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RESEARCH ARTICLE

SERUM FETUIN- A LEVEL AND RENAL IMPAIRMENT INDUCED BY OBESITY IN COMPARISON WITH RENAL IMPAIRMENT INDUCED BY GENTAMICIN IN RATS.

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Abstract

Aim of the work: is to investigate the relation between serum fetuin-A level and renal impairment induced by obesity in comparison with that induced by gentamicin in rats and to determine the effect of fenofibrate treatment. Materials and Methods: 58 male rats were divided into four groups Control group: 15 rats were fed standard chow diet. Obese group 13 rats were fed a High fat diet (HFD). Then, albumin/ creatinine ratio (ACR) was done and according to the result of this test, this group was diagnosed as obese group with renal impairment. Fenofibrate treated obese group: 15 rats were fed a HFD along with fenofibrate. Gentamicin induced renal impairment group: 15 rats were fed stander chow diet. In the last eight days they were injected intraperitoneally with gentamicin. Body mass index (BMI) was measured. Urine was collected albumin, creatinine were measured in urine, blood was collected and serum urea, creatinine, fetuin-A, adiponectin, tumor necrosis alpha (TNF-α), malondialdehyde (MDA), reduced glutathione, fasting glucose, insulin, HOMA IR, lipid profile, calcium, phosphorus and solubility product were measured. Results: Fetuin –A was significantly increased in obese group with renal impairment. This group also showed significant increase in ACR, serum urea, creatinine and phosphorus. Also, there was positive correlation between serum fetuin-A and BMI, ACR with a negative correlation between it and adiponectin. Also, it showed significant decrease in adiponectin, reduced glutathione and HDL and significant increase in BMI, TNF-α, MDA, Insulin, HOMA IR, triglyceride, LDL. However, fenofiberates intervention was associated with significant correction of all these parameters. In gentamicin induced renal impairment group, there was only significant increase in ACR, serum urea, creatinine, TNF-α, MDA and significant decrease in reduced glutathione. Fetuin-A shows no significant change when compared to control group. Conclusion: Fetuin-A may be associated with obesity-related renal impairment. So, more researches should done to investigate whether reduction of serum fetuin-A may hamper the development and progression of obesity-related complications or not. Also fenofibrate is found to be protective to renal impairment with obesity.

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Introduction:-

There are few studies investigating the pathophysiology of obesity and its early effects on kidney structure and function ***(Kovesdy et al., 2017)***. Fetuin-A or α2 Heremans-Schmid glycoprotein (AHSG) is a glycoprotein secreted by the liver and adipose tissue. Fetuin-A is a systemically-acting inhibitor of ectopic calcification. There are very limited data available concerning fetuin-A in obesity and especially in obese with chronic kidney disease (CKD) ***(Wagner et al., 2017)***. Clinically relevant cut points of Fetuin-A concentration in obesity and CKD are currently unknown ***(Trepanowski et al., 2015)*** Also the mechanisms that underlie the relationship between Fetuin-A and obesity-related renal complications is not fully investigated ***(Hunley et al., 2010)***.

Understanding the mechanisms linking obesity and CKD is important not only because of the societal health burden of both conditions but also to understand underlying mechanisms that may lead to new strategies to treat or prevent CKD and its associated co-morbidities ***(Targher et al., 2011)***. Therefore, the aim of this work is to investigate the relation between serum fetuin-A level and renal impairment induced by obesity in comparison with that induced by gentamicin in rats and to determine the effect of treatment with fenofiberates.

Materials and Methods:-

The present work was carried out on 58 male Albino rats of local strain aged (24-28 weeks) weighted (150-170g). The rats were housed in an isolated animal cages, in a standard animal laboratory room with free access to water and food all over the period of the work and kept at room temperature (22-25)°c, at 12-h light-dark cycle. All procedures were accepted by ethical committee of faculty of medicine by code no (30549/10/15), Tanta University.

