected (M.O, R.K) groups and treated M.O+R.K group was detected when compared to normal control. However when comparing renal PON activity in the protected and treated groups with TAA (positive control), a high significant increase was observed. With respect to compare the treated and protected groups with their controls, non-significant decrease were observed (Table 3).

Table (4) has shown high significant elevation in hepatic MDA and NO concentrations in TAA and protected (M.O, R.K, M.O+R.K) groups as compared to normal control. However when comparing hepatic MDA and NO levels in the protected and treated groups with TAA (positive control), a high significant decrease was observed in treated M.O+R.K group, while a significant decrease observed with protected M.O, M.O+R.K groups and treated R.K group. By comparing hepatic MDA and NO levels in the treated and protected groups with their controls,

significant increases were observed in both.

A high significant decrease in hepatic PON activity was detected in TAA group and protected (M.O, R.K, M.O+R.K) groups as compared to normal control. However when comparing hepatic PON activity in the protected and treated groups with TAA (positive control), a high significant increase (*p*-values≤0.001) was observed in treated M.O+R.K group. Also significant increase observed with treated (M.O, R.K) groups, while non-significant increase was observed in protected (M.O, R.K, M.O+R.K) groups. By comparing hepatic PON activity in the treated and protected groups with their controls significant decreases were observed (Table 4).

Histopathological results of hepatic tissues

Hepatic sections of control (Fig.1a), M.O control (Fig.1c), R.K control (Fig.1d) and M.O+ R.K control (Fig.1e) groups showed a normal construction of the liver lobule. The central vein (CV) placed at the center of the lobule enveloped by cords of hepatocytes (HC). The hepatocytes shows strongly eosinophilic granulated cytoplasm and round nuclei. Between the strands of hepatocytes the hepatic sinusoids are often seen (HS). In TAA treated group, there were progressive modifications in several hepatocytes, irregular dense fibrotic septa and focal necrosis. The nuclei show different form of injury including; pyknosis, karyohexsis or karyolysis (Fig.1 (b1). On the other hand, disruption of the liver lobules, hydropic deterioration and vacuoles in the hepatocytes are noticed (Fig.1 (b2)

Liver sections of protected M.O group were nearly like normal (Fig.1 f). While, protected R.K group showed congested portal tract associated with inflammatory infiltration (Fig.1 g). In protected M.O+R.K group, microscopic examination of liver sections showed disruption of the hepatic lobules and focal necrosis of the hepatocytes (Fig.1 h). Microscopic examination of liver sections of treated rats given M.O, R.K and M.O+ R.K after TAA showed the hepatic lobules nearly like normal (Fig.1(i, j, k respectively)

Histopathological results of kidney tissues

Kidney sections of control (Fig.2a), M.O control (Fig.2c), R.K control (Fig.2d) and M.O+ R.K control (Fig.2e) groups showed the normal architecture of the renal corpuscles and renal tubules. Kidney section of TAA-treated rat showed some cellular debris and haemorrhagic areas in the dilated interstitial spaces. The glomeruli showed hypercellularity with wide urinary spaces. On the other hand, the cells of the renal tubules showed many progressive alterations with karyolytic nuclei (Fig.2 b).

Kidney sections of protected M.O and protected M.O+RK groups showed the architecture of the renal corpuscle and renal tubules appeared like normal (F.g.2 f and h respectively). While protected R.K group showed swelling of the renal corpuscles associated with wide urinary spaces, edema, and inflammatory infiltration in the interstitial space (Fig.2 g). Administration of thioacetamide to rats then given M.O only, R.K only or M.O+ R.K exhibited that the architecture of the renal corpuscles and renal tubules appeared nearly like normal (Fig.2 (i, j, k respectively).

