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# Quercetin impact against psychological disturbances induced by fat rich diet

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**Abstract**: Obesity is a metabolic disease which promotes by consuming fat rich diet. The consequences may include leptin resistant and initiation of metabolic syndromes. Beside the fact, obesity has psychological impacts it act as a signal of depression by stimulating endogenous stress stimulation. Quercetin is a natural polyphenol, considered as nutraceutical agent which produce antioxidant effects. It is reported to promote energy expenditure and protective mechanism against obesity. This presented work was designed to observe the effects of quercetin on high fat diet treated obese animals with impaired psychological behavior. The study comprised on 36 animals, divided in to different groups as follow: I Normal Diet and II. High fat diet. After the induction of obesity both groups divided in to further three subgroups as control, Quercetin and sertraline. Food intake and body weight along with behavioral analysis for four weeks were done for the assessment of anti-obesity and antidepressant effects of quercetin. The results showed the effective treatment of quercetin in obese animals, it significantly reduced the food intake and body weight of animals. The behavioral test showed the increased locomotor activity in the activity box and improved psychological behavior in quercetin-treated rats in the open field and light-dark box. It is concluded from the present study that quercetin exhibits the ameliorative effects against obesity and associated neuroendocrine alterations.

**Keywords**: Energy expenditure, obesity, psychological behavior, quercetin.

## INTRODUCTION

Obesity is now becoming a common consequence of the emerging fat-rich diet trend in developing countries which promotes leptin resistance and leads to initiating metabolic syndromes. Many studies demonstrate the psychological impacts of obesity and considered it as the signal of depression as it induces endogenous stress and promotes the HPA axis alteration (Farhan *et al.,* 2015). One of the most considerable strategies to treat depression is to inhibit monoamine oxidase and enhance the level of monoamine neurotransmitters such as serotonin and dopamine. However this artificially high level of neurotransmitters can cause alteration in metabolic activities (Cascade *et al.,* 2009). Laboratory animals are helpful to evaluate the impacts of dietary composition and impacts on metabolism and neuro-endocrinology. There are numerous strains of animals including Albino Wistar, Sprague-Dawley and Long-Evans. Many studies use these animals to produce a model of obesity for the evaluation of the responsiveness of obesity to different drug-induced mechanisms (Kobori *et al.,* 2011; Lai *et al.,* 2011).

Quercetin is assumed to produce a protective mechanism against adipogenesis, it is found as a stimulating agent of energy expenditure (Stewart *et al.,* 2008). It is reported to reduce lipogenesis and fat accumulation by suppressing hyperinsulinemia and dyslipidemia (Rivera *et al.,* 2008). Quercetin is also effective in the suppression of inflammatory interleukin markers. Many reports are supporting evidence of the effectiveness of quercetin in reducing weight gain however this effect is contrary as Wein, 2010, reported no changes in body weight after the treatment of quercetin. Therefore, the current study is intended to observe the efficacy of quercetin on High fat diet-treated rats as well as on high fat diet-induced psychological disturbances including anxiety and depression in comparison with sertraline; a standard antidepressant drug.

## MATERIALS AND METHODS

The study was designed on 36 male Albino Wistar rats weighing from 180 to 200 gm and approved by Board of Advanced Studies and Research (BASR), University of Karachi with letter number 03346/Sc. They were acclimatized for three days under control environment including 22±2ºC temperature, 12 hour light-dark cycle, and 30-35% humidity. All animals were housed individually and received normal standard diet before experimentation then they were allocated in two groups as Normal fat diet (NFD) and High fat diet (HFD). Animals of high fat diet group received fat rich diet for 5 weeks to produce obesity model (table 1). After the induction of obesity both groups divided in to further three groups i) NFD control ii) NFD Quercetin iii) NFD sertraline iv) HFD control v) HFD Quercetin vi) HFD sertraline. Quercetin was given at the dose of 100 mg/kg orally and the doses of sertraline were 5mg/kg by the same route of

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administration. Behavioral analysis was done for a period of 4 weeks to the assessment of anti-obesity and antidepressant effects of quercetin.

***Food intake and body weight***

Food intake and body weight described earlier (Farhan *et al.,* 2015) were recorded every week of treatment.

***Activity box test***

Ambulatory behavior was tracked in a familiar environment, or Home cage described previously (Haleem *et al.,* 2007). Each animal was introduced in the center of activity cage, and the count of cage crossovers was recorded over a 10-minute period.

