SUBACUTE EFFECTS OF TRPV1 AGONIST ON ENERGY METABOLISM IN RATS

By

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ABESTRACT

Background: Red peppers are used as a spice for enhancing the palatability of food. Capsaicinoids are responsible for 90% of total pungency of pepper fruits. They enhance energy metabolism and thermogenesis. However, there is a little information about the effect of capsaicinoids on lipolysis and carbohydrate metabolism. **Aim of the work:** the present study was designed to study the effects of CAP on the serum glucose, free fatty acids (FFA) and glycerol concentrations in rats. **Method:** 20 healthy, adult, male albino rats weighing 240-250 gm were divided into 2 equal groups (n=10): Control group and CAP treated group. CAP (dose 3mg/kg body weight / day) was administered via a S.C. injection for consecutive 10 days.. **Results:** CAP increased markedly serum glucose concentration on day 1-10 as compared with the control group while CAP increased serum FFA and glycerol concentrations on day 3-10 as compared with the control group. CAP did not change the relative weight of white (perirenal and periepididymal) adipose tissues and brown (interscapular) adipose tissue to body weight during the experimental period as compared with the control group. **Conclusions:** CAP markedly elevated serum glucose, FFA and gylecrol without significant changes in the relative weight of white (perirenal and periepididymal) and brown (interscapular) adipose tissues in rats.

Keywords: Capsaicin, energy metabolism.

INTODUCTION

Red peppers are used as a spice for enhancing the palatability of food and drugs such as counterirritant on stomach medicines in many countries [(1), (2), (3)]. The pungent principle of red pepper is a group of compounds called capsaicinoids, which possess a variety of biological properties and capsaicinoids are a family of natural products isolated from the dried fruits of chili peppers [(1), (4), (5)]. Capsaicin (CAP: (E)-N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-amide) and dihydrocapsaicin (DHC: N (4-hydroxy-3-methoxybenzyl)-8-methaylnonamide are responsible for 90% of total pungency of pepper fruits [(1)].

It is generally accepted that capsaicinoids enhance energy metabolism through catecholamine secretion from the adrenal medulla as a result of the activation of the central nervous system and which was mediated through thermo sensitive transient receptor potential (TRP) channels, vanelloid 1 (TRPV1) [(6)]. The TRPV1 is activated by volatile pungent foods such as hot pepper (capsaicin), black and white pepper (piperine) and ginger (gingerol). Furthermore, low or high temperatures also affect TRPV1 (<18ºC) and TRPV1 (>43ºC)[(7)].

Activation of TRPV1 plays a role not only in transmission of the pungent or pain sensation but also enhancement of capsaicinoids-induced energy consumption and thermogenesis [(7)]. Capsaicinoids enhance energy metabolism via adrenalin secretion from the adrenal medulla through activation of sympathetic nervous system in rats [(8), (9)]. In addition, the effects capsaicinoids on body heat production , lipid and energy metabolism, swimming endurance capacity, antioxidant activity and perspiration have been reported by many studies [(1), (2), (3), (8), (9), (10), (11)]. However, subacute effects of capsaicinoids on blood glucose, free fatty acids (FFA) and glycerol levels are not fully elucidated [(9)]. Study of these parameters is of critical importance to understand the mechanism of capsaicinoids-induced responses in energy metabolism. Therefore, this research was designed to study the subacute effect of CAP on plasma glucose, FFA and glycerol concentration in adult male rats. The subacute effect of CAP

on the weight of perirenal and periepididymal white adipose tissues, and interscapular brown adipose tissue were also examined

MATERIALS AND METHODS

Animals: 20 healthy, adult, male albino rats weighing 240-250 gm were used that were bred in the animal house. The rats were kept in steel wire cages under hygienic conditions in physiology research laboratory in faculty of medicine Zagazig University. Animals were kept on normal diet that consisted of mixed commercial rat laboratory chow had free access to water, kept at room temperature and were maintained on a 12 h light/dark cycle. The rats were accommodated to new conditions for 5 days before the experiments going on.