Discussion

Thioacetamide (TAA) is a sulfur containing, carcinogenic agent [22] and producing hepatic failure [23]. Several reports exhibited that the contact to TAA caused hepatic damage, fibrosis and cirrhosis in experimental rats [24, 25]. Therefore, it is essential to improve the function of natural antioxidants in organ toxicity inhibition in different researches. M. oleifera was a multipurpose plant that could be used as a medicinal plant possesses antitumor, anti-inflammatory, antiulcer, antihypertensive, antioxidant,

antidiabetic, hepatoprotective and anti-microbial activities [26]. M. oleifera seeds have protective effect on liver, anti-inflammatory and anti-fibrotic properties [27]. Also, Raspberry Ketone (R.K) is an aromatic compound present in raspberry fruits; it is promoted as a dietary supplement, as a product to help promote weight loss [28].

The current study showed a non-significant elevation in both SGPT and SGOT levels in control (M.O, R.K, M.O+R.K) and treated (M.O+R.K) groups when compared to normal control. This reflects a safe effect on the gastrointestinal tract physiology and/or food assimilation; consequently did not damage the organs physiology. This finding is matched with that of Geleta *et al*., [29], Waterman *et al*., [30] and Bibi *et al*., [31]. Our data obtained high significant elevation in SGPT and SGOT activities in TAA group in comparison with normal control, this result is consistent with findings of Al-Hashem *et al*., [32] who stated that the modifications stimulated by TAA in liver enzymes may be due to the oxidative damages of liver cells. Also, Mustafa *et al*., [33] found that TAA induced animals showed significant elevation in activities of SGPT and SGOT.

In our results, when comparing the protected and treated groups with TAA (positive control), a significant decrease in SGPT and SGOT were observed. This indicated the hepatoprotective effect of M.O and R.K and both of them against TAA, also our results showed that M.O and R.K and M.O+R.K in the treated groups (administered after TAA) give better results than those of protective groups (M.O and R.K administered before TAA), this result is consistent with findings of Wang *et al*., [12] who indict the hepatoprotective effect of RK. Also, confirmed with that of Bahr& Farouk [34].

In the present result a high significant increase was detected in serum AFP in TAA group comparable with normal control this may be due to increase in tumor development. This finding agreed with that of Hessin *et al*., [35]. Alternatively, treated groups exhibited significant decreases in AFP in compare to TAA. However, MO extract was more effective than RK extract or a mixture of both to improve AFP in TAA gp. These results matched with Hessin *et al*., [35] who stated that decrease in AFP level after M.O administration may be because of declines in the rate of tumor generation.

With regard to renal function, our research showed a non-significant alteration in serum creatinine and urea concentrations in M.O, R.K and M.O+R.K control groups in comparison with control. Our finding approved the nephroprotective influence of these medicinal plants. However a highly significant elevation was detected in TAA group as compared to normal control group, our study consistent with Begum *et al*., [36] and Kadir *et al*., [37] who stated that the high levels of serum urea and creatinine reveal kidney damage. Meanwhile in this study, a high significant decrease in levels of urea and creatinine was detected in the protected and treated groups (M.O, R.K and M.O+R.K) as compared with TAA group this proved the protective effect of M.O and R.K. This study is in agreement with Karthivashan *et al*. [38].

Conversely, the treated groups in this study showed obvious reversion (more than protected group) of the hepatic and renal markers almost close to that of the control group.

In our study, TAA group induced significant increase of NO and MDA in liver tissue this finding agrees with that of Mustafa et *al*., [33]. In addition, in RK treated group there was a significant reduction in MDA concentration in comparison with TAA group, our finding matched with that of Wang *et al*., [12]. However, administration of MO in treated groups significantly decreased the levels of MDA and NO in comparison with the TAA group. Karthivashan *et al*., [38] demonstrated that the existence of flavonoids such as kaempferol, apigenin,

quercetin, and multiflorin in the MO extract responsible for advancing antioxidant potential. While significant elevation in the level of NO was detected in treated (M.O, R.K, M.O+R.K) groups as compared to TAA gp.

