The effect of olive oil on ibuprofen induced Renal toxicity in female rats.

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Key words: Ibuprofen, Renal toxicity, Olive oil, Rats.

Abstract:

Ibuprofen is an effective, cheap, and it is one of the most commonly non-steroidal anti-inflammatory drug, which are among the most frequent prescribed medications worldwide **.**The aim of this study is to investigate the protective effect of olive oil against ibuprofen-induced nephrotoxicity female albino rats. In this study we used 24 female white rats and divided them into 4 equal groups. Each experimental group consisted of six animals. group1, control they were fed on diet and water without any treatment, group2, ibuprofen given at dose 40 mg/kg/day orally by gastric tube for 30 days, group3,olive oil 2 ml/kg/day (oral administration) , group4, ibuprofen at dose of 40 mg/kg/day and olive oil 2 ml/kg/day (oral administration).Treatments were administered once daily for 30 days. After 30 days, biochemical and histopathological analysis were conducted to evaluate nephrotoxicity. Serum levels of urea, creatinine, calcium, glucose, phosphorus and amylase were measured. Animals creatinine treated with ibuprofen alone showed a significant increase in serum levels of urea, and glucose and significant decrease in calcium. Treatment of rats with olive oil showed significant improvement in kidney function, presumably as a result of decreased boichemical parameters associated with ibuprofen-induced nephrotoxicity. Histopathological examination of the rats kidney confirmed these observations. Therefore olive oil may protect against ibuprofen-induced nephrotoxicity.

أثر زيت الزيتون على التسمم الكلوي المستحث باعطاء الايبوبروفين لأناث الجرذان

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الكلمات المفتاحية :الايبوبروفين، التسمم الكلوي، زيت الزيتون، الجرذان.

الخلاصة:

الايبوبروفين هو فعال ورخيص، هو احد الادوية غير الستيرويدية المضادة للالتهابات الاكثر شيوعا، وهي من بين الأدوية الموصوفة في كثير من الأحيان في جميع أنحاء العالم. الهدف من الدراسة هو التحقيق من الاثار الوقائية لزيت الزيتون ضد التسمم الكلوي الذي يسببه الايبوبروفين في الجرذان. في هذه الدراسة أستخدمنا 24 انثى من الجرذان البيضاء و قسمناها الى 4 مجاميع متساوية على النحو التالي: المجموعة الاولى: الضابطةعلى الغذاء والماء دون أي علاج ، المجموعة الثانية :الايبوبروفين 24 ملغم/كغم/يوم يعطى شفويا بواسطة انبوب المعدة، المجموعة الثالثة: زيت الزيتون 4 مل/كغم/يوم يعطى بواسطة انبوب المعدة، المجموعة الرابعة: ايبوبروفين 40 ملغم/كغم/يوم، زيت الزيتون 4 مل/كغم/يوم

يعطى شفويا بواسطة انبوب المعدة، تم إعطاء العلاجات مرة واحدة يوميا لمدة 30 يوما. بعد 30 يوما، تم إجراء تحليل بيوكيميائي ونسيجي لتقييم التسمم الكلوي. تم قياس مستويات مصل اليوريا، الكرياتينين، الكالسيوم، الكلوكوز، الفوسفيت والأميليز. أظهرت الحيوانات المعاملة مع الإيبوبروفين زيادة كبيرة في مستويات مصل الدم من اليوريا والكرياتينين والجلوكوز وانخفاض كبير في الكالسيوم. وأظهرت معاملة الفئران بزيت الزيتون تحسنا كبيرا في وظائف الكلى، المعاملات البيوكيميائية المرتبطة بالتهاب الكلية الناجم عن الايبوبروفين. ولذلك قد يحمي زيت الزيتون من التسمم الكلوي الناجم عن الإيبوبروفين.

Introduction:

Ibuprofen is an effective, cheap, and it is one of the most commonly non-steroidal anti-inflammatory drugs, which are among the most frequently prescribed medications worldwide [1-2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are cyclooxygenase enzyme inhibitors used widely and frequently as analgesics, antipyretics and anti-inflammatory agents [3-4]. However, its frequent use is limited by a risk of serious side effects such as hepatotoxicity and nephrotoxicity. Previous studies have shown the adverse effects of different NSAIDs to the kidney [5-6].

