

Effect of Functional Yoghurt Fortified with Manuka Honey and Anulin on Liver and Kidney Function in Laboratory Rats

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Annotation: The study was carried out at Department of Food Science laboratories and the animal house of College of Veterinary Medicine, Tikrit University using the official number 1601A301C011 during the period from 9/1/2024 to 10/1/2024, and that this study demonstrates also the importance of functional yoghurt with manuka honey and inulin of the liver and kidney function in the experimental Animal (rat). The findings also revealed a significant reduction in the liver enzymes (AST, ALT). Levels of ALP were increased in the treated groups, particularly the yogurt group containing Manuka honey, which point out a protective response to oxidative stress and inflammation. Kidney function parameters (urea and creatinine) were also markedly reduced, suggesting that an increased renal filtration rate was taking place.

This is likely to be contributed to the high antioxidant and anti-inflammatory properties of Manuka honey which is rich in strong phenolic compounds and flavonoids which play a role in the suppression of inflammatory cytokines and protect the

stability of the liver and kidney cells. Inulins, on the other hand, as a prebiotic fiber, stimulate the proliferation of beneficial bacteria and maintain microbial balance in the intestine, which can enhance the status of the gut-liver-kidney axis and decrease the accumulation of nitrogen wastes.

These data suggest that enrichment of yoghurt by natural compounds as manuka honey and inulin is a novel dietary approach in the protection against hepatic and renal dysfunctions. It further manifests that functional foods are an effective mean to promote public health and prevent diet-related diseases through a variety of biological mechanisms.

Keywords: Functional yogurt, Manuka honey, linolenic acid, liver function, kidney function.

Introduction:

In the last decades, functional foods have attracted great attention as an essential part of the health and chronic diseases prevention. Of these, yoghurt is most pertinent since, in addition to high nutrition, the health effects of its probiotic bacteria (as well as their fermentation products), make it an excellent food that is beneficial to gut health and the immune system (**Al-Khafaji et al., 2021**). Recently, the development of so-called "functional yoghurt" has attracted much attention wherein natural materials having a bio-effect such as manuka honey and inulin is added to yoghurt to increase its ability to prevent diseases and to promote the function of a vital organ.

Manuka honey has been observed to have antimicrobial and anti-inflammatory activities, and the contents of phenolic compounds and flavonoids are high due to which this has a strong potential in reducing oxidative stress and protecting liver and kidney cells from injury (**Khurrami et al., 2022; Srinivasan et al., 2024**). Inulin is a prebiotic fiber which also kinetically favours the growth of healthy bacteria within the GI track and helps balance the gut microbiome- all positive effects that contributes towards better gut/liver/kidney axis health, reducing inflammation, and optimising nutrient absorption (**She et al., 2024**).

Recent researches indicates that intake of this compounds-enriched yoghurt can reduce blood levels of liver enzymes (ALT, AST, ALP), urea and creatinine, showing the preventive effect of against oxidant and inflammatory injuries related to liver and kidney disease (**Naguib et al., 2021**). Therefore, studies that examine the effect of yoghurt containing manuka honey and inulin on function are essential in order to demonstrate any potential biological mechanisms of action and therapeutic value, as we have done using an animal model, which is an important first step before undertaking further clinical trials

Materials and working methods

Vital Experience:

Laboratory Animals Prepared: Male Albino, aged 2 - 3 months, and weight (190 - 210) g were used in this study (Collected from Faculty of Veterinary Medicine/Tikrit University) were placed in plastic cages with metal shielded lid floor covered with sawdust sterilizing the sawdust daily and change it through 4 times per week were given orally injection with Yogurt, Yogurt fortified with inulin and yogurt fortified with Manuka with dose as concentration were used in experiment.

Experience Design:

The experimental animals were randomly divided into four groups of five animals.

C Control Group, K Yougart Group, M Yogat Group, Fortified with Manuka Honey 5%, A Group of Yogat Fortified with Inulin5%

Biochemical blood test: 0.5 ml blood was collected in tubes with EDTA to avoid coagulation for analysis. The tube was gently mixed and centrifuged, and the measurement of urea and creatinine was carried out by a Spanish Hematology analyzer for kidney function study.