Furthermore, animals groups treated with TAA and M.O recorded a significant improvement in hepatic levels MDA, NO, and PON in compare to the animals group intoxicated with TAA alone; the highest improving potential was recorded with regard animal group treated with M.O in combination with TAA. This finding is confirmed with that of Yassa & Tohamy [39]; Bahr & Farouk, [34] and Bibi *et al*., [31]. Our data revealed a high significant decrease in hepatic PON activity was detected in TAA group and protected (M.O, R.K, M.O+R.K) as compared to normal control group. This finding matched with that of Marsillach *et al*., [40] who stated that with hepatitis or cirrhosis, the concentration of PON-1 in plasma is decreased by 50% and the decrease is proportional to the degree of tissue injury. Also, in our results a significant decrease was observed in the control (M.O, M.O+R.K) and treated (M.O, R.K) groups against normal control. These results prove the protective action of M.O and R.K against liver tissue damage.

Our study showed that severe histopathological changes in liver tissue following TAA administration that vary from irregular dense fibrotic septa and focal necrosis of the hepatocytes. The nuclei showed different form of injury. These injuries were in the form of pyknosis, karyohexsis or karyolysis. Also, hepatic lobules disruption, hydropic deterioration and vacuoles in the liver cells were detected. This is consistent with Al-Attar and Al-Rethea, [41].

The microscopic examinations in the TAA+ M.O, TAA+ R.K and TAA+ M.O+ R.K protected group showed reduced complexity of microscopic modifications in comparison with the TAA group. On the other hand all treated groups (TAA+ M.O, TAA+ R.K and TAA+ M.O+ R.K) showed improvement of the hepatic lobules looked like normal. It has been observed that the antioxidant and hepatoprotective effect of M. O leaves extract with R.K as it was also confirmed by the histopathological picture of the liver. This find is agreement with Yassa &Tohamy [39] who reported that hepatic sections of rats treated with Moringa extract only exhibited normal histological construction of the hepatic lobules and central vein compared to normal animals.

In our results the histopathological examination of kidney section of TAA-treated rat showed some cellular debris and haemorrhagic areas in the dilated interstitial spaces. The glomeruli showed hypercellularity with wide urinary spaces. On the other hand, the renal tubules cells showed many degenerative alterations with karyolytic nuclei. This result was confirmed with several studies [42, 37]. Administration of thioacetamide to rats then given M.O only, R.K only or M.O+ R.K exhibited that the architecture of the renal corpuscles and renal tubules appeared more or less like normal. This proves the protective action of these medicinal plants on renal tissue.

In conclusion: Regarding the histopathological and biochemical examination of liver and kidney tissues, it was noticed that the administration of TAA result in severe damage in the tissue which can be resolved by administration of M.O alone or better with both M.O and RK as natural antioxidants. Briefly; this study clearly demonstrated a strong evidence for the treatable action of hydroethanolic extract of M.O pods and aqueous solution of R.K for hepatic and renal tissue damage, and anti-toxic efficiencies of both.

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Table1: SGPT & SGOT activities and AFP levels in the serum of different rat groups