Groups: After adaptation period, the animals were randomly divided into 2 groups.

- **Control group (CON) (n=10)**
- **Capsaicin treated group (CAP) (n=10)**

Experimental protocol: the experimental protocol is shown in **fig. (1)**. CAP (Sigma–Aldrich.CH-9471Buchs, Germany) was prepared in 2% ethanol containing 0.9% NaCl as a vehicle to obtain 0.1% solution of CAP [(4)]. CAP (dose 3 mg/kg body weight / day) was administered to rats via subcutaneous (s.c.) injection from the cervical portion of the back (9:00-9:30 a.m.) for consecutive 10 days [(5)]. In the control group, an equivalent volume of CAP- free 0.9% NaCl solution containing 2% ethanol and was administered to rats in the same manner.

Blood samples (1-2 ml) were collected from tail vein 3 hours post s.c. injection.

Food intake (gm/day), water intake (gm/day), body weight gain (gm/10 days) and food efficacy (= ratio of the body weight gain to total food intake) were calculated in the studied groups [(9)].

The perirenal and periepididymal white adipose tissues and interscapular brown adipose tissue were isolated and weighed on the next day of final administrations.

Plasma glucose, FFA and glycerol assays: The blood samples were centrifuged for 20 minutes at approximately 500 rpm [(12)]. The separated serum was stored at -20° C. Repeated freezing and thawing was avoided. Serum glucose, FFA and glycerol concentrations were assayed on day 0, 1, 3, 7 and 10 [(13)]. Serum glucose was assayed by enzymatic colorimetric method, ENDPOINT (Joaquim Costa, 18, 2a planta. 08390 Montagat- Barcelona-Spain). Serum FFA was assayed by quantitative colorimetric method using enzymatic ™ FFA Assay Kits supplied by (Bioassay systems, USA). Serum glycerol was assayed by quantitative colorimetric method using enzymatic ™ Glycerol Assay Kits supplied by (Bioassay systems, USA).

Statistical analysis: The data obtained in the present study were expressed as mean ± SD for quantitative variables and statistically analyzed according to the methods described by **Kirkwood (1989) [(14)].** The statistical analysis is done by using SPSS program (version 17) (SPSS Inc. Chicago, IL, USA). The effects of CAP on the studied parameters in rats were evaluated by **the student "t" test** for comparison of means of two independent groups. Test was considered significant at P values < 0.05.

RESULTS

Effects of CAP on food intake, water intake, body weight gain and food efficacy:

As shown in **table 1 and fig 2,** administration of CAP decreased significantly the body weight gain ***(P<0.001)*** and food efficacy ***(P<0.001)*** as compared with that of the control group while CAP did not change food intake and water intake during the experimental period as compared with the control group ***(P>0.05)***. **.**

Effects of CAP on relative weight of adipose tissues:

As seen in **table 2,** CAP did not change the relative weight of white (perirenal and periepididymal) adipose tissues and brown (interscapular) adipose tissue to body

weight during the experimental period as compared with the control group ***(P>0.05)***. **.**

Effects of CAP on serum glucose concentration:

As seen in **table 3 and fig 3,** CAP (3 mg/kg BW/ day) increased serum glucose concentration on day 1 ***(P<0.001)***, 3 ***(P<0.001)***, 7 ***(P<0.001)*** and 10 ***(P<0.001)*** as compared with the control group. **.**

Effects of CAP on serum FFA concentration:

As seen in **table 3 and fig 4,** CAP (3 mg/kg BW/ day) increased serum FFA concentration on day 3 ***(P<0.01)***, 7 ***(P<0.01)*** and 10 ***(P<0.001)*** as compared with the control group**.**

Effects of CAP on serum glycerol concentration:

As seen in **table 3 and fig 5,** CAP (3 mg/kg BW/ day) increased serum glycerol concentration on 3 ***(P<0.05)***, 7 ***(P<0.01)*** and 10 ***(P<0.001)*** as compared with the control group**.**