The rats were divided randomly into four main groups as follow:

1. ***Control group*** (15 rats): were fed standard chow diet for 24 weeks.
2. ***Obese group*** **(**13 rats**)**: Were fed a HFD for 24 weeks ***(Buettner et al., 2007)*** After that, rats of this group were exposed to preliminary test (urine albumin to creatinine ratio **(**ACR≥30mg albumin/g creatinine) and according to the result of this test, this group was diagnosed as ***obese group with renal impairment***
3. ***Fenofibrate treated obese group*** (15 rats): were fed a high fat diet along with fenofibrate **(100 mg/kg/d)** (by oral gavage) for 24 weeks ***(Kostapanos et al., 2013)***.
4. ***Gentamicin induced renal impairment group*** (15 rats): were fed stander chow diet for 24 weeks. In the last eight days they were injected intraperitoneally with Gentamicin at dose **(80 mg/kg/d)** ***(Kumar et al., 2000)***. After that, rats of this group were exposed to preliminary test (urine albumin to creatinine ratio **(ACR).** Renal impairment was determined by ACR≥30mg albumin/g creatinine.

At the end of the experiment: urine was collected in a metabolic cage. Urinary albumin ***(Baure, 1982)*** and Urinary creatinine ***(Baure, 1982)*** concentration were measured and *urine albumin to creatinine ratio (ACR)* was calculated
Then, rats were anaesthized by 0.1 ml intraperitoneally of 1% sodium barbiturate. Body weight and body length (nose-anus length) were measured for all groups then Body mass index (BMI): was calculated ***(Novelli et al., 2007)***. The anaesthized rats were sacrificed by decapitation and blood samples were collected in clean plastic test tubes, and centrifuged at 3000 rpm for 15 minutes and the separated sera were then transferred into clean cuvette tube stored at -30^{o}C for estimation of the following parameters:

Serum creatinine level ***(Bartles et al., 1972)***, Serum Urea level ***(Fawcett and Soctt, 1970)***, Serum fetuin-A **(*Roos et al., 2010)***, Serum adiponectin levels ***(Tsao et al., 2003)***, Serum TNF-α level ***(Brouckaert et al.,1993)***, Serum malondialdehyde (MDA) ***(Ohkawa et al.,1979)***, Serum reduced glutathione level ***(Beutler et al., 1963),*** Serum fasting glucose level ***(Tietz 1986),*** Serum fasting insulin level ***(Chevenne et al., 1994)***, HOMA IR was calculated ***(Matthews et al., 1985),*** Serum HDL ***(Grove, 1979),*** Serum LDL***(Assmann et al., 1984)***, Serum triglycerides ***(McGowan et al., 1983)***, Serum phosphorus level ***(Daly and Ertingshausen, 1972)***, Serum calcium level ***(Harold et al., 1966)*** and Solubility product was calculated ***(Gimenez et al., 1987)***. Then, the sacrificed animals were packed in special package according to safety precautions and infection control measures.

Statistical analysis:-

Results were expressed as Mean ± SD and all statistical comparisons were made by means of one-way ANOVA test, followed by Tukey's post hoc analysis, and p values less than 0.05 were considered statistically significant. Analysis was performed by statistical package for the social science software (SPSS version 22.0). **Pearson correlation**

coefficient (Pearson r test): was calculated to measure the strength and relation between variables. **r** = $(-1\ to\ +1)$.

1. -1 means there is a strong negative correlation
2. +1 means that there is a strong positive correlation
3. 0 means that there is no correlation (this is also called zero order correlation).

Results:-

The result of this work revealed that: Fetuin –A was significantly increased in ***Obese group with renal impairment***. Together with significant decrease in adiponectin, reduced glutathione and HDL and significant increase in BMI, TNF-α, malondialdehyde (MDA), Insulin, HOMA IR, triglyceride, LDL, however the fasting glucose and calcium levels are not affected.

In addition to the above factors contributing to obesity ***obese group with renal impairment*** showed significant (P≤0.05) increase in ACR, serum creatinine, urea, phosphorus and solubility product Also, there was positive correlation between serum fetuin-A and BMI, ACR with a negative correlation between it and adiponectin figure (1,2,3).

On other hand***, fenofibrate treated obese group*** showed significant increase in serum adiponectin, reduced glutathione and serum HDL and significant decrease in BMI, TNF-α, malondialdehyde, insulin, HOMA IR, LDL and triglycerides and significant decrease in serum fetuin-A as compared to ***obese group with renal impairment.***
Also, ***fenofibrate treated obese group*** showed significant decrease in ACR, serum creatinine, urea, phosphorus and solubility product as compared to ***obese group with renal impairment*** and ***gentamicin induced renal impairment group***. Also, there was positive correlation between serum fetuin-A and BMI, ACR with a negative correlation between it and adiponectin figure (4,5,6).