Olive oil is the main origin of fat in the Mediterranean food, and its regular intake is thought to have many useful effects on human health [7]. It is have antioxidant properties, hypotensive, hypoglycemic and cardiovascular, nephro and hepato protective effects. At the same time, it was also known for its antimicrobial activity and anti- inflammatory properties [8-9].

The aim of this study is to investigate the protective effects of olive oil against Ibuprofen induced renal toxicity in female rats by biochemical assaying and histopathology of kidney tissues.

Materials and Methods:

Chemicals:

Ibuprofen was obtained from the essential drug company (Baghdad, Iraq), (Each 5ml contains 100mg) and given as orally at dose of 40 mg/kg body weight as previously described by Sydney[10]. Olive oil was purchased from local market (Kerbala, Iraq), provided by ZER Company/ Turkey .Olive oil was given by gavages at a dose of 2 ml/kg as described by necib[11].

Experimental animals:

In this study, we used 24 Wister albino 230-240 g female brought from the University of Babylon College of Science. Rats were left in our laboratory for seven days before beginning the experiment. The rats were housed in wire bottom cages, free diet, tap water and with a 12 h light / dark cycle for 30 days. The experimental protocol and procedures used in this study were approved by the Ethics Committee of the Kerbala University , Kerbala , Iraq for the care and use of laboratory animals .The animals were randomly divided into four groups. Each experimental group consisted of six animals:

Group1. Control group (n=6): They were given only normal saline for 30 days.

Group2. Ibuprofen (n=6): Animals of this group were given Ibuprofen as given orally by gavage at a dose level of 40 mg / kg body weight, every day for 30 days.

Group3. Olive oil (n=6): Animals of this group were given olive oil via gavage at a dose level of 2 ml/kg body weight, every day for 30 days.

Group4. Ibuprofen + Olive oil-treated group: Rats were treated with Ibuprofen (40 mg / kg) and Olive oil (oral administration) (2 ml / kg) daily for 30 days.

At the end of the experiment rats were given ketamine 10 for anesthesia and were sacrificed 24 h after the last olive oil and ibuprofen received, and blood samples were collected in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at (60 RPm) for (7 min). The serum was collected in plastic tubes and stored in a frozen at -8^{0} for biochemical analysis.

Histopathological examination:

The animal was killed under anesthesia, the kidneys were excised and the specimens were fixed in formalin 10% solution . After fixation, the tissues were washed under running tap water and dehydrated with concentrated ethanol. After the application of xylol, the specimens were made into paraffin blocks. 5-6 micron thick sections were rehydrated and dyed with eosin and hematoxylin and examined under light (Olympus BX51) microscope.

Biochemical analysis:

When measuring biochemical analysis, an automatic device (veterinary chemo analyzer) was used for this task. Several parameters were measured and included kidney function tests (creatinine, urea) and other biochemical analysis (glucose, calcium, phosphorus and amylase).

Statistical analysis:

The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means ± MSE and $P < 0.05$ was considered to be statistically significant.

Results:

Table (4.1) shows the effect of olive oil on the renal functions among the different groups. Ibuprofen administration influenced the renal function as assessed by significant ($P<0.05$) increase in the concentrations of serum urea and creatinine when compared with the corresponding values of the control group. Olive oil administration significantly improved ($P<0.05$) renal dysfunction as assessed by significant decreased serum urea and creatinine concentrations of (ibuprofen+ olive oil) group.

On another hand, there was no changes in concentrations of urea and creatinine in the serum of olive oil- treated group as compared to control group as mentioned in table (4.1).

Table (4.1) Effect of olive oil on concentrations of serum urea and creatinine of ibuprofen- treated and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen+ olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Urea (mmol/L)	5.96±1.64	16.33±3.55*	5.4±1.05	6.03±1.05
Creatinine (µmol/L)	41.16±4.44	100.5±8.84*	40.66±3.5	42.16±3.86

There was significant increase (P<0.05) in concentration of fasting blood glucose of ibuprofen- treated group when compared to control group, but such change was ameliorated by olive oil giving that led to significant decrease (P<0.05) in concentration of blood glucose of (ibuprofen+ olive oil)- treated group when compared to ibuprofen- treated group. As shown in table (4.2).