Table (1): Effects of CAP (3 mg/ kg body weight) on food intake, water intake, body weight gain and food efficacy

Parameters ($\bar{x}$±SD)	CON (n=10)	CAP (n=10)	T test	P value
Food intake (gm/day)	26±1.8 (23-28)	26.3±2 (24-30)	0.35	>0.05(NS)
Water intake (gm/ day)	47±3.5 (42-52)	48.1±4.1 (43-56)	0.64	>0.05(NS)
Body weight gain (gm/ 10 days)	71.3±2.7 (68-76)	61.4±2 (59-65)	9.77***	<0.001(S)
Food efficacy	0.27±0.015 (0.25-0.3)	0.23±0.017 (0.2-0.25)	5.78***	<0.001(S)

Table (2): Effects of CAP (3 mg/ kg body weight) on relative weight of white adipose tissues (perirenal and periepididymal) and brown adipose tissue (interscapular) to body weight in rats

Parameters ($\bar{x}$±SD)	CON (n=10)	CAP (n=10)	T test	P value
Body weight (gm)	312±3.8 (308-318)	300.9±1.5 (298-303)	8.96***	<0.001(S)
Perirenal white adipose tissue/BW (mg/g)	3.4±0.09 (3.2-3.5)	3.44±0.09 (3.3-3.6)	1.808	>0.05(NS)
Periepididymal white adipose tissue/BW (mg/g)	3.78±0.14 (3.5-4)	3.68±0.1 (3.4-3.8)	0.89	>0.05(NS)
Interscapular brown adipose tissue/BW (mg/g)	1.46±0.12 (1.2-1.6)	1.4±0.1 (1.3-1.5)	1.156	>0.05(NS)

(***) significant when compared with control group (P<0.001).

Table (3): Effects of CAP (3 mg/ kg body weight) on serum glucose, FFA and glycerol concentrations in rats.

Parameters ($\bar{x}$±SD)	Experimental day				
	0	1	3	7	10
Serum glucose (mg/dl)					
CON (n=10)	99.5±8.4 (89-109)	91.7±13.4 (70-113)	90.1±8.5 (79-102)	92.8±13.1 (71-115)	96±11.9 (79-112)
CAP (n=10)	100.4±10.3 (80-113)	145.4±5.7 (138-152)	137.4±5.3 (131-145)	129.3±4.6 (119-133)	126±6.4 (115-137)
T test	0.214	11.68 ***	14.96***	8.3***	6.98***
(p)	>0.05(NS)	<0.001(S)	<0.001(S)	<0.001(S)	<0.001(S)
Serum FFA (µMol)					
CON (n=10)	203±9.5 (190-218)	200.9±8.5 (189-215)	202.7±7.6 (194-212)	201.3±9.5 (191-214)	202.1±10.2 (188-217)
CAP(n=10)	200.1±9.1 (186-215)	203±8.4 (192-216)	217.6±12.8 (199-231)	218.3±11.7 (200-235)	229.6±11.7 (215-249)
T test	0.696	0.556	3.16**	3.57**	5.59***
(p)	>0.05(NS)	>0.05(NS)	<0.01(S))	<0.01(S)	<0.001(S)
Serum glycerol (mg/dl)					
CON (n=10)	8.6±0.7 (7.5-9.9)	8.6±0.7 (7-10.3)	8.8±0.8 (7.9-10.5)	9±0.9 (7.9-11)	9.4±1.1 (8.3-12)
CAP (n=10)	8.7±0.7 (7.9-10.1)	9.1±0.8 (82-10.8)	9.9±1.2 (8.3-11.4)	11.9±2.2 (9.5-16)	19.6±3.5 (16-25)
T test	0.24	1.49	2.57 *	3.82 **	8.803 ***
(p)	>0.05(NS)	>0.05(NS)	<0.05(S)	<0.01(S)	<0.001(S)

(*) significant when compared with control group (P<0.05).
(**) significant when compared with control group (P<0.01).
(***) significant when compared with control group (P<0.001).

Figure (1): Experimental protocol.