Gentamicin induced renal impairment group showed significant increase in ACR, serum TNF-α and malondialdehyde, serum creatinine, urea, phosphorus, solubility product, and significant decrease in serum reduced glutathione as compared to control group. Fetuin-A shows no significant change when compared to control group.

Table 1:- Serum creatinine, urea and ACR among studied groups (Mean value ±SD)

Groups / ***Parameters***	***Control group (n=15)***	***Obese Group with renal impairment (n=13)***	***Fenofibrate treated obese group(n=15)***	***Gentamicin induced renal impairment group (n=15)***	***F value***	***P value***
ACR (mg albumin/g creatinine)	5.0±0.80	129.15±19.63[ac]	6.53±1.01[bd]	128.65±15.53[ac]	513. 55	0.00 (P≤0.05)
Serum Creatinine (mg/dl)	0.55±0.08	1.78±0.24[ac]	0.62±0.07[bd]	1.87±0.14[ac]	337.79	0.000 (P≤0.05)
Serum Urea (mg/dl)	25.20±5.34	75.31±6.18[ac]	26.93±1.48[bd]	77.33±4.28[ac]	501.88	0.000 (P≤0.05)

1. [a] P≤0.05 versus control group
2. [b] P≤0.05 versus obese group with renal impairment.
3. [c] P≤0.05 versus fenofibrate treated obese group
4. [d] P≤0.05 versus gentamicin induced renal impairment group.

Table 2:- BMI, Fetuin-A, Adiponectin, TNF-α, Malondialdehyde and reduced glutathione among studied groups (Mean values ±SD)

Groups / ***Parameters***	***Control group (n=15)***	***Obese group with renal impairment (n=13)***	***Fenofibrate treated obese group (n=15)***	***Gentamicin induced renal impairment group(n=15)***	***F value***	***P value***
BMI (g/cm2)	0.56±0.07	0.82±0.08[acd]	0.63±0.04[b]	0.57±0.08[b]	41.96	0.000 (P≤0.05)
Fetuin-A (ng/ml)	87.97±3.06	110.78±5.85[acd]	89.62±4.24[b]	83.89±3.74[b]	84.00	0.000

						(P≤0.05)
Adiponectin (mg/l)	23.78±2.66	11.75±1.09[acd]	23.60±2.76[b]	23.49±3.01[b]	84.535	0.000 (P≤0.05)
serum TNF α (ng/l)	2.81±0.42	18.19±1.12 [ac]	2.75±0.39[bd]	17.26±1.55 [ac]	891.33	0.000 (P≤0.05)
Serum Malondialdehyde (mg/dl)	1.37± 0.16	2.68± 0.30 [ac]	1.57± 0.33[bd]	2.48± 0.21 [ac]	69.30	0.000 (P≤0.05)
Serum reduced glutathione (mg/dl)	2.88±0.35	1.36±0.35 [ac]	2.69±0.56[bd]	1.42±0.18 [ac]	61.426	0.000 (P≤0.05)

[a] P≤0.05 versus control group.
[b] P≤0.05 versus obese group with renal impairment.
[c] P≤0.05 versus fenofibrate treated obese group
[d] P≤0.05 versus gentamicin induced renal impairment group

Table 3:- Fasting Glucose, Insulin and HOMA IR among studied groups (Mean values ±SD)

Groups ***Parameters***	***Control group (n=15)***	***Obese group with renal impairment (n=13)***	***Fenofibrate treated obese group (n=15)***	***Gentamicin induced renal impairment group (n=15)***	***F value***	***P value***
Fasting Glucose (mg/dl)	79.59±8.54	82.84±3.77	76.47±7.59	77.07±6.67	2.85	0.031
Fasting insulin level (µIu/ml)	11.92±1.93	26.66±3.44[acd]	11.62±2.08[b]	10.60±1.32[b]	162.89	0.000 (P≤0.05)
HOMA IR	2.34±0.50	5.44±0.69[acd]	2.18±0.41[b]	2.02±0.31[b]	176.64	0.000 (P≤0.05)

[a] P≤0.05 versus control group.
[b] P≤0.05 versus obese group with renal impairment.
[c] P≤0.05 versus fenofibrate treated obese group
[d] P≤0.05 versus gentamicin induced renal impairment group