Table (4.2) Effect of olive oil on concentration glucose in serum of ibuprofen- treated and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen +olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Glucose (mmol/L)	5.31±0.48	10.03±1.44*	4.18±0.35	5.28±1.02

As shown in table (4.3), there was significant increase (P<0.05) in concentration of amylase of ibuprofen- treated group when compared to control group, but such change was ameliorated by olive oil giving that led to significant decrease (P<0.05) in concentration of amylase of (ibuprofen+ olive oil)- treated group when compared to ibuprofen- treated group. we noticed that there was decrease in concentration of amylase of olive oil-treated group when compared to control group as in table (4.3).

Table (4.3) Effect of olive oil on concentration amylase in serum of ibuprofen- treated and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen +olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Amylase (U/L)	1291.33±126.07	2343.83±126.56*	1234±120.7	1263.66±39.34

As shown in table (4.4), there was significant decrease (P<0.05) in concentration of calcium and significant increase (P<0.05) of phosphorus of ibuprofen- treated group when compared to control group, but such change was ameliorated by olive oil giving that led to significant increase (P<0.05) in concentration of calcium and significant decrease (P<0.05) in phosphorus of (ibuprofen+ olive oil)- treated group when compared to ibuprofen- treated group.

Table (4.4) Effect of olive oil on concentration calcium and phosphorus in serum of ibuprofen- treated and control rats.

	Control group	Ibuprofen group	Olive oil group	(Ibuprofen +olive oil) group
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Calcium (mmol/L)	2.76±0.15	1.26±0.18*	2.77±0.13	2.62±0.15
Phosphorus (mmol/L)	1.55±0.2	2.8±0.17*	1.6±0.21	1.51±0.14

Histopathological examination of kidney tissue:

Histopathological examination showed no changes of glomerular structure in section of the kidney tissue (stained with eosin and hematoxylin) of olive oil group when compared with kidney tissue of the control group (figures 4.1 and4.2). On the other hand, we founded that treatment with ibuprofen led to sever congestion, diffuse hydropic degeneration, intraluminal secretion with focal acute tubular necrosis, no significant inflammatory and mild glomerular congestion seen (figure 4.3). But these changes were treated by giving olive oil with ibuprofen (Figure 4.4).

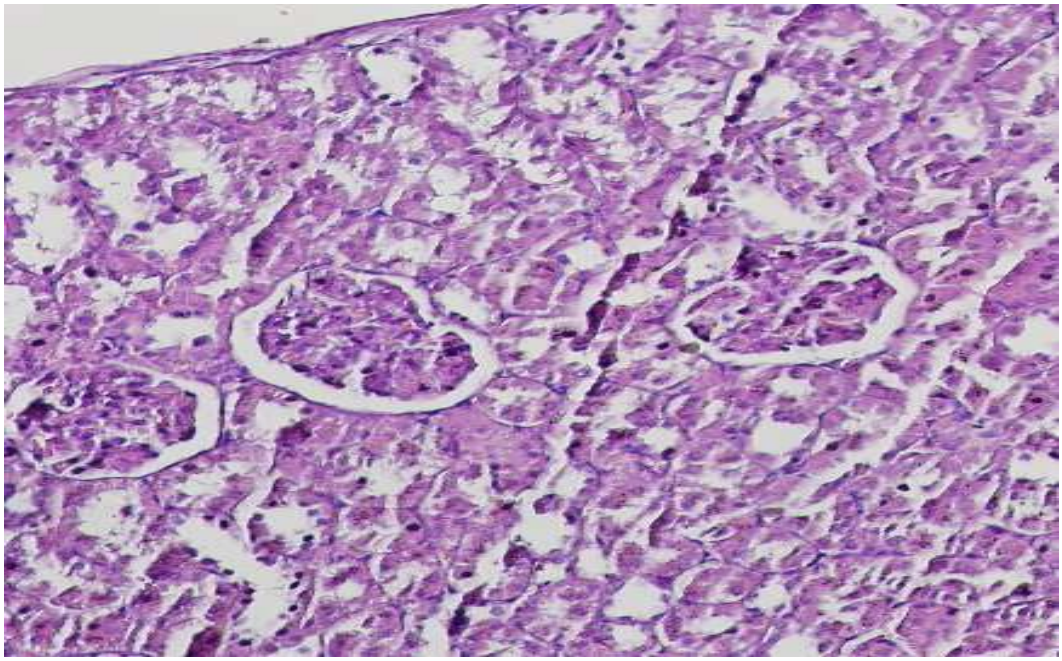

Figure (4.1): Photomicrograph of kidney from a control rat showing normal histologicalappearance, no significant changes. Magnification (200x).