Group \ Parameters		GPT (ALT) (U/l)	GOT (AST) (U/l)	AFP (Pg/ml)
Group 1 N. control	Range Mean ± SD	13.9 – 27.9 20.03 ± 5.6	41.9 – 130.9 85.2 ± 30.9	25.1 – 29.8 27.2 ± 1.6
Group 2 M.O control	Range Mean ± SD p- value	22.9 – 26.5 23.6 ± 1.4 0.453	86.2 – 89.5 87.7 ± 1.36 0.871	23.2 – 28 25.3 ± 1.9 0.777
Group 3 R.k control	Range Mean ± SD p- value	7 – 31.4 21.2 ± 8.1 0.808	59.4– 156 98.05 ± 44.3 0.414	18 – 30 24.1 ± 5.03 0.646
Group 4 M.O+R.k control	Range Mean ± SD p- value	22.5 – 32.2 27.8 ± 4.6 0.109	75.3 – 79.4 77.96 ± 1.54 0.644	20.2 – 26.1 23.4 ± 2.39 0.575
Group 5 TAA control	Range Mean ± SD p- value	39.5 - 45 41.67 ± 1.94 0.000	199 – 221 210.7 ± 9 0.000	130.2 – 210 172.3 ± 29.2 0.000
Group 6 M.O protected	Range Mean ± SD p- value p^a- value p^b- value	33.2 – 39 36 ± 2.4 0.001 0.24 0.012	115.2 – 143.2 128.4 ± 9.5 0.008 0.000 0.012	95.9– 130 113.2 ± 12.2 0.000 0.000 0.000
Group 7 R.k protected	Range Mean ± SD p- value p^a- value p^c- value	26.2 – 49.8 37.7 ± 10.27 0.000 0.40 0.001	137 – 155.4 146.2 ± 7.7 0.000 0.000 0.003	99.2– 136.2 120.7 ± 13.17 0.000 0.000 0.000
Group 8 M.O+R.k protected	Range Mean ± SD p- value p^a- value p^d- value	23.5 – 39.2 31.6± 5.56 0.018 0.04 0.425	106 – 115 110.16 ± 3.1 0.115 0.000 0.044	95– 126 106.5 ± 10.9 0.000 0.000 0.000
Group 9 Treated M.O	Range Mean ± SD p- value p^a- value p^b- value	15.7– 47.1 29.9± 12.9 0.042 0.018 0.189	77.3 – 193.8 115.07 ± 41.7 0.061 0.000 0.086	79– 104.6 90.5 ± 9.6 0.000 0.000 0.000
Group 10 Treated R.k	Range Mean ± SD p- value p^a- value p^c- value	16.6 – 67.6 33.5± 17.5 0.007 0.093 0.012	62.8– 214.8 159.2 ± 55.44 0.000 0.002 0.000	88– 102 95.1 ± 5.3 0.000 0.000 0.000
Group 11 Treated M.O +R.k	Range Mean ± SD p- value p^a- value p^d- value	23.2 – 30.4 26.28± 2.47 0.196 0.002 0.752	99.5 – 120 109.3 ± 7.02 0.129 0.000 0.05	86.2– 109 98.7 ± 7.9 0.000 0.000 0.000

p value versus normal (negative control)
$p^{(a)}$ value versus TAA (positive control). $p^{(b)}$ value versus M.O control.
$p^{(c)}$ value versus R.K control. $p^{(d)}$ value versus M.O+R.K control.
$p > 0.05$ is non-significant, $p \leq 0.05$ is significant

Table 2: Urea and Creatinine levels in the serum of different rat groups

Parameters / Group		Urea (mg/dl)	Creatinine (mg/dl)
Group 1 N. control	Range Mean ± SD	21.7 – 29 25.9 ± 3.03	0.778 – 0.9 0.83 ± 0.056
Group 2 M.O control	Range Mean ± SD p- value	18.3 – 26.2 21.93 ± 2.5 0.01	0.5 – 0.89 0.7 ± 0.17 0.315
Group 3 R.k control	Range Mean ± SD p- value	26.7 – 30.2 29.03 ± 1.36 0.04	0.9 – 1.02 0.95 ± 0.04 0.372
Group 4 M.O+R.k control	Range Mean ± SD p- value	18.8 – 28.8 24.2 ± 4.2 0.28	0.51 – 0.79 0.64 ± 0.11 0.098
Group 5 TAA control	Range Mean ± SD p- value	57.7 - 61 59.25 ± 1.16 0.000	2 – 2.9 2.5 ± 0.36 0.000
Group 6 M.O protected	Range Mean ± SD p- value p^a- value p^b- value	40.4 – 46.2 43.05 ± 2.4 0.000 0.000 0.000	1.9 – 2.55 2.2 ± 0.29 0.000 0.022 0.000
Group 7 R.k protected	Range Mean ± SD p- value p^a- value p^c- value	47.6 – 52 48.6± 1.67 0.000 0.000 0.000	1.88 – 2.5 2.1 ± 0.23 0.000 0.002 0.000
Group 8 M.O+R.k protected	Range Mean ± SD p- value p^a- value p^d- value	37.7 – 40.7 38.9± 0.98 0.000 0.000 0.000	1.9 – 2.3 2.06 ± 0.13 0.000 0.001 0.000
Group 9 Treated M.O	Range Mean ± SD p- value p^a- value p^b- value	36.4– 42 39.2± 1.86 0.000 0.000 0.000	1.5 – 2.3 1.92 ± 0.33 0.000 0.000 0.000
Group 10 Treated R.k	Range Mean ± SD p- value p^a- value p^c- value	34– 43.2 38.66± 3.02 0.000 0.000 0.000	1.6 – 1.93 1.7 ± 0.12 0.000 0.000 0.000
Group 11 Treated M.O +R.k	Range Mean ± SD p- value p^a- value p^d- value	30.4 – 41.3 35.3± 4.1 0.000 0.000 0.000	1.39 – 1.79 1.59 ± 0.14 0.000 0.000 0.000