Table 4:- HDL, LDL and TG among studied groups (Mean values ±SD)

Groups ***Parameters***	***Control group (n=15)***	***Obese group with renal impairment (n=13)***	***Fenofibrate treated obese group (n=15)***	***Gentamicin induced renal impairment group (n=15)***	***F value***	***P value***
HDL (mg/dl)	50.18±2.04	34.20±5.43[acd]	50.67±2.83[b]	52.79±3.33[b]	110.63	0.000 (P≤0.05)
LDL (mg/dl)	89.17±3.65	118.46±3.49[acd]	90.85±2.20[b]	89.51±3.76[b]	371.13	0.000 (P≤0.05)
TG (mg/dl)	146.74±8.45	168.53±4.85[acd]	146.12±5.48[b]	145.63±5.54[b]	60.86	0.000 (P≤0.05)

[a] P≤0.05 versus control group.
[b] P≤0.05 versus obese group with renal impairment.
[c] P≤0.05 versus fenofibrate treated obese group
[d] P≤0.05 versus gentamicin induced renal impairment group

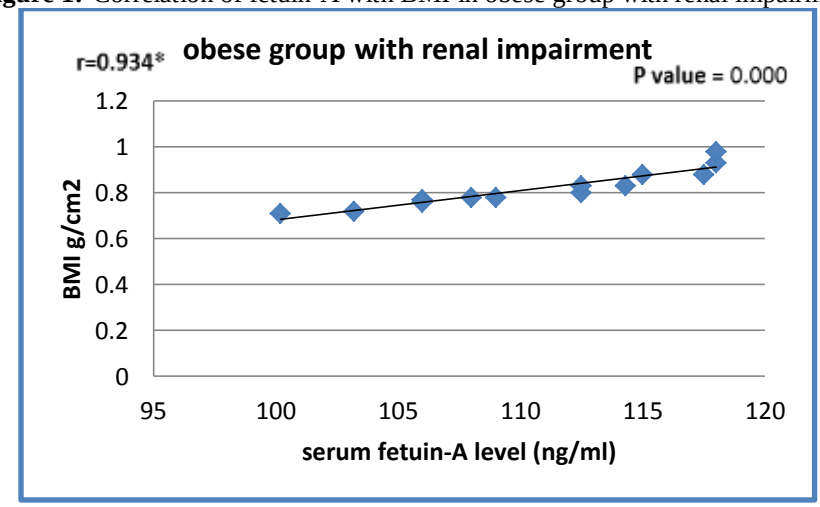
Table 5:- Serum phosphorus, serum calcium and solubility product among studied groups (Mean values ±SD)

Groups ***Parameters***	***Control group (n=15)***	***Obese group with renal impairment (n=13)***	***Fenofibrate treated obese group (n=15)***	***Gentamicin induced renal impairment group (n=15)***	***F value***	***P value***
Serum phosphorus (mg/dl)	3.60 ± 0.54	11.36 ± 1.59[ac]	3.56 ± 0.65[bd]	12.84 ± 2.80[abd]	131.65	0.000 (P≤0.05)
Serum calcium	8.88 ±	9.11 ± 0.47	8.93 ± 0.33	8.92 ± 0.74	0.377	0.258

(mg/dl)	0.60					
Solubility product (CaxP)	31.89 ± 4.29	103.52 ± 15.18[ac]	31.76 ± 5.67[bd]	115.39 ± 31.05[abd]	97.27	0.000 (P≤0.05)