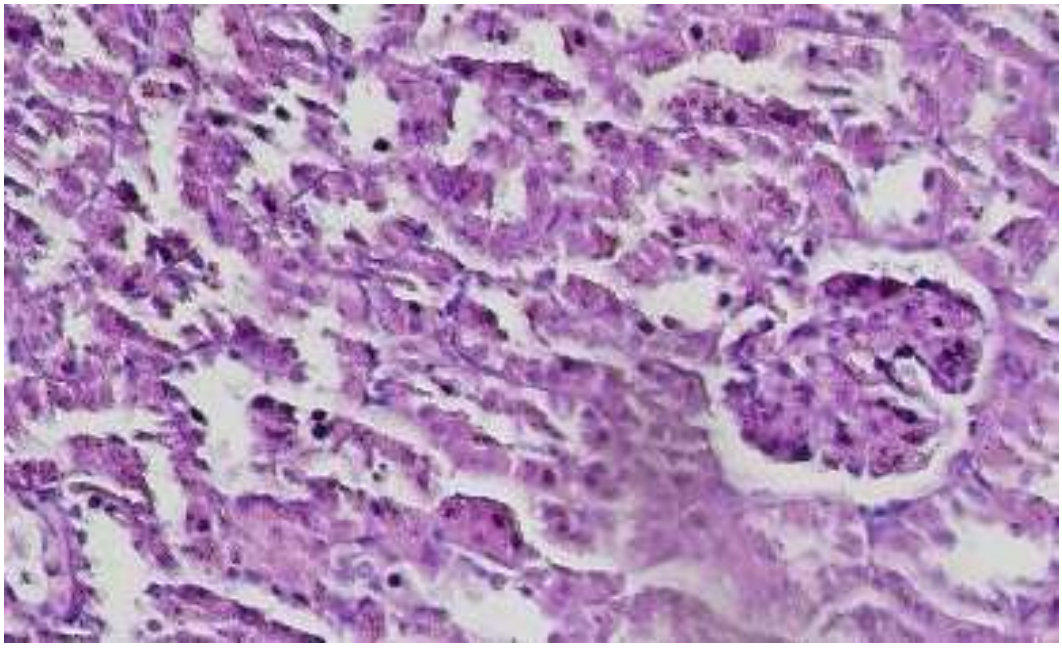

Figure (4.2): Photomicrograph of kidney from olive oil- treated rat showing normal histological as compared to control rat. Magnification is (200x).