p value versus normal (negative control)
$p^{(a)}$ value versus TAA (positive control). $p^{(b)}$ value versus M.O control.
$p^{(c)}$ value versus R.K control. $p^{(d)}$ value versus M.O+R.K control.
$p > 0.05$ is non-significant, $p \leq 0.05$ is significant

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Table 3: Stress related biomarkers in kidney tissue of different rat groups

Parameters / Group		Renal MDA (nmol/g. tissue)	Renal NO (µmol/L)	Renal PON (kU/L)
Group 1 N. control	Range	22.3 – 31	12 – 23	36 – 54
	Mean ± SD	27± 2.8	18± 4	44± 6.7
Group 2 M.O control	Range	21 – 33.2	14 – 22	39 – 46
	Mean ± SD	29± 4.3	18± 2.7	43± 2.5
	p- value	0.451	1.00	0.699
Group 3 R.k control	Range	26 – 34	16– 23	38 – 46
	Mean ± SD	30± 3.4	19± 2.8	42± 3.15
	p- value	0.259	0.644	0.441
Group 4 M.O+R.k control	Range	21 – 31.2	12– 23	36 – 46
	Mean ± SD	28± 3.67	18± 3.35	41± 4.01
	p- value	0.705	1.00	0.249
Group 5 TAA control	Range	42 – 60	23– 36	26 – 39
	Mean ± SD	53± 6.4	29± 4.4	31± 4.6
	p- value	0.000	0.000	0.000
Group 6 M.O protected	Range	42 – 53	19– 34	33 – 44
	Mean ± SD	47± 3.8	25± 5.1	39± 3.56
	p- value	0.000	0.002	0.057
	p^a- value	0.027	0.068	0.003
	p^b- value	0.000	0.002	0.126
Group 7 R.k protected	Range	29 – 53	22– 26	36 – 45
	Mean ± SD	45± 8.3	24± 1.5	40± 3.5
	p- value	0.000	0.007	0.126
	p^a- value	0.004	0.024	0.001
	p^c- value	0.000	0.024	0.441
Group 8 M.O+R.k protected	Range	38.4 – 45.3	19– 26	30 – 46
	Mean ± SD	42.05± 2.6	23± 2.9	37± 5.96
	p- value	0.000	0.024	0.009
	p^a- value	0.000	0.007	0.024
	p^d- value	0.000	0.024	0.126
Group 9 Treated M.O	Range	29 – 46	17– 26	31 – 41
	Mean ± SD	40± 6.2	22.6± 3.2	36± 3.2
	p- value	0.000	0.034	0.003
	p^a- value	0.000	0.005	0.057
	p^b- value	0.000	0.034	0.009
Group 10 Treated R.k	Range	40.4 – 46.2	16– 31	27 – 44
	Mean ± SD	43.05± 2.4	25± 5.4	37± 5.6
	p- value	0.000	0.002	0.009
	p^a- value	0.000	0.068	0.024
	p^c- value	0.000	0.007	0.057
Group 11 Treated M.O +R.k	Range	37.7 – 40.7	18– 28	33 – 46
	Mean ± SD	38.9± 0.9	22± 3.6	39± 4.3
	p- value	0.000	0.068	0.057
	p^a- value	0.000	0.002	0.003
	p^d- value	0.000	0.068	0.441

p value versus normal (negative control)
$p^{(a)}$ value versus TAA (positive control). $p^{(b)}$ value versus M.O control.
$p^{(c)}$ value versus R.K control. $p^{(d)}$ value versus M.O+R.K control.
$p > 0.05$ is non-significant, $p \leq 0.05$ is significant