***Open field test***

Monitoring of exploratory activity in novel environment is generally known as Open field activity test. The design of the apparatus was described by Haleem *et al.,* 2007, the number of squares crossed was recorded over 5 minutes.

***Light dark box test***

To observe the anxiolytic and antidepressant effects of quercetin, the light-dark box test was applied which was described earlier (Imaizumi *et al.,* 1994). The number of entries and the time taken with all four paws in the light chamber were tracked to determine a cutoff period of 5 minutes.

***Estimation of total cholesterol***

To determine the level of cholesterol enzymatic hydrolysis can be done by using quinonamine indicator which can be produced from hydrogen peroxide and 4-aminoantiprine. The reaction process in the presence of phenol and peroxidase. 10µl sample and 10µl standard added in separate test tubes.10µl distilled water, all tubes were then added with 1000µl reagent. Tubes were then mixed well and incubated at 20-25ºC for approximately 10 minutes. The observed were checked at 500nm against blank.

***Estimation of high-density lipoprotein***

The addition of phosphotungstic acid and magnesium ions can be used to precipitate the fraction of LDL-c, VLDL-c and chylomicrons upon centrifugation. The level of HDL-c in remaining fraction can be determined. The reagents were used as R1: tungstophosphoric acid (0.55 mmol/l) + $MgCl_2$ (25 mmol/l). R2: Dilution R1 with distilled water (4:1). The sample and standard (200µl) were separately taken in test tubes. R2 was added in each tube with volume 500µl, mixed well and left for 10 minutes at room temperature. Centrifugation was done at 4000 rpm for 10 minutes. Supernatant was collected and measurement was done as described in total cholesterol estimation method (Friedewald, 1972).

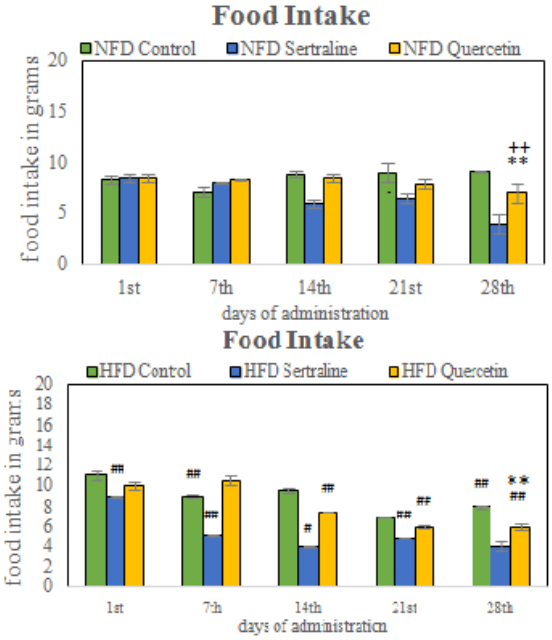
## STATISTICAL ANALYSIS

Results are expressed as mean ± SD. Differences between the individual mean values of different groups were analyzed by three-way ANOVA (analysis of variance) of SPSS (Version 21). Comparison between mean value of different groups was analyzed by Newman-Kuels test, $p<0.05$ and $p<0.01$ were considered as significant.

## RESULTS

***Effects of quercetin on food intake***

The effects of repeated monitoring ($F=24.806$; df=4, 33; p0.01), medications ($F=14.432$; df= 1, 33; $p<0.01$), the effects of the HFD ($F=107$; df= 1, 33; $p<0.01$) and the interaction between all the components ($F=26.08$; df=4, 33; $p<0.01$) were all significant in a three-way ANOVA (repeated measures design). Sertraline treatment significantly reduces food intake in both normal and HFD rats, according to a post-hoc study using the Newman-Keuls test. The hypophagic effects of quercetin was also seen after 3 weeks administration however the effects was not significant (fig.1).




Values are presented as mean ± SD (n=6). Data analyzed by three- way ANOVA and Newman-Kuels test. Significant differences are expressed as **p<0.01 from control, ++p<0.01 from 1st dose, #p<0.05, ##p<0.01 from respective normal diet group.