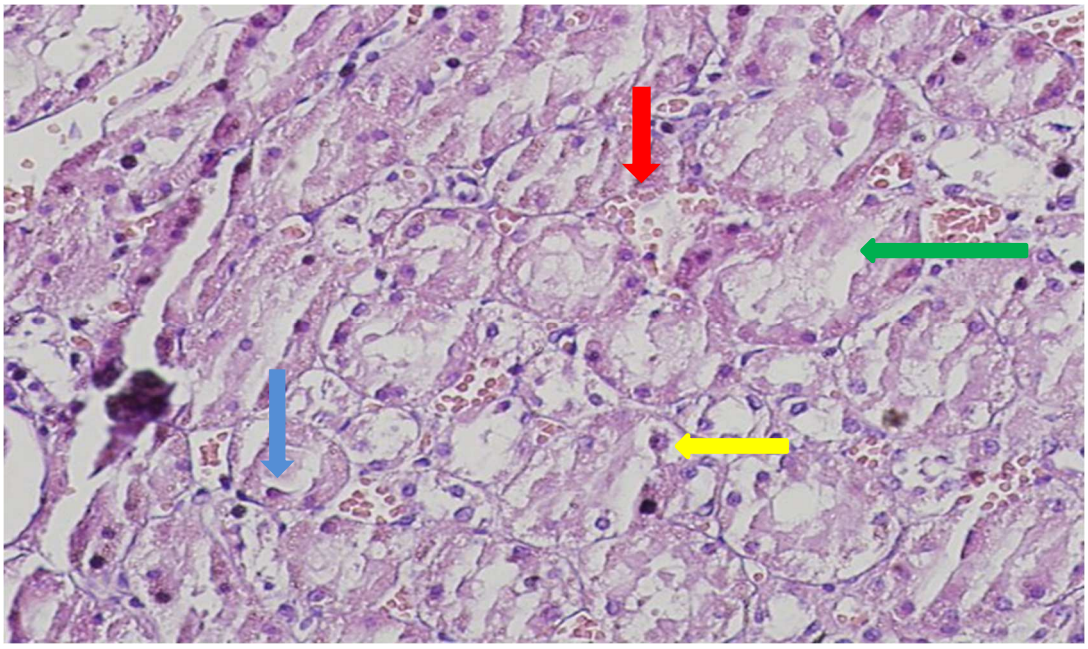

Figure (4.3): Photomicrograph of kidney from rat ibuprofen- treated showing mild congestion (red indicator), tubular necrosis (green indicator), hydropic degeneration (yellow indicator), intraluminal secretion (blue indicator). Magnification is (400x).

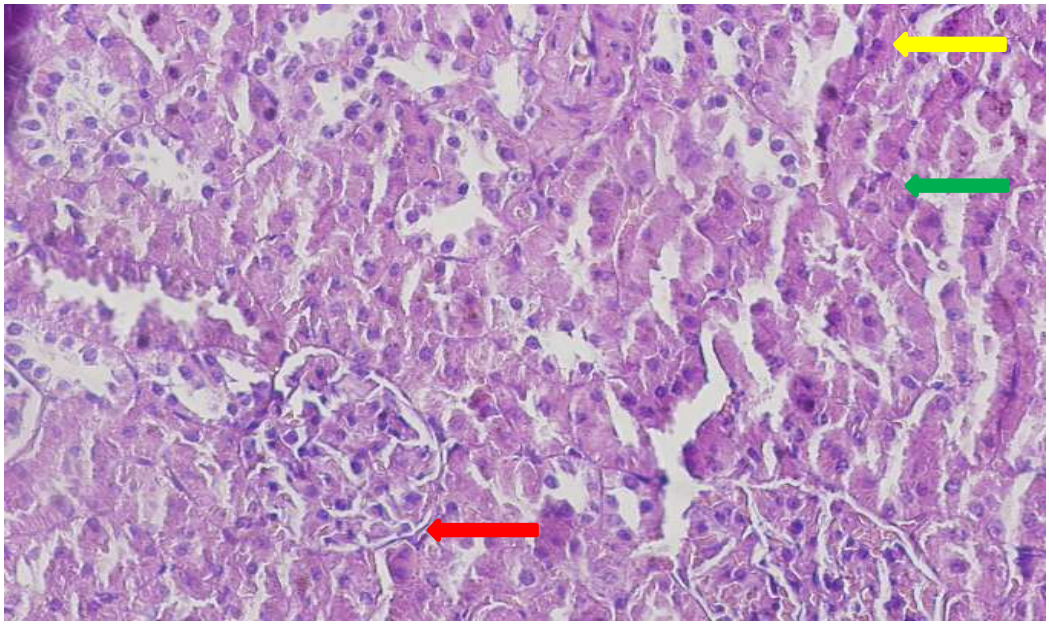

Figure (4.4): Photomicrograph of kidney from rat (ibuprofen + olive oil)- treated showing decrease in congestion (red indicator), mild decrease in degeneration (green indicator), mild decrease in necrosis (yellow indicator). Magnification (400x).

Discussion:

This study aimed to investigate the protective effects of olive oil against Ibuprofen - induced nephrotoxicity in male white rats. In this study, we investigated kidney function tests by measurement of serum urea, creatinine, calcium, phosphate and amylase concentrations.

In this study showed that ibuprofen administration resulted in elevation of creatinine and urea levels in serum as compared with control group that means giving of ibuprofen caused renal dysfunction. That deal with study reported by [12].Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys [13], and their serum concentrations are commonly used as markers of renal toxicity[14-15].

Ibuprofen induced nephrotoxicity by two mechanism: The first mechanism of acute kidney injury (AKI) from NSAIDs (Ibuprofen) is due to reduced renal plasma flow caused by a decrease in prostaglandins, which regulate vasodilation at the glomerular level. NSAIDs disrupt the compensatory vasodilation response of renal prostaglandins to vasoconstrictor hormones released by the body [16]. Inhibition of renal prostaglandins results in acute deterioration of renal function after ingestion of NSAIDs. The second mechanism of AKI is acute interstitial nephritis (AIN), which is characterized by the presence of an inflammatory cell infiltrate in the interstitium of the kidney[17-18].