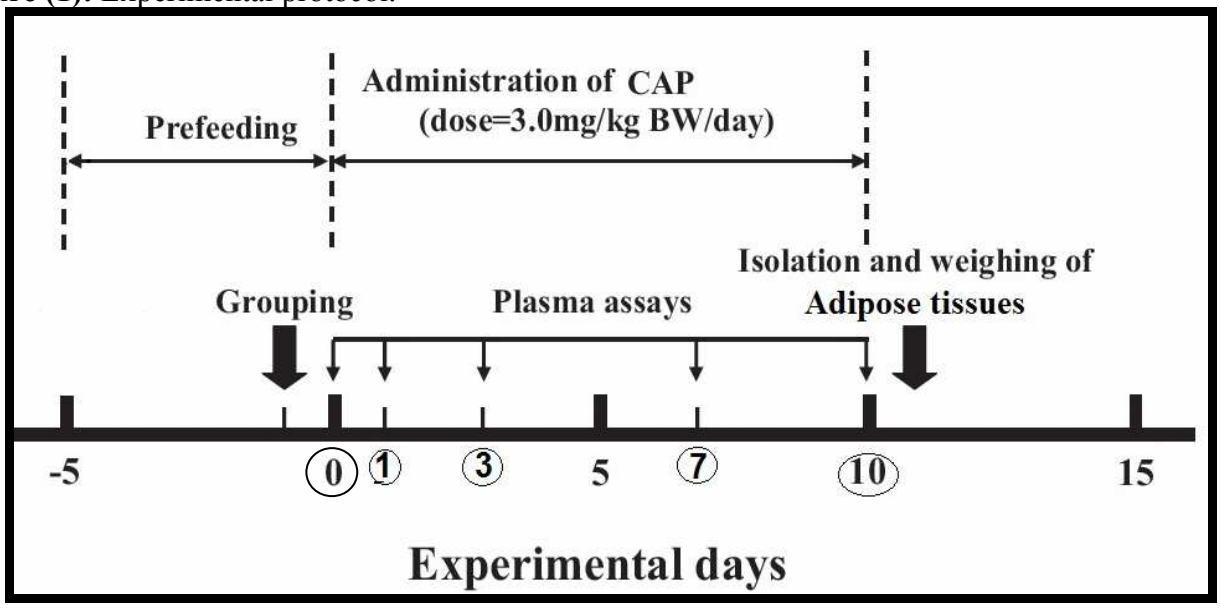

Figure (2): Effects of CAP (3 mg/ kg body weight) on food intake, water intake, body weight gain and food efficacy.

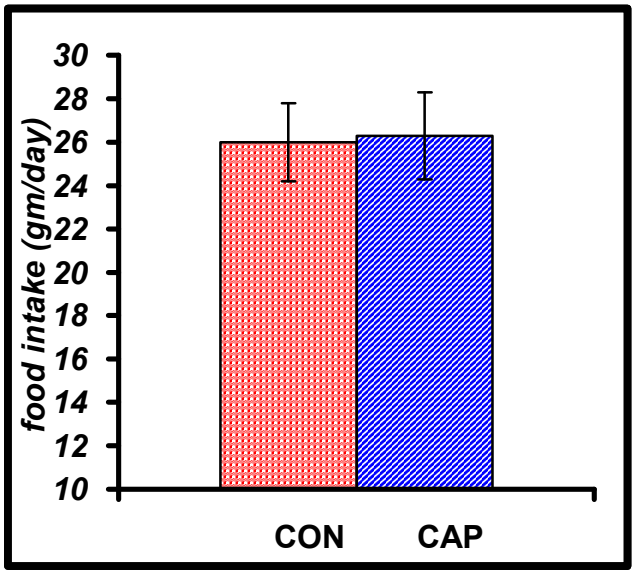

Fig (2a): Comparison between food intake (gm/day) in the studied groups throughout the experimental period