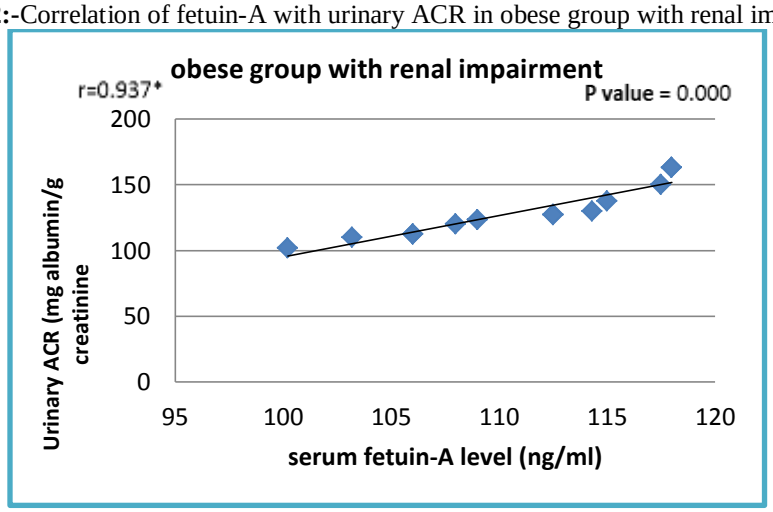
[a] P≤0.05 versus control group.
[b] P≤0.05 versus obese group with renal impairment.
[c] P≤0.05 versus fenofibrate treated obese group
[d] P≤0.05 versus gentamicin induced renal impairment group

Figure 1:-Correlation of fetuin-A with BMI in obese group with renal impairment



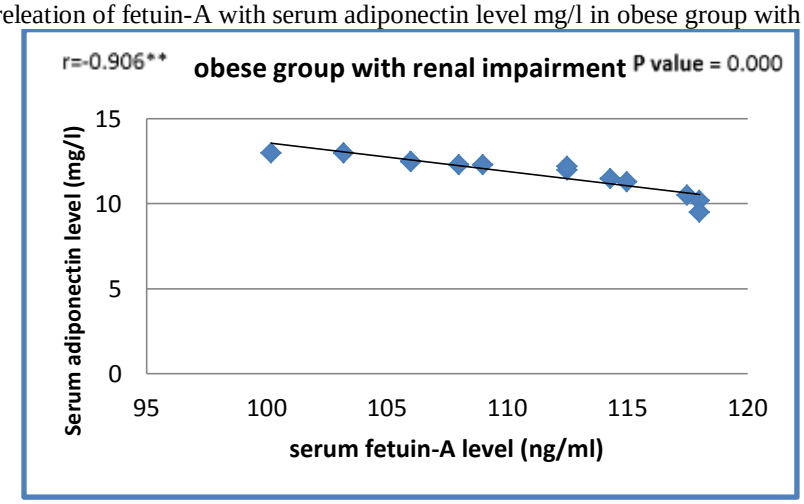
*denotes statistical significance at P≤0.05 (positive correlation)

Figure 2:-Correlation of fetuin-A with urinary ACR in obese group with renal impairment



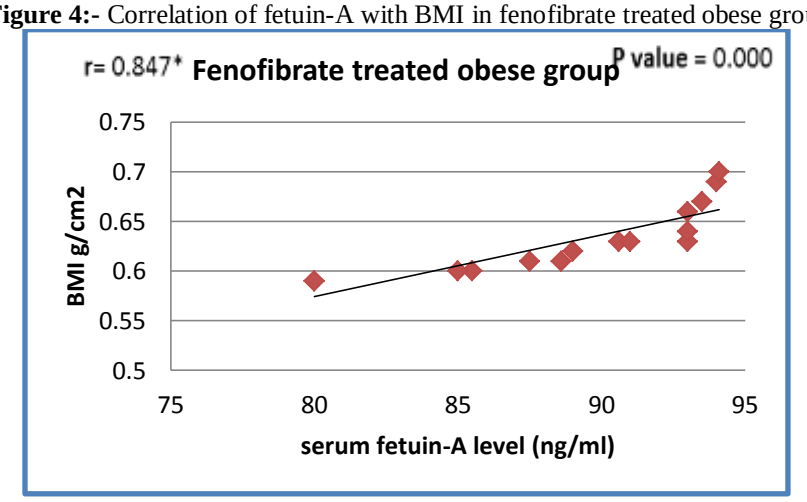
*denotes statistical significance at P≤0.05 (positive correlation)

Figure 3:- Correleation of fetuin-A with serum adiponectin level mg/l in obese group with renal impairment