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Table 4: Stress related biomarkers in liver tissue of different rat groups

Group \ Parameters		Hepatic MDA (nmol/g. tissue)	HepaticNO (µmol/L)	Hepatic PON (kU/L)
Group 1 N. control	Range Mean ± SD	22 – 37 28± 4.8	11 – 22 15± 4.3	35 – 42 38± 2.5
Group 2 M.O control	Range Mean ± SD p- value	21 – 33 29± 4.27 0.770	10 – 29 18± 6.8 0.297	26.2 – 40.3 33± 5.5 0.05
Group 3 R.k control	Range Mean ± SD p- value	26 – 34 30± 3.38 0.559	12 – 23 17± 4.15 0.485	28.3 – 46 34± 6.5 0.123
Group 4 M.O+R.k control	Range Mean ± SD p- value	21 – 31 28± 3.67 1.00	12 – 23 16± 4.08 0.727	28 – 36 32± 3.1 0.023
Group 5 TAA control	Range Mean ± SD p- value	30 – 61 48± 12.2 0.000	20 – 40 30± 6.9 0.000	18 – 29.3 24± 3.8 0.000
Group 6 M.O protected	Range Mean ± SD p- value p^a- value p^b- value	24– 53 41± 9.8 0.000 0.044 0.001	22 – 41 29± 7.1 0.000 0.727 0.000	21 – 31 26± 3.9 0.000 0.438 0.008
Group 7 R.k protected	Range Mean ± SD p- value p^a- value p^c- value	38– 45 42.5± 2.6 0.000 0.11 0.001	25 – 31 28± 2.26 0.000 0.485 0.000	21.2 – 31 25± 3.4 0.000 0.698 0.001
Group 8 M.O+R.k protected	Range Mean ± SD p- value p^a- value p^d- value	29– 46 40± 6.2 0.001 0.022 0.001	21 – 32 27± 3.6 0.000 0.297 0.000	21 – 31.2 28± 3.67 0.000 0.124 0.124
Group 9 Treated M.O	Range Mean ± SD p- value p^a- value p^b- value	36– 43 39± 2.6 0.002 0.11 0.005	20 – 30 24± 3.9 0.003 0.04 0.04	26 – 34 30± 3.4 0.003 0.023 0.247
Group 10 Treated R.k	Range Mean ± SD p- value p^a- value p^c- value	35– 42 38± 2.6 0.005 0.05 0.022	20 – 26 23± 2.03 0.007 0.017 0.04	26 – 36 31± 4.2 0.008 0.008 0.247
Group 11 Treated M.O +R.k	Range Mean ± SD p- value p^a- value p^d- value	31– 40 35± 3.1 0.044 0.000 0.044	13 – 29 22± 5.7 0.017 0.007 0.040	26.2 – 43 34± 6.6 0.123 0.000 0.438

p value versus normal (negative control)
$p^{(a)}$ value versus TAA (positive control). $p^{(b)}$ value versus M.O control.
$p^{(c)}$ value versus R.K control. $p^{(d)}$ value versus M.O+R.K control.
$p > 0.05$ is non-significant, $p \leq 0.05$ is significant