In histopathological examination of kidney tissue showed that administration of ibuprofen caused that sever congestion, diffuse hydropic degeneration, intraluminal secretion with focal acute tubular necrosis, inflammatory and glomerular congestion seen, this results in agreement with many studies [19-20].

Treatment with olive oil had a nephroprotective effect and that was showed by amelioration of kidney function as evidenced by this study results such improving was investigated by a significant decrease in levels of serum urea and creatinine and these results deal with study by [21].

Our study showed that there was a significant increase in serum glucose concentration of ibuprofen group rats and that is showed ibuprofen stimulation of gluconeogenesis that is agree with study , Hyperglycemia obligates large amounts of additional water to extracellular spaces by osmotic-induced intracellular water loss. This glucose-induced increase in extracellular water dilutes plasma sodium such that there is a 1 mEq/L decrease in plasma sodium concentration for every 62 mg/dL increase in serum glucose concentration. The serum glucose levels were decreased by giving of olive oil. It contains (MUFA), which may regulate blood glucose level by enhancing secretion of glucagon-like peptide-1 (GLP-1) from intestinal cells. GLP-1 is the potent anti-hyperglycemic hormone, which stimulates the proliferation and differentiation of insulin secreting β-cells, glucose - dependent insulin secretion, restores glucose sensitivity of pancreatic β-cells and also suppress glucagon secretion [22- 23.]

In our study we noticed that there was significant increase in serum amylase that deal with study. Serum amylase is not specific to pancreatitis and can also be increased in other abdominal pathologies such as small bowel obstruction, mesenteric ischaemia, perforated gastric ulcer, renal failure and even ectopic pregnancies[24]**.** But the serum amylase levels improved by giving of olive oil and that was deal with several studies[25].

In our study we showed that giving of ibuprofen caused renal toxicity resulting in renal function damage that was caused decrease in level of serum calcium. Serum calcium can be decreased as result of renal failure[26].

Olive oil was increased level of serum calcium and that deal with, olive oil was effective in preventing ovariectomy-induced hypocalcemia in the Olive-OVX rats. Olive oil enhances intestinal absorption of calcium[27]**.**

In this study, we observed a high level of phosphatase due to the renal function damage in kidney that agree with (DiPalma et al., 1996)[28]. But the level was decreased by giving olive oil with ibuprofen and that deal with (Mohammed et al., 2017) [29].

Conclusion:

In the present study urea, creatinine, calcium, phosphate, and amylase concentration , it was concluded that olive oil has significant nephroprotective effect against ibuprofen-induced renal toxicity in female rats.

References:

1. Aprioku, J. S., Uche F. I.Renal. (2013); Effects of non-steroidal anti -inflammatory drugs in Albino Rats. British Journal of pharmaceutical Research 3(3): 314- 325.

2. Burke A., Smyth E. M., FitzGerald G. A. (2006); Analgesic-antipyretic agents: pharmacotherapy of gout. In: Brunton L. L., Lazo J. S., Parker K. L., editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill; Medical Publishing Division, 11: 629- 651.

3. Vane JR. (1971); Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nature New Biol. 231: 232-5.

4. Reynolds EF. (1982); Aspirin and similar analgesic and anti-inflammatory agents. Martindale. The Extra Pharmacopoeia. 28 Ed, 15: 234-82.

5. Neugarten J, Kozin A, Cook K. (1989); Effect of indomethacin on glomerular permselectivity and hemodynamics in nephrotoxin serum nephritis. Kidney Int. 36:51-6.

6. Efrati S, Berman S, Siman-Tov Y, Lotan R, Averbukh Z, Weissgarten J, Golik A. (2007); Nacetylcysteine attenuates NSAID-induced rat renal failure by restoring intrarenal prostaglandin synthesis. Nephrol Dial Transplant. 22:1873-81.

7. Waterman E, Lockwood B. (2007); Active components and clinical applications of olive oil. Alternative Medicine Review. 12:331-43.

8. Gilani AH, Khan AU, 1. Shah AJ. (2005); Blood pressure lowering effect of olive is mediated through calcium channel blockade. Int J Food Sci Nutr 56:613- 620.