Urea was estimated using the analysis kit prepared by Biolabo, as urea is hydrolyzed into carbon dioxide and ammonia gas according to the method used by (Wang et al. 2023). And it was calculated according to the following law

Difference in the absorption of the sample

Creatine concentration = -----×standard solution concentration 20mg/dl))

Difference in the absorption of the standard solution

Results and Discussion:

Effect of Functional Yogurt Fortified with Manuka Honey and Anulin by 5% on Liver Enzymes

The results of the table indicate that there are significant differences ($p \leq 0.05$) between the groups in liver enzyme markers (ALT, AST, ALP), which are sensitive indicators of liver cell health. The control group (T1) recorded the highest ALT value (31.66 ± 1.45 U/L), followed by the Yoghurt group (T2) (28.66 ± 0.66 U/L), the inulin group (T3) (25.66 ± 1.20 U/L), and the Manuka group (T4) has the lowest value (20.33 ± 0.33 U/L), demonstrating a clear protective effect of Manuka in reducing liver cell damage.

For AST, the control group (T1) recorded the highest value (36.33 ± 2.72 U/L), followed by the Yoghurt group (T2) (31.00 ± 1.52 U/L), the inulin group (T3) (25.66 ± 1.20 U/L), and the Manuka group (T4) has the lowest value (24.00 ± 1.15 U/L), reflecting improved liver function under subsidized treatments.

In terms of ALP enzyme, the control group (T1) recorded the highest value (90.66 ± 1.76 U/L), followed by the Yoghurt group (T2) (80.33 ± 1.76 U/L), and the inulin group (T3) (68.66 ± 1.20 U/L). The Manuka group T4 has the lowest value (60.33 ± 1.45 U/L), indicating a stabilization of hepatocyte activity under the influence of biocompounds, especially Manuka honey.

The enhanced liver performance following the administration of Manuka honey is related to its high content of phenol contents and flavonoids that decreases oxidative stress and suppresses inflammatory cytokines. Furtherore, honey enhances the activity of endogenous anti-oxidant enzymes, such as SOD and glutathione peroxidase, which helps to protect the stability and function of hepatocellular membranes (Khormi et al., 2022).

These results are consistent with what (Saddiqa et al., 2022) noted in their mouse study of inulin-rich garlic extract (added to yogurt), the reduction in the liver enzymes ALT, AST and ALP of the treated groups with UC. Inulin's hepatoprotective effect is due to the modulation of the "gut-liver

axis", leading to decreased transport of bacterial toxins from the gut to the liver with less inflammation and less cell injury due to intestinal infections.

These findings are also consistent with what (Miller et al., 2020) as it has been reported that diabetes presents a significant rise of liver and kidney enzymes (ALT, AST, urea, creatinine) in response to oxidative stress and inflammation. However, inulin fortified synbiotic yogurt consumption brought these markers near to normal and it was an indication of an improved liver and renal function. This is due to the promotion of the growth of probiotic bacteria by inulin and the production of short-chain fatty acids that lower inflammation and enhance integrity in the gut-liver-kidney axis.

These findings are also consistent with what (Obaid et al., 2020) Δ have also shown that daily consumption of probiotic-fermented yogurt resulted in significantly reducing liver enzymes' activity (ALT and AST) in hypercholesterolemia mice, suggestive for a better liver function and less oxidative damage. This action is presumed to be due to the capacity of probiotics to modulate the gut microbiome, diminish cholesterol absorption and stimulate the production of short-chain fatty acids which have a positive impact on the liver and diminution of inflammation.

These findings were also confirmed by (Naguib et al., 2021). whose research revealed that consumption of inulin-fortified yogurt plus encapsulated probiotic was associated with significant decrease in levels of AST and ALT in diabetic mice, signifying enhancement of liver function and decrease of damage by oxidative stress(22). This may be due to the enhanced integrity of the gut microbiome and the production of short-chain fatty acids induced by inulin, as inulin serves as a fuel to good gut bacteria and enhances the production of short-chain fatty acids, which reduce inflammation and improve nutrient absorption, regulate blood sugar and blood lipids.