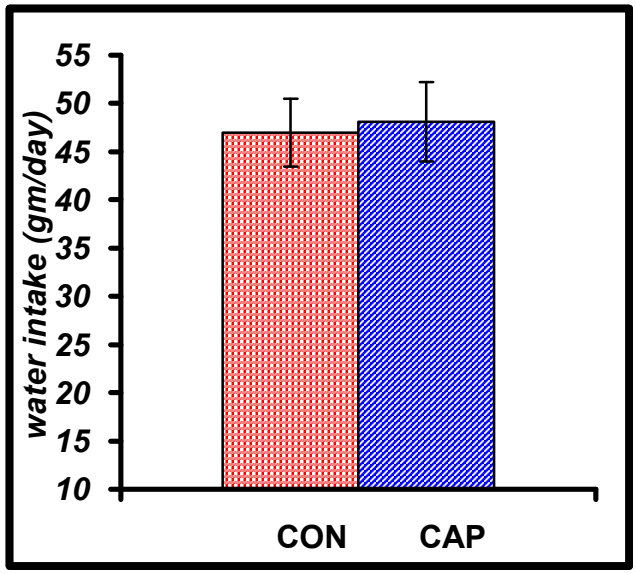

Fig (2b): Comparison between water intake (gm/day) in the studied groups throughout the experimental period

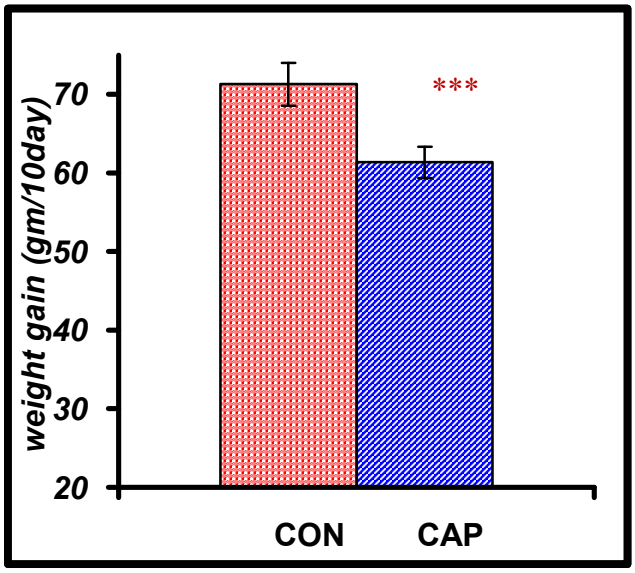

Fig (2c): Comparison between body weight gain (gm/10day) in the studied groups throughout the experimental period

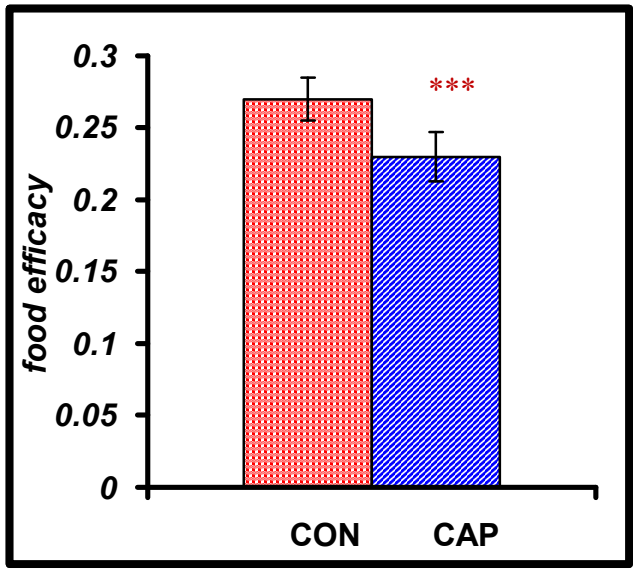

Fig (2d): Comparison between food efficacy in the studied groups throughout the experimental period

(***) significant when compared with control group (P<0.001).

Figure (3): Effects of CAP (3 mg/ kg body weight) on serum glucose concentrations (mg/dl) in rats throughout the experimental period.