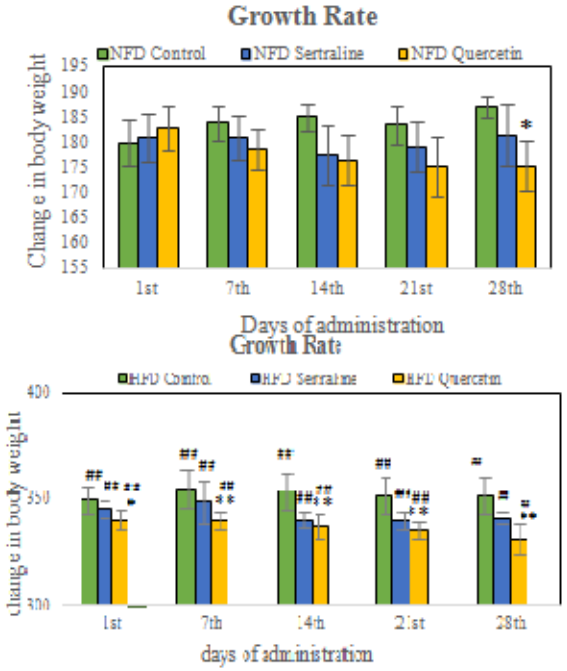
**Fig. 1**: Effects of quercetin on food intake

***Effects of quercetin on growth rate***

The impact of daily administration ($F=10.927$; df= 4, 33; $p<0.01$), medicines ($F=156.89$; df= 1, 33; $p<0.01$), HFD ($F=8128.6$; df= 1, 33; $p<0.01$), and the interaction between all factors ($F=426.855$; df= 4, 33; $p<0.01$) was significant. In NFD rats, post-hoc analysis using the Newman-Keuls test revealed no significant difference in body weight change following quercetin treatment. However, in HFD rats, administration of quercetin significantly reduced the body weight of rats as compared to control animals (fig. 2).

**Table 1**: The composition of diet was based on the recommendation of American Institute of Nutrition (National Research Council, 1995).

| Dietary profile | 10 kcal% fat (control) | | | 60 kcal% fat (experimental Animals) |
|---|---|---|---|---|
| | gm% | Kcal % | Gm % | Kcal% |
| Protein | 19 | 20 | 6 | 20 |
| Carbohydrate | 67 | 70 | 6 | 20 |
| Fat | 4 | 10 | 5 | 60 |
| Total | | 100 | | 100 |




Values are presented as mean ± SD (n=6). Data analyzed by three- way ANOVA and Newman-Kuels test. Significant differences are expressed as *p<0.05, **p<0.01 from control, #p<0.05, ##p<0.01 from respective normal diet group.

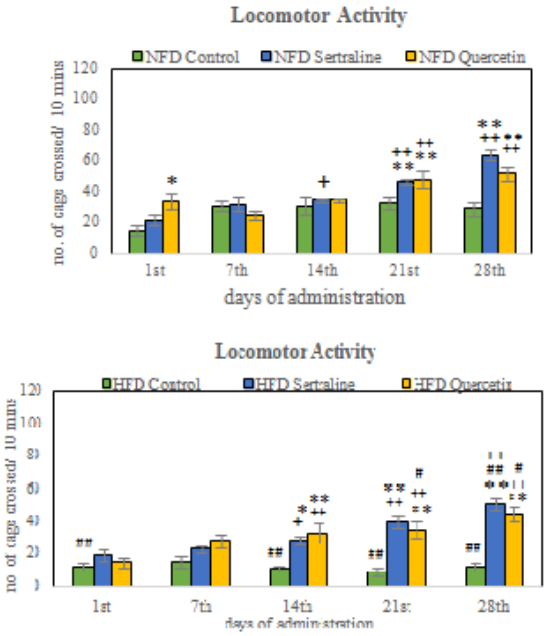
**Fig. 2**: Effects of quercetin on growth rate

***Effects of quercetin on locomotor activity***

The significant effects of daily administration (F=3.883; df= 4, 33; p<0.05), medicines (F=61.389; df=1, 33; p<0.01), HFD induction (F=42.288; df= 4, 33; p<0.05) and the interaction between all these factors (F=19.698; 4, 33; p<0.01) were seen in statistical analysis. Newman-keuls showed that administration of quercetin gradually increased the ambulatory activity of normal rats and high fat diet rats. Significant effects was found after 21st administration in NFD while 14th administration in HFD rats as compared to control group as well as from 1st day administration. The results of quercetin is quite comparable than antidepressant sertraline (fig. 3).