The table shows the effect of functional eukert fortified with 5% manuka honey and inulin on liver enzymes

Proteins			Transactions
ALp	AST	ALT	
1.76 ± 90.66 a	2.72 ± 36.33 a	1.45 ± 31.66 a	T1
1.76 ± 80.33 b	1.52 ± 31.00 off	0.66 ± 28.66 off	T2
1.20 ± 68.66 c	1.20 ± 25.66 bc	1.20 ± 25.66 b	T3
1.45 ± 60.33 d	1.15 ± 24.00 c	0.33 ± 20.33 c	T4

*Different lowercase letters within one column indicate significant differences ($p \leq 0.05$) between the effects of the coefficients

T1: Control Group

T2: Yoghurt

T3: Yoghurt + Inulin 5%

T4: Yoghurt + Manuka 5%

The effect of functional yoghurt fortified with Manuka honey and inulin by 5% on kidney function

The results of the table indicate that there are significant differences ($p \leq 0.05$) in kidney function indices (Urea, Creatinine), which are used as an indicator of the efficiency of kidney filtration. The control group (T1) recorded the highest urea concentration of (31.66 ± 3.28 mg/dL), followed by the yoghurt group (T2) (28.33 ± 0.88 mg/dL), and the inulin group (T3) (23.33 ± 1.76 mg/dL).

In comparison, the Manuka group (T4) recorded the lowest value (20.33 ± 0.33 mg/dL), indicating a significant improvement in kidney function as a result of the consumption of fortified yoghurt.

The control group (T1) had the highest value (1.20 ± 0.15 mg/dL), followed by the Yoghurt group (T2) (1.06 ± 0.08 mg/dL), the inulin group (T3) (0.86 ± 0.03 mg/dL), and the Manuka group (T4)) at the lowest concentration (0.73 ± 0.03 mg/dL), which augments Manuka's protective effect on the kidney by lowering nitrogen waste products. The betterment in renal function could be a result of the effective biological activity of honey due to its high natural phenol content (phenolic compounds and antioxidants) and protective effect that helps to minimize oxidative stress and improve the balance in cells of the renal tissues (Srinivasan et al., 2024). (Nerli et al., 2023) have indicated that these properties enable honey to lower urea and creatine levels, making it an adjunct in preventing or alleviating kidney disease. This was confirmed by the results of (Khormi et al., 2022), who also reported that manuka honey treatment resulted in a marked reduction in the concentration of urea and creatinine in the serum of treated mice as compared to diazinon only group, nearing the normal values of those of normal physiological states. These findings confirm earlier reports in which proportions of particular blood (Prokisch et al., 2022) have noted, functional yogurt added with honey has been observed to be capable of reducing blood sugar; this effect might indirectly contribute to the protection of the kidney. This might be due to the beneficial contents of phenolic and antioxidant compounds in honey which may protect against oxidative stress related to renal function decline.

The results are also consistent with (Miller., 2020) who said the inulin-fortified yogurt also had the effect of lowering urea and creatine, adding that the compound could help improve kidney function by growing good bacteria while bringing down inflammation of the gut-liver-kidney axis. It is thought that the prebiotic effect of inulin in retaining blood urea and creatine levels (14-16) is due to its FOS properties, enhancing the growth of LA bacteria via fibre, and thus inducing a shift in the gut microbiome and reducing systemic inflammation (He et al., 2024).

The table shows the effect of functional eukert fortified with manuka honey and inulin by 5% on kidney function

Attributes		Transactions
Creatine	urea	
0.15 ± 1.20 a	3.28 ± 31.66 A	T1
0.08 ± 1.06 off	0.88 ± 28.33 Off	T2
0.03 ± 0.86 bc	1.76 ± 23.33 Bc	T3
0.03 ± 0.73 c	0.33 ± 20.33 C	T4

*Different lowercase letters within one column indicate significant differences ($p \leq 0.05$) between the effects of the coefficients

T1: Control Group

T2: Yoghurt

T3: Yoghurt + Inulin 5%

T4: Yoghurt + Manuka 5%

Conclusion: Recent scientific studies have established that yogurt supplementation with active natural products, like Manuka honey and inulin, is an emerging trend in the field of functional foods, with valuable beneficial effects in the maintenance of general health and the prevention of liver and kidney disorders. Research showed that Manuka honey antioxidant and anti-

inflammatory activities and the prebiotic effect of inulin had a great impact on decreasing the oxidative stress, modulating the balance of the gut-microbial and boosting the performance of the intestinal-liver-kidney axis. These results reinforce the movement toward a bio-fortified diet as a practical, safe, effective way to prevent chronic diseases and promote quality of life.

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