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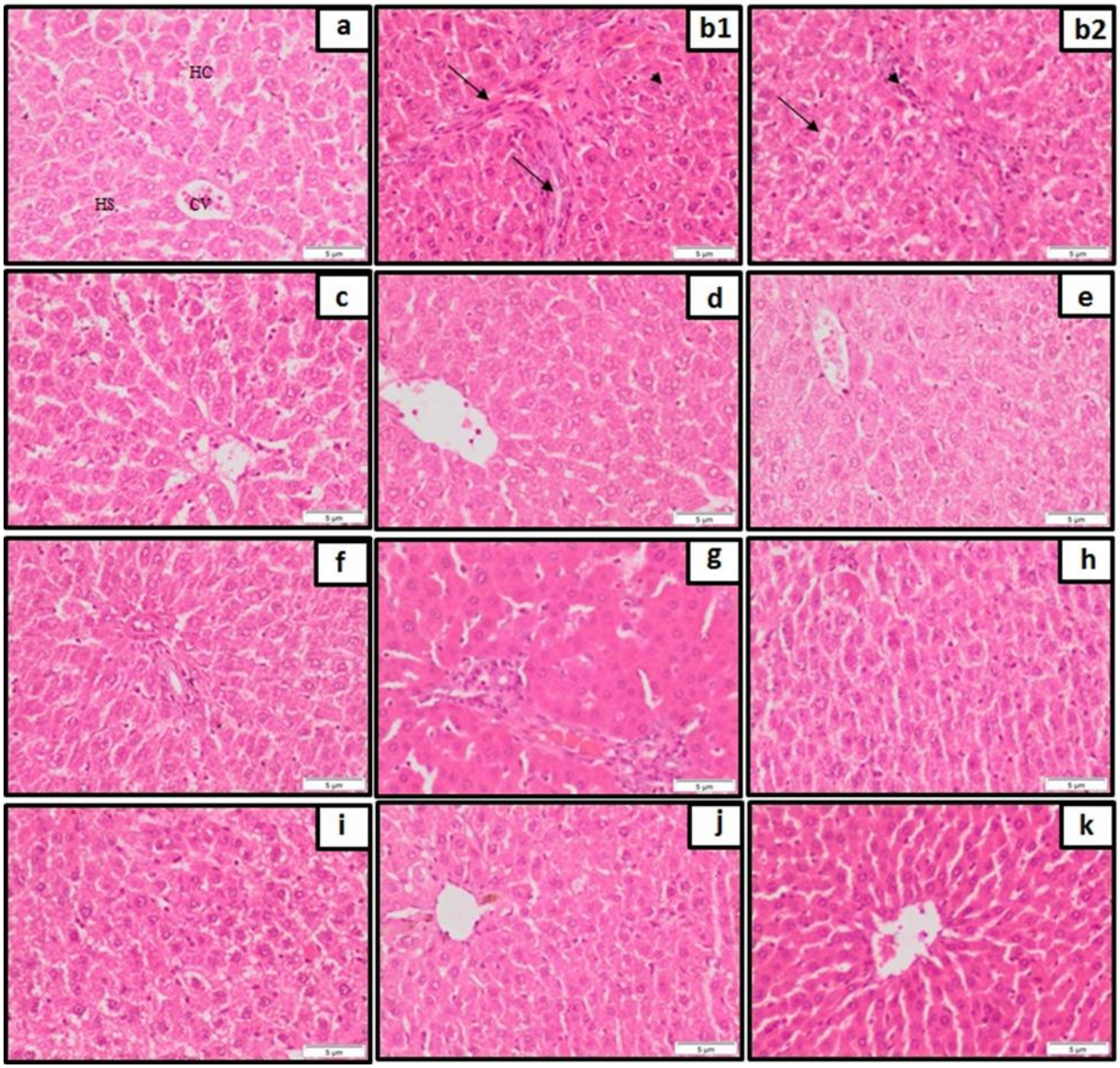

Fig.1. A photomicrograph of the liver of control rats (a) M.O control (c), R.K control (d) and M.O+ R.K control (e) groups showed normal hepatocyte histological structure. (b1,b2): Showed liver sections of TAA group degenerative changes in numerous hepatocytes, irregular dense fibrotic septa and focal necrosis. Protected M.O group (f) showed normal like architecture. Protected R.K group (g) showed congested portal tract associated with inflammatory infiltration. Protected M.O+R.K group (h) showed disturbance of the hepatic lobules. Treated rats given M.O, R.K and M.O+ R.K (i, j and k) showed normal like architecture.

