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Effect of Piracetam, resveratrol and propylene glycol extract of Royal jelly on behavioral functions and markers of oxidative stress in rats with experimental metabolic syndrome

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Abstract
Background: Oxidative stress (OxS) is a disturbance associated with the pathogenesis of diabetes/pre-diabetes, obesity, cancer, ageing, inflammation, apoptosis, neurodegenerative disorders and cardiovascular diseases. Metabolic syndrome (MetS), also called insulin-resistance syndrome, is a complex of disorders, each of which contributes to cardiovascular risk, associated with imbalance between production and inactivation of reactive oxygen species. In the last years the potential role of OxS in MetS is rapidly evolving.
Methods: The present study was designed to investigate the effect of Piracetam, Resveratrol and propylene glycol extract of Royal jelly on behavioral functions and OxS markers in serum of fructose-drinking rats.
Results: MetS induced by 60% fructose solution in rats leads to impairment of cognitive functions, based on the development of OxS. Royal jelly extract and Resveratrol show pronounced antiamnesic activity in rats with MetS. All studied drugs inhibit reactions of free radical oxidation in varying degree.
Conclusions: This study indicates an important role of OxS in the development of cognitive disorders that appear in consequence of MetS. In experimental model of MetS the largest antiamnesic and antiradical efficacy was observed for propylene glycol extract of Royal jelly.

Keywords: *Metabolic syndrome, Piracetam, Resveratrol, propylene glycol extract, Royal jelly, high fructose fed rats.*

Introduction
Metabolic syndrome (MetS) has become a leading global health problem owing to its association with a high incidence of different diseases, and has significantly contributed to morbidity and mortality around the world. It represents a clustering of metabolic disorders and cardiovascular risk factors including: insulin resistance, hyperinsulinemia, central obesity, glucose intolerance or diabetes mellitus, atherogenic dyslipidemia, hypertension, endothelial dysfunction, prothrombotic and proinflammatory states [1, 2, 3]. In animal models high-fructose diets induce features of MetS including weight gain, insulin resistance, hypertriglyceridemia, and hypertension [4, 5, 6].

Based on different studies, it has been suggested that oxidative stress (OxS) is a common pathway through which various risk factors for several diseases exert their deleterious effects. OxS is a well-recognized mechanism playing important role in many pathological conditions [7], and several human diseases have been closely related to oxidative stress [8]. OxS is associated with pathogenesis of diabetes/pre-diabetes, obesity, cardiovascular diseases, neurodegenerative disorders, cancer, ageing, inflammation, apoptosis, etc. In diabetes OxS decreases insulin secretion from pancreatic β-cells and impairs glucose uptake in skeletal muscles and fat tissues [9]. Violation of intracellular glucose balance due to chronic hyperglycemia promotes intensification of free radical oxidation and inhibition of antioxidant defensive system in brain cells. This could serve as an independent factor that causes damage of neurons or their death. Thus, we can assume that formation of chronic OxS by diet or by genetic alterations may lead to cognitive decline.

Pharmacological therapy is a critical step in management of patients with MetS when lifestyle modifications fail to achieve the therapeutic goals. In this study we examined whether Piracetam, phytoalexin Resveratrol and propylene glycol extract of Royal jelly could ameliorate fructose-induced cognitive impairment and OxS in rats with experimental MetS.
Piracetam (PIR) is one of the most common neuroprotective drugs. In neurological practice it

is prescribed for atherosclerosis, vascular Parkinsonism, and other diseases with symptoms of chronic cerebrovascular insufficiency, which manifest themselves in impaired memory, learning, brain metabolism, and mental capacity [10, 11].

Royal jelly (RJ) is a natural bee product that has beneficial properties for human body (antimicrobial, anti-inflammatory, antiviral, antispasmodic, regenerative, bio stimulating, tonic, anti-aging). RJ is frequently used to treat cardiovascular diseases: ischemia, hypotension, anemia and hypertension. It has positive effects in treatment of atherosclerosis and coronary heart disease [12, 13, 14].

Resveratrol (RVT) is a phytoalexin contained in grapes, Japanese knotweed *Polygonum*, cowberry, peanut, and it exists mainly as a *trans*-form in nature. RVT is a powerful antioxidant that improves insulin sensitivity, prevents decline in cognitive functions, inhibits oxidative stress, etc [15, 16]. Indeed, RVT consumption has been shown to exert beneficial metabolic effects, regulate glucose homeostasis, and protect against chronic metabolic diseases, including diabetes [17, 18].