***Effects of quercetin on open field test (Number of square crossed per 5 minutes)***

The statistical results showed that effects of daily administration (F=11.437; df= 4, 33; p<0.01) and drugs (F=38.398; df= 1, 33; p<0.01), treatment (F=56.888; df= 1, 33; p<0.01) and the interaction between all these factor (F=45.288; df= 4, 33; p<0.01) were significant. Post-hoc analysis by Newman-Keuls test showed that quercetin increases the square crossed after 14th and 7th administration in normal and high fat diet rats respectively, the results are comparable with anxiolytic effects of sertraline as there was no significant differences found between sertraline and quercetin, however the results are more significant in HFD rats (fig.4).



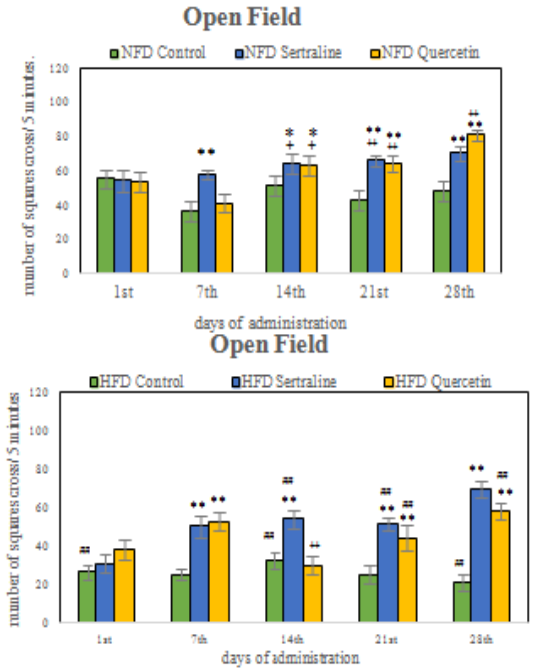

Values are presented as mean ± SD (n=6). Data analyzed by three- way ANOVA and Newman-Kuels test. Significant differences are expressed as *p<0.05, **p<0.01 from control, +p<0.05, ++p<0.01 from 1st dose, #p<0.05, ##p<0.01 from respective normal diet group.

**Fig. 3**: Effects of quercetin on locomotor activity

***Effects of quercetin in light dark box (number of entries)***

The effects of daily administration (F=4.689; df= 4, 33; p<0.05) and medications (F=8.765; df= 1, 33), the effects of HFD (F=67.177; df= 1, 33; p<0.01), and the interaction between all of these factors (F=36.125; df= 4, 32; p<0.01) were all significant, according to the statistical results. Post-hoc test showed that quercetin increases the number of entries after repeated administration but results are

significant in HFD group after 14th administration (fig. 5a).




Values are presented as mean ± SD (n=6). Data analyzed by three- way ANOVA and Newman-Kuels test. Significant differences are expressed as *p<0.05, **p<0.01 from control, +p<0.05, ++p<0.01 from 1st dose, ##p<0.01 from respective normal diet group.

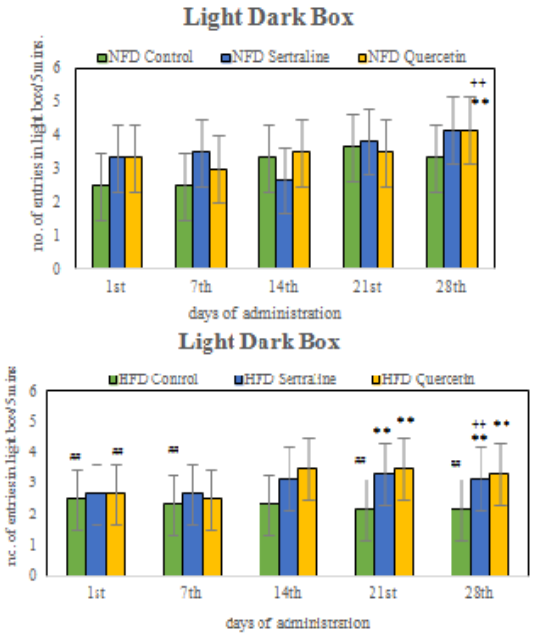
**Fig. 4**: Effects of quercetin on open field

***Effects of quercetin in light dark box (time spent in light box)***

The statistical results showed that effects of daily administration (F=0.415; df= 4, 33) was non-significant, however the effects of drugs (F=42.357; df= 4, 33; p<0.01), treatment (F=131.659; df=1, 33; p<0.01) and the interaction between all factors (F=18.01; df= 4, 33; p<0.01) were significant. Post-hoc analysis revealed SSRI as standard drug for anxiety increases the time spent but the effects are quercetin in quite comparable with antidepressant after 14th administration in both NFD and HFD group. However the normal rats showed more anxiolytic behavior than fat rich diet rats (fig. 5b).