9. Owen RW, Giacosa A, Hull WE. (2000); Olive oil consumption and health: the possible role of antioxidants. Lancet Oncol , 1:107-112.

10. McQuay, H. J.; Moore, R.A.(2007); Dose– response in direct comparisons of different doses of aspirin, ibuprofen and paracetamol (acetaminophen) in analgesic studies, British Journal of Clinical Pharmacology, 63(3), 271-278.

11. Bouchefra, A. and T. Idoui. (2012); Nutritional effect of virgin olive oil on growth performance, plasma lipids and endogenous microflora of wistar rats. Techniol. Laboratoire, 7: 1-7.

12. Jonah, S. A., Lucky, L. N., Cecilia, N. A. (2014); Evaluation of Toxicological Profile of Ibuprofen in Wistar Albino Rats, American Journal of Biomedical Sciences 6(1), 32-40.

13. Gaspari, F.; Perico, N.; Matalone, M.; Signorini, O.; Azzollini, N.; Mister, M.; Remuzzi, G. (1998); Precision of plasma clearance of iohexol for estimation of GFR in patients with renal disease, Journal of American Society of Nephrology, 9:310-313.

14. Perrone, R.; Madias, N.; Levy, A. (1992); Serum creatinine as an index of renal function: New insights into old concepts, Clinical Chemistry, 38:1933-1953.

15. Traynor, J.; Geddes, C. C.; Fox, J. G. (2006); How to measure renal function in clinical practice, British Medical Journal, 333:733-737.

16. Whelton, A. (1999); Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiological foundations and clinical implications. The American Journal of Medicine, 106:

17. Ulinski, T.; Guigonis, V.; Dunan, O.; Bensman, A. (2004); Acute renal failure after treatment with nonsteroidal anti-inflammatory drugs. Eur. J. Pediatr, 168: 148- 150.

18. Dixit, M.; Nguyen, C.; Carson, T.; Guedes, B.; Dixit, N.; Bell, J.; Wang Y.(2008); Non-steroidal antiinflammatory drugs-associated acute interstitial nephritis with granular tubular basement membrane deposits. Pediatr Nephrol, 23: 145-148.

19. Gokcimen A, Akdogan M and Karaoz E. (2000); Structural and biochemical changes in liver and renal tissues induced by an acute high dose of diclofenac sodium in rats. *Bio Med Res*., 11; 293 – 302.

20. Gokcimen A, Aydin G, Karaoz E, Malas MA and Oncu M. (2001); Effect of Diclofenac sodium administration during pregnancy in the postnatal blood *Fetal Diagn Ther*16; 417 – 422.

21. Rashid F, Kaleem M, Sheema, Bano B. (2005); Comparative effect of olive oil and fish oil supplementation in combating gentamicin induced nephrotoxicity in rats. Indian J Clin Biochem.,20(1):109-14.

22. Doyle ME, Egan JM. (2007); Mechanisms of action of glucagon-like peptide 1 in the pancreas. Pharmacol Ther., 113(3):546-93.

23. Nauck MA, Meier JJ. (2005); Glucagon-like peptide 1 and its derivatives in the treatment of diabetes. Regul Pept., 128(2):135-48.

24. Philip S. P., Parveen J. (2012); Clinical data interpretation for medical finals .core surgical, Royal national or thopedic .

25. José L. Quiles M. Carmen Ramírez-Tortosa and Parveen Y. (2006); Antioxidant Properties of Olive Oil Phenolics, Olive Oil and Health 1: 112-116.

26. Catherine C. G., John H. Ronaldo T. (2013); Different Diagnosis for physical therapists, screening for referral. 978-0- 323-47849-6.

27. Campos MS, López-Aliaga I, Barrionuevo M, Lisbona F, Coves F. (1989); Nutritive utilization of calcium in rats: effects of dietary fat components and vitamin D3 on intestinal resected rats. J Nutr Sci Vitaminol. 35:511–521.

28. DiPalma JA, Buckley SE, Warner BA. (1996); Biochemical effects of oral sodium phosphate. Dig Dis Sci 41(4):749–53.

29. Mohammed A, Shaik R, Bilal A.T. (2017); Antiurolithic effect of olive oil in a mouse model of ethylene glycol-induced urolithiasis. National Institutes of Health, 58(3): 210–216.