Therefore, a pre-clinical study was conducted to determine the effects of orally administered PIR, RJ and RVT on cognitive functions and oxidative stress markers in an animal model of diet-induced MetS.

Materials and methods
Experimental Animals
A total of 50 healthy male albino rats (6 weeks old) weighing 180-220 g were used in this study. They were housed in regular cages situated in an animal room at constant temperature (22±2 °C) with 12 h light/dark cycle and had free access to standard pellet diet and water (*ad libitum*).

All animal studies were conducted according to European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose (Strasbourg, 1986), Directive 2010/63/EU on the protection of animals used for scientific purposes, and were approved by the Institutional Animal Care Committee.

Chemicals and Reagents
Fructose was obtained from Galam Ltd. (Israel), 5% solution of Piracetam was obtained from Galychfarm (Ukraine), Resveratrol (Evelor) was purchased from Supplements Ltd, Cyprus, and propylene glycol extract of Royal jelly was purchased from LLC "Scientific and Production Company "Vilarus" (Ukraine)

Study design
The animals were randomly assigned into 5 experimental groups of ten each, as given below:
1) Group-I: intact rats (passive control), fed with standard pellet diet and having free access to water.
2) Group-F: rats with MetS induced by 60% fructose solution (instead of water) during 8 weeks (active control).
3) Group-F+PIR: animals with experimental MetS that were treated with Piracetam (500 mg/kg/day).
4) Group-F+RJ: rats with experimental MetS that were treated with propylene glycol extract of Royal jelly (1, 5 mg/kg/day).
5) Group-F+RVT: animals with experimental MetS that were treated with Resveratrol (20 mg/kg/day).

All studied drugs (PIR, RJ, RVT) were administered orally for 14 days during last 2 weeks of MetS induction.

Behavioral Testing
Behavioral testing was conducted under the room illumination (60W light) between 12:00–17:00 daily following a 1 hr period during which the rats were habituated to the testing room.

Passive avoidance test (PAT) is a widely accepted simple and rapid means of memory testing. Passive avoidance response was determined using an apparatus, which consisted of an illuminated and dark compartments adjoining each other through a guillotine door. The test was conducted for 2 consecutive days at the same time each day. On the first day (learning trial) each rat was placed in the illuminated compartment facing away from the dark compartment. Briefly, during the training sessions 180 sec adaptation and aversion trials in the apparatus were achieved. Once the rat enters completely into the dark compartment, it receives 5 strokes with electric shock (1 mA, 1 sec) were delivered at 5-second intervals through the stainless steel grid floor. The amount of time it took for the rat to enter into the dark compartment was recorded automatically, and described as step-through latency or latent period (LP).

After 24 hr, following the training sessions, the avoidance of the dark shock-associated compartment and the latency to enter the dark compartment were recorded to present as memory retention trial within 180 sec. Nonetheless, a latency of 180 sec was given to those animals that did not enter the dark compartment within the experimentation period. Fraction of time spent in dark and bright compartment for each rat was noted [19, 20].

Oxidative Stress Markers
Venous blood (5 ml) was collected from each animal after over-night fasting (>10 hr). Serum was then obtained, frozen and stored at −20 °C for further analysis. Analysis was performed immediately after defrosting of the samples.

State of the antioxidant defensive system in given experimental conditions was estimated by determining the activity of superoxide dismutase (SOD) [21]. The principle of the total superoxide dismutase (EC 1.1.15.1.1) enzyme activity method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by O_2^- generated by the xanthine/xanthine oxidase system. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate [21]. Total SOD activity was expressed as units per milligram protein (U/mg protein).

The intensity of lipid peroxidation was measured by estimating serum malondialdehyde (MDA) levels. This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid (TBA) producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm. An MDA standard was used to construct a standard curve against which readings of the samples were plotted [22].

Oxidative modification of proteins in serum was assessed by early (aldehydephenylhydrazones, APH, λ=270 nm) and late (ketophenylhydrazones, KPH, λ=363 nm) markers of oxidative degradation of proteins using standard spectrophotometric method [22]. APH and KPH levels were expressed as standard units per milligram protein.