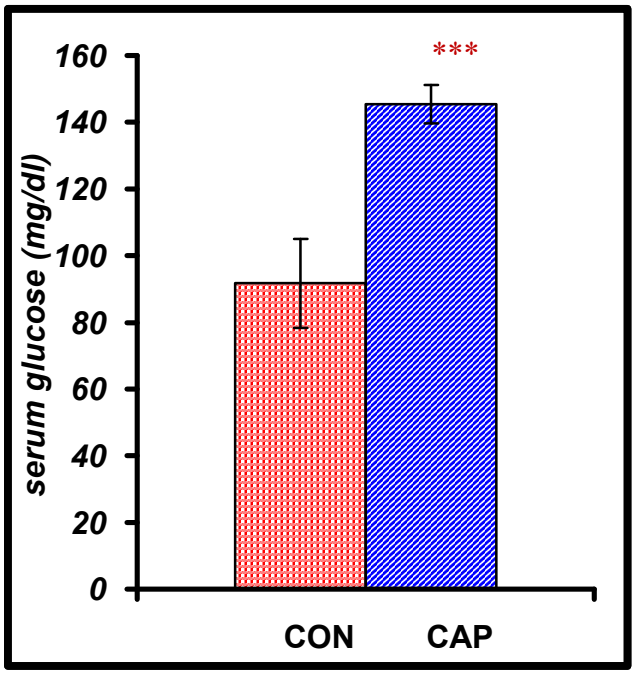

Fig (3a): Comparison between glucose concentration (mg/dl) in the studied groups at day 1 of experimental period

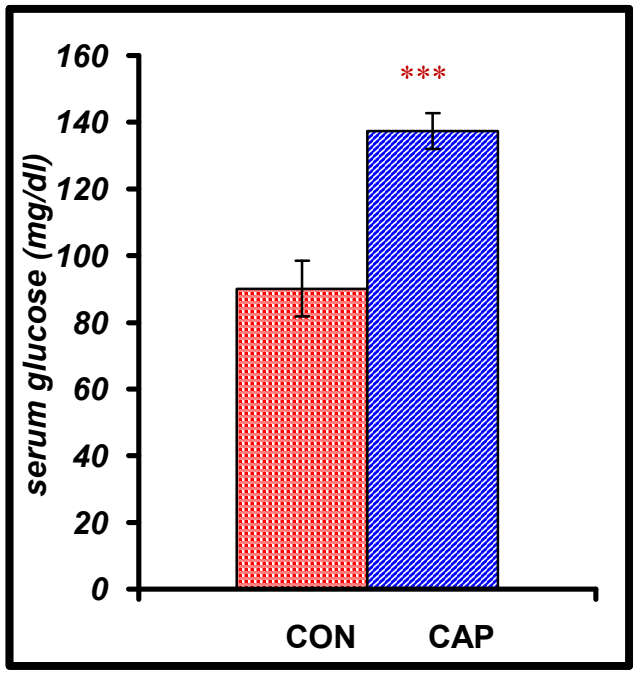

Fig (3b): Comparison between glucose concentration (mg/dl) in the studied groups at day 3 of experimental period