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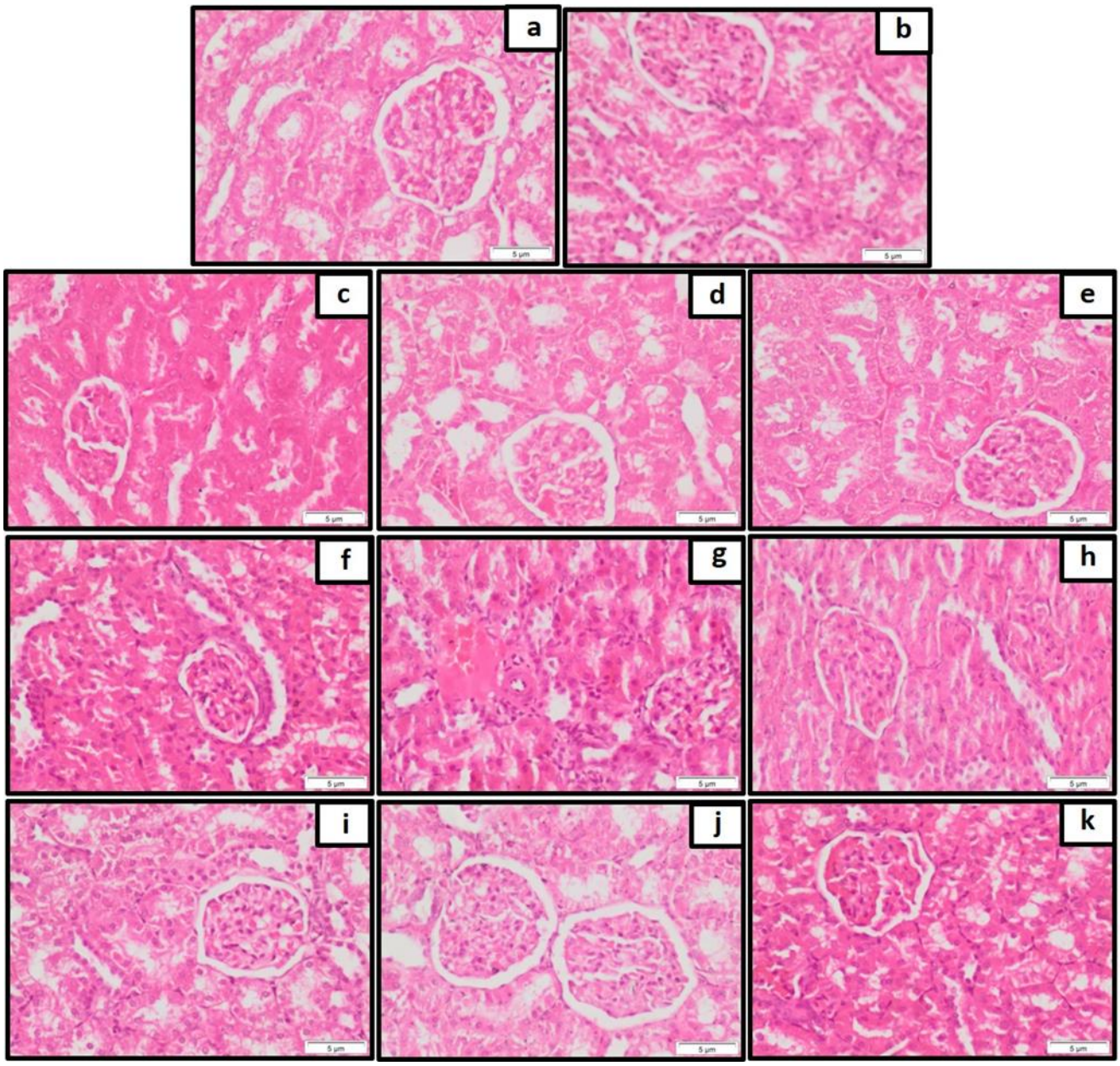

Fig.2. A photomicrograph of the kidney of control rats (a) M.O control (c), R.K control (d) and M.O+ R.K control (e) groups showed normal architecture of the renal corpuscles and renal tubules. (b): Showed kidney sections of TAA group cellular debris and haemorrhagic areas, glomeruli showed hypercellularity. Protected M.O group (f) showed normal like architecture. Protected R.K group (g) showed swelling of the renal corpuscles associated with wide urinary spaces, edema, and inflammatory infiltration. Protected M.O+R.K group (h) showed normal like architecture. Treated rats given M.O, R.K and M.O+ R.K (i, j and k) showed normal like architecture.