Statistical Analysis
The results were analyzed with Stat Plus 2006 software (Analyst Soft; www.analystsoft.com). Data were expressed as
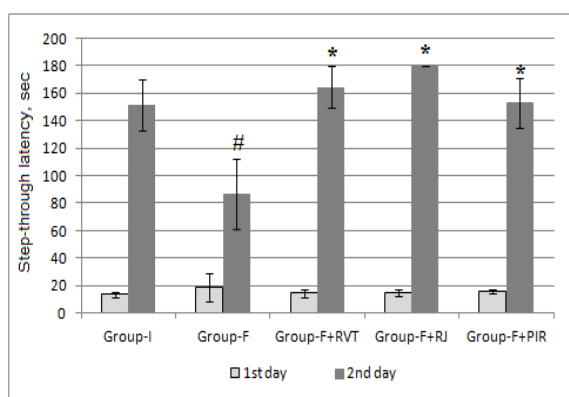
means ± S.E.M. (n = 10). The statistical significance of differences (p) was evaluated by nonparametric Mann–Whitney U-test. The differences between the samples were significant at $p < 0.05$.

Results
Effects on Behavioral Testing
During the passive avoidance test there was no significant difference between all treatment groups in the initial latent period (LP) to enter the dark compartment, time spent in dark and bright compartments. Therefore any differences seen subsequently are a reflection of differences in the retrieval and are not related to the initial baseline activities.

However, there were some differences in this parameter between Group-I and Group-F while testing the animals 24 hours after initial testing (Fig. 1). LP in Group-I significantly exceeded initial values ($p < 0.05$), and it was an evidence of effective learning of rats in this group. However, LP in Group-F was less by 42.8% ($p < 0.05$) as compared to Group-I.

– statistically significant difference from Group-I ($p < 0.05$); * – statistically significant difference from Group-F ($p < 0.05$)
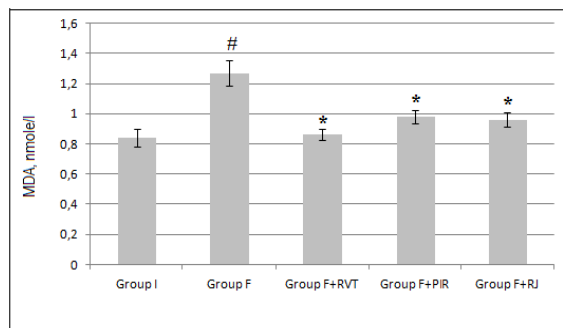
Fig 1: Effect of PIR, RVT and RJ on step-through passive avoidance test

It should be noted that during passive avoidance test, rats treated by RVT, RJ and PIR took longer time to enter the dark compartment as compared to rats with MetS. Oral administration of all studied drugs in rats with MetS results in significant increase of step-through latency of passive-defensive reflex on the second day of the test (Fig. 1). RJ, RVT and PIR increased the duration of LP in the range 76.7–107.6% ($p < 0.05$) as compared to a group of animals with MetS (Fig. 1). Longer latency indicates good retrieval of learned behavior.
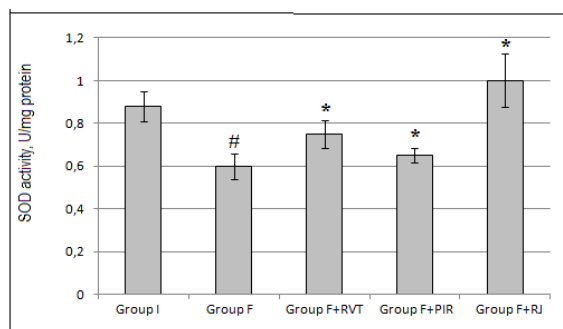
Oxidative Stress Markers
It was established that deterioration of memory as an evidence of cognitive deficit in animals with MetS was accompanied by a significant activation of lipid peroxidation and oxidative modification of proteins in rat serum (Fig. 2, 3).
Hyper activation of lipid peroxidation and development of oxidative stress was confirmed by the growth of serum MDA level in Group-F by 50.9% ($p < 0.05$) as compared to Group-I (Fig. 2). The levels of APH and KPH were also increased by 15.8% ($p < 0.05$) and 15.5% ($p < 0.05$) in comparison with group of intact animals (Fig. 4, 5). Along with activation of lipid peroxidation and oxidative modification of proteins in rats with MetS there was a decrease by 31.6% ($p < 0.05$) in activity of a key antioxidant enzyme (SOD) (Fig. 3).
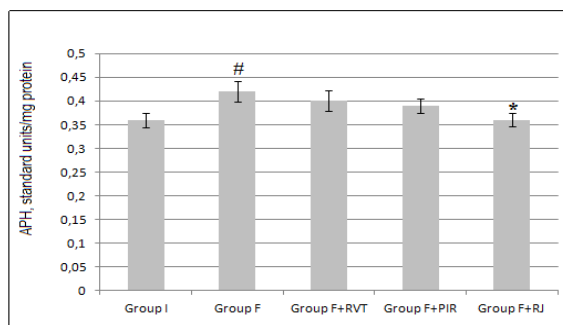

– statistically significant difference from Group-I ($p < 0.05$); * – statistically significant difference from Group-F ($p < 0.05$)

Fig 2: Effect of PIR, RVT and RJ on MDA level in serum of fructose-drinking rats.