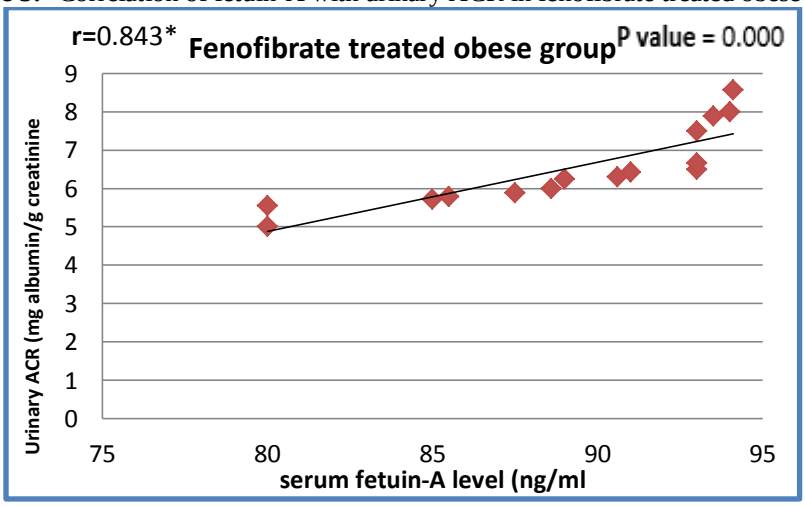
**denotes statistical significance at P≤0.05 (negative correlation)

Figure 4:- Correlation of fetuin-A with BMI in fenofibrate treated obese group



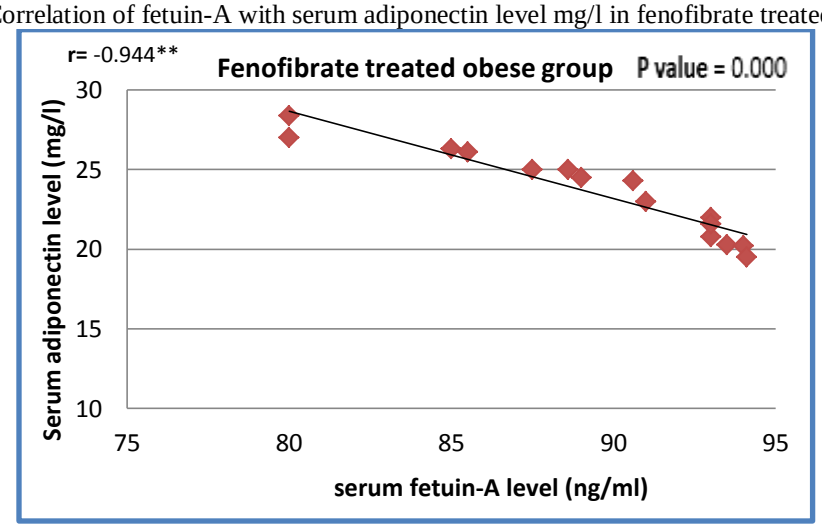
*denotes statistical significance at P≤0.05 (positive correlation)

Figure 5:- Correlation of fetuin-A with urinary ACR in fenofibrate treated obese group



*denotes statistical significance at P≤0.05 (positive correlation)

Figure 6:- Correlation of fetuin-A with serum adiponectin level mg/l in fenofibrate treated obese group



**denotes statistical significance at P≤0.05 (negative correlation)

Discussion:-

There are many pathways by which obesity might contribute to renal disease. The mechanisms leading to both may be interrelated through crosstalk between fat, kidney and liver via fetuin-A ***(Ismail et al., 2012)***.

The factors that link the association between obesity and renal impairment may include increase in hepatokines as Fetuin-A which is significantly increased in ***obese with renal impairment***, fetuin-A make a connection between fat cells and the kidney ***(Huddam et al., 2013)*** this is evidenced by significant positive correlation between fetuin-A and ACR, also the level of fetuin-A is not affected in renal disease induced by gentamicin in (non -obese) animals, indicated that fetuin-A play a role as a co-factor in development of renal impairment associated with obesity and not renal diseases alone.

The relation between fetuin-A and obesity may be bidirectional. Fetuin-A knockout mice are resistant to weight gain on a HFD, this speculates that high Fetuin-A levels may lead to obesity ***(Ismail et al., 2012)***.

High Fetuin-A concentrations observed in this group could be caused by adipocyte dysfunction and fatty liver which may be considered as a cause for the elevated serum Fetuin-A ***concentration (Celebi et al., 2015)*** or it could be due increase in fetuin-A mRNA expression in the liver ***(Ramsay et al., 2017)***. In addition other factors may contribute to renal impairment of this work show significant decrease in adiponectin, this hypoadiponectinemia could be due to significant increase in TNF-α observed in this work reducing the level of adiponectin ***(Henninge et al., 2008)***.