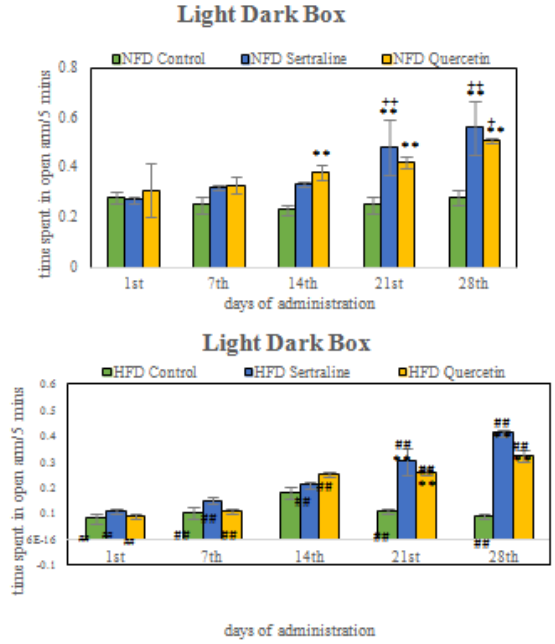
***Effects of quercetin on lipid profile***

Table 2 presents the results of lipid profile. The results of one-way ANOVA shows significant effects of treatment on cholesterol (F=57.388; df= 5, 30; p<0.01), HDL (F=8.434; df= 5, 30; p<0.05). Tukey's test revealed that quercetin significantly (p<0.05) reduced the level of cholesterol in NFD rats as compared to sertraline treated rats. However, the suppressing effects were more significant in HFD group, where the reducing cholesterol level were significant as compared to the control and sertraline group as well as respective NFD treated groups. However, the results of HDL after the administration of quercetin were increased in both NFD and HFD.




Values are presented as mean ± SD (n=6). Data analyzed by three- way ANOVA and Newman-Kuels test. Significant differences are expressed as **p<0.01 from control, +p<0.05, ++p<0.01 from 1st dose, #p<0.05, ##p<0.01 from respective normal diet group.

**Fig. 5a**: Effects of quercetin on light dark box




Values are presented as mean ± SD (n=6). Data analyzed by three- way ANOVA and Newman-Kuels test. Significant differences are expressed as **p<0.01 from control, +p<0.05, ++p<0.01 from 1st dose, #p<0.05, ##p<0.01 from respective normal diet group.

**Fig. 5b**: Effects of quercetin on light dark box.

**Table 2**: Effects of repeated quercetin administration (100mg/kg) on Cholesterol and HDL

| | Cholesterol | HDL |
|---|---|---|
| NFD Control | 73.33±5.2 | 46.16 ± 5.7 |
| NFD Sertraline | 78±6.8 | 50.5 ± 11.5 |
| NFD Quercetin | 69.16 ± 3.7[a] | 58.3 ± 4.2 [a] |
| HFD Control | 98.16 ± 0.75 | 36.1 ± 5.6 |
| HFD Sertraline | 98 ± 0.7 | 42.8 ± 2.4 |
| HFD Quercetin | 82.8 ± 2.5 [a] | 52.16 ± 5.8 [a] |

Values are presented as mean±SD (n=6). Data analyzed by three- way ANOVA and Newman-Kuels test. Significant differences are expressed as [a]$p<0.05$ from control.

## DISCUSSION

Obesity is a serious metabolic illness and despite extensive effort to understand the underlying mechanism, its frequency continues to rise (Gallagher *et al.,* 2000) with other health-related concerns. Psychological behavior has a strong association with dietary nutrients thus, a poor energy dense diet has major impact on emotions and it increases the risk of several mental illness such as anxiety and depression. HPA axis is one of the neuroendocrine system which regulates the responses of stress by regulating the levels of glucocorticoids. Hypothalamus is the main regulator of energy homeostasis, as adipogenesis causes endogenous stress it alters the energy homeostasis and elevate the risk of HPA axis hyper-activation and increasing the risk of depression (Silvestro *et al.,* 2021). Being a most frequent diagnostic psychiatric condition, depression have been extensively studied for evaluation of better treatment modalities. Although there are wide range of available antidepressants to treat depressive symptoms by acting on neurotransmitters metabolism including serotonin dopamine and noradrenaline (María *et al.,* 2013). The action behind this therapeutic action may be the inhibition of high affinity reuptake receptors or by reducing the activity of monoamine oxidases. Unfortunately, most of them have delayed onset of therapeutic action with visible side effects. A valid number of growing evidences have investigated natural compounds with antidepressant potential (Vgontzas *et al.*, 2007).