– statistically significant difference from Group-I ($p < 0.05$); * – statistically significant difference from Group-F ($p < 0.05$)

Fig 3: Effect of PIR, RVT and RJ on SOD activity in serum of fructose-drinking rats.
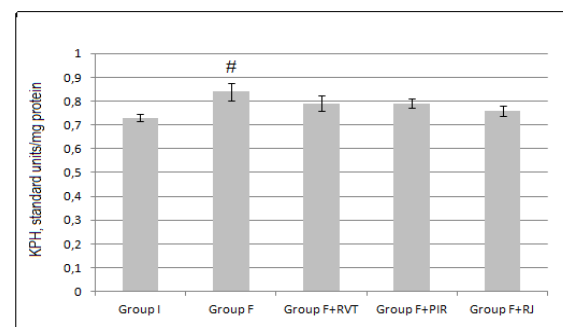

– statistically significant difference from Group-I ($p < 0.05$); * – statistically significant difference from Group-F ($p < 0.05$)

Fig 4: Effect of PIR, RVT and RJ on APH level in serum of fructose-drinking rats.

– statistically significant difference from Group-I ($p < 0.05$)

Fig 5: Effect of PIR, RVT and RJ on KPH level in serum of fructose-drinking rats.

Oral administration of the studied drugs during 14 days resulted in suppression of activity of free radical oxidation of lipids and proteins. Significant reduction in serum MDA levels was noted in all experimental groups (Fig. 2). Moreover, the most expressive diminution of this marker by 32.4% ($p<0.05$) was observed in the group of animals receiving Resveratrol. Less decrease of MDA level by 24.3% ($p<0.05$) and 23.0% ($p<0.05$) were noted in Group-F+RJ and Group-F+PIR as compared to Group-F (Fig. 2). It has been also ascertained that after administration of propylene glycol extract of Royal jelly, Piracetam and Resveratrol the content of a late marker of oxidative proteins degradation (KPH) in rat serum was reduced by diminished by 9.2%, 6.5% and 5.4% respectively, but these changes were not significant (Fig. 5). Regarding the early marker of oxidative proteins degradation (APH) in rat serum, only RJ extract was shown to decrease it by 14.6% ($p<0.05$). However, other drugs did not significantly influence APH content in serum of rats with metabolic syndrome (Fig. 4).

It has been also established that PIR, RVT and RJ increase activity of a key antioxidant enzyme (SOD) in rat serum by 7.6% ($p>0.05$), 25.1% ($p>0.05$) and 66.3% ($p<0.05$) respectively as compared to Group-F (Fig. 3).

Discussion
Thus, experimental modeling of MetS in rats leads to impairment of training and retrieval of learned behavior. Our results indicate that administration of Piracetam, propylene glycol extract of Royal jelly and phytoalexin Resveratrol is capable to prevent accelerated development of cognitive deficits in rats with MetS.

Inhibition of cognitive functions in animals with experimental MetS was accompanied by development of oxidative stress, which manifested in the form of excessive lipids/proteins peroxidation and inhibition of antioxidant protection. In turn, the lack of a considerable increase of APH along with significant rise of KPH in rats with MetS may indicate an absence of acute destructive changes in this condition and be the evidence of its chronic course. All studied drugs can suppress reactions of free radical oxidation of lipids and proteins that are activated in rats with experimental MetS and may influence its course and development of complications.

This study indicates an important role of oxidative stress in development of cognitive disorders that appear in consequence of metabolic syndrome. At the same time, inhibition of free radical oxidation reactions in experimental MetS by studied drugs may play a key role in their cerebroprotective properties.

Conclusion
Experimental MetS induced by 60% fructose solution in rats leads to impairment of cognitive functions (deterioration of learning process), based on development of oxidative stress. Propylene glycol extract of Royal jelly and Resveratrol show pronounced antiamnesic activity in rats with MetS. All studied drugs inhibit reactions of free radical oxidation in varying degree. According to obtained data, the largest antiamnesic and antiradical efficacy is typical for propylene glycol extract of Royal jelly.

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