Stefan et al., (2016) reported that fetuin-A strongly induces increase pro inflammatory cytokines TNF-α. ***Tzanavari et al., (2010)*** demonstrated that the liver-derived fetuin-A induced low-grade inflammation with increase in TNF-α. Interestingly the relation between the increase in TNF-α and Fetuin-A are interrelated. Increase TNF-α may also cause increase in fetuin-A ***(Stefan et al., 2016).***

Increase in TNF-α and Fetuin-A may predispose to hyperinsulinemia and significant increase in HOMA IR ***Li et al., (2013)*** concluded that Fetuin-A is considered as an important promoter of insulin resistance, Also pro-inflammatory molecules as TNF-*α* produced by adipose tissue may contribute to the development of insulin resistance

Oxidative stress in obese animals as evidenced by significant decrease in reduced glutathione and increase malondialdehyde could be another possible mechanism inducing insulin resistance ***(Han, 2016)***. It is believed that insulin resistance leads to hyperinsulinemia ***(Ye, 2013)*** when β-islets expand to produce a large amount of insulin in an effort to reduce the elevated blood glucose ***(Sears et al., 2015)***. Insulin resistance and increase pro inflammatory cytokines may lead to dyslipidemia ***(Jung et al., 2014)***. Also fetuin A may be associated with the derangement of visceral adiposity and consequently, dyslipidemia ***(Chen et al., 2009)***. Hypertriglyceridemia is one of the most common quantitative lipid abnormalities in patients with CKD ***(Vaziri and Moradi, 2006).***

In the present work, ***fenofibrate treated obese group showed*** significant improvement of all parameters studied. This could be explained as fibrates increase the oxidation of fatty acids in liver and muscle and hence increase lipoprotein lipase activity and TG clearance ***(Rosenson, 2009)***. fiberats decreased transfer of cholesterol from HDL to VLDL ***(McKeage and Keating, 2011)***. Fenofibrate may also inhibit hydroxyl-methyl-glutaryl coenzyme A reductase activity, thus reducing cholesterol synthesis ***(Evans and Rees, 2002)***; moreover, cholesterol excretion into the bile is also increased by fenofibrate ***(Bijland et al., 2010).***

Also, fenofibrate administration increase adiponectin (insulin sensitizer) in adipose tissue ***(Tarantino et al., 2017).*** Moreover, fenofibrate can affect inflammation, oxidation and apoptosis probably because it is an agonist of PPAR-α (the transcription factor which regulates genes involved in lipid metabolism ***(Varga et al., 2011)*** When fenofibrate activates PPARα, TG hydrolysis and HDL synthesis increase, also it causes improvement insulin resistance ***(Yan et al., 2014)***.

In the present work, the results of ***gentamicin induced renal impairment group*** showed significant increase in ACR, serum TNF-α and malondialdehyde, serum creatinine, urea, phosphorus, solubility product, and significant decrease in serum reduced glutathione as compared to *control group*.

However, it showed no significant changes in BMI, adiponectin, fetuin-A, insulin, HOMA IR, HDL, LDL and serum triglycerides, as compared to *control group.*

Gentamicin induces oxidative stress in renal tubular cells ***(Keiko, 2016)***. Gentamicin interferes with the phosphorylation process and diminishes levels of ATP in renal tubular cells. This leads to reactive oxygen species(ROS) induced cell death ***(Oung et al.,2015).***The interaction of ROS with cellular components may result in damage to DNA, cellular proteins and lipids by inhibition of the electron transport chain and subsequent ATP production ***(Keiko,2016)***.Moreover, increased hydrogen peroxide generation and lipid peroxidation, concomitant with a decreased glutathione level in the renal cortex, were observed in gentamicin-treated rats ***(Jaikumkao et al., 2016)***.The nephrotoxic effects caused by gentamicin are characterized by augmentation of urea and creatinine levels along with severe proximal renal tubular necrosis which ultimately leading to renal failure ***(Mardani et al., 2013).***

Conclusion:-

Fetuin-A may be associated with obesity-related renal impairment. Also fenofibrate is found to be protective to renal impairment with obesity

Recommendation:-

More researches should done to investigate whether reduction of serum fetuin-A may hamper the development and progression of obesity-related complications or not.

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