The increased level of cortisol in depression may be the consequence of increased level of cholesterol in obese individual. Lipids are involve in steroid hormone synthesis, energy storage and they also predominantly regulate the inflammatory markers, they are vulnerable to target by free radicals. The destruction by free radical is appear as lipid peroxidation, which may induce various pathologic events. Quercetin is a bioflavonoid of the flavonol subgroup and a potent antioxidant it is reported to produce many biological effects, initially known to exhibit antimicrobial activity as it inhibits the growth of staphylococcus aureus and showed resistance against many strains of gastrointestinal, respiratory and urinary tract bacteria (Sakakibara *et al.,* 2006). Quercetin possesses the most powerful antioxidant activity among all the flavonoids and resist the progression of Reactive oxygen species (ROS). It also reported to produce defense mechanism against adipogenesis and the inverse psychological effects of adipogenesis such as endogenous depression (Rivera *et al.,* 2008). Hyper-triglycemia in connection with lower level of HDL may induce the pathologic lipid metabolism. Hyper-triglycemia in connection with lower level of HDL may induce the pathologic lipid metabolism. The mechanism of action behind the hypolipidemic effects of quercetin may be the increased energy expenditure and anti-inflammatory effects of the doses. It is also reported to exert immune modulating effects in murine models of autoimmunity (Pothion *et al.,* 2004). Preclinical trials also suggest the ameliorative effects of quercetin on high-fat diet indicating that it has potential action against visceral adipose TNF-α production (Cryan and Sweeney, 2011). The effects of quercetin (100mg/kg) were investigated on high-fat diet as well as high-fat diet induced psychological changes such as depression in comparison with a standard antidepressant. The study was designed on Albino Wistar rats pretreated with high-fat diet for five weeks, after the progression of obesity and depressive symptoms they were treated with quercetin for the next four weeks for the investigation of antidepressant effects of quercetin in comparison with sertraline. The outcomes of the present study revealed that the treatment of quercetin is significantly comparable to results with antidepressants i.e. sertraline. However, the treatment of quercetin is not sufficient to produce effects on food consumption and body weight (fig.1-2). The enhancing effects on ambulatory activity show the significant results of quercetin (fig. 3).

To focus on the anxiolytic activity different models with good validity were used to understand the anxiolytic effects of quercetin. Anxiolytic agents of quercetin has been shown in open field and light dark box (fig.4-5) where the performance is quite comparable with sertraline. These behavioral models are valid for the investigation of anxiety (O'Leary and Cryan, 2013), where the effects of quercetin is reduced the anxiogenic behavior it might be due to the increased level of serotonin and dopamine (Pothion *et al.,* 2004) of animals and the performance are significantly comparable to anti-depressive drug such as sertraline indicated that the quercetin has strong potential to alleviate stress induced behavioral deficits.

Obesity is prone to induced dyslipidemia. The supplementation of quercetin treatment improved the altered lipid level of obese rats (table 2) as compared to control animals. These effects of quercetin are supported by other studies using animal model of the polycystic

ovarian syndrome, dyslipidemia patients and carbon tetrachloride induced hepatotoxic effects in rodents (Jahan *et al.,* 2018).

## CONCLUSION

It is concluded from the present study that quercetin exhibits the ameliorative effects against obesity and associated neuroendocrine alterations. It has significant hypolipidemic effects on obese rats as it reduced the cholesterol levels in obese animals. The energy expenditure is also increased by the treatment of quercetin indicates the modification of the altered obesogenic mechanism. The improved performance of animals in behavioral tests ensure the antidepressant activity of quercetin which is quite comparable with antidepressant medications. Further studies required on clinical and molecule levels for a better understanding of the possible mechanism of quercetin to produce its pharmacological effects. Based on the significant antioxidative and anti-depressive results of quercetin in HFD-induced stress, the later experiments are designed for further evaluation of therapeutic effects of quercetin of different oxidative stress models.

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