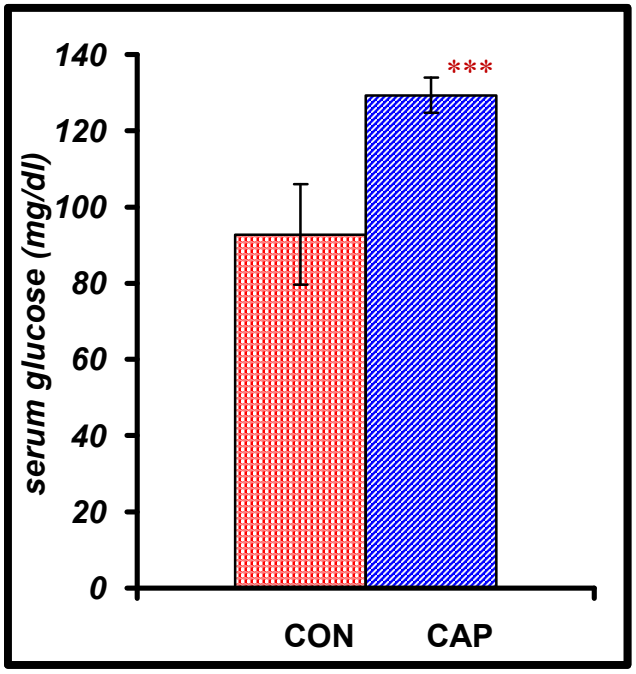

Fig (3c): Comparison between glucose concentration (mg/dl) in the studied groups at day 7 of experimental period

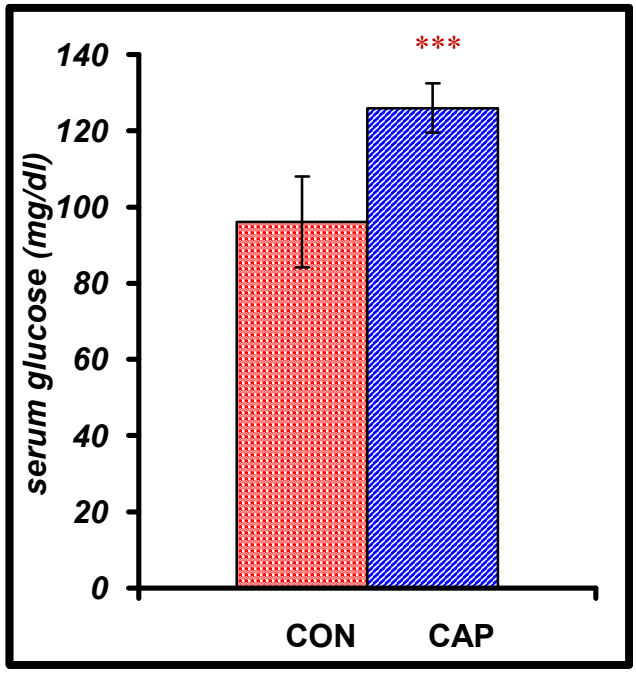

Fig (3d): Comparison between glucose concentration (mg/dl) in the studied groups at day 10 of experimental period

(***) significant when compared with control group (P<0.001).

Figure (4): Effects of CAP (3 mg/ kg body weight) on serum FFA concentrations (µMol) in rats throughout the experimental period.

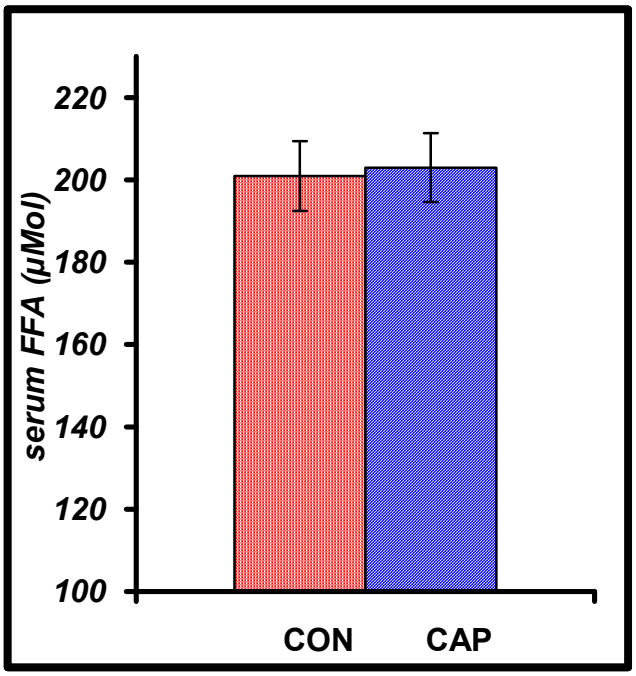

Fig (4a): Comparison between FFA concentration (µMol) in the studied groups at day 1 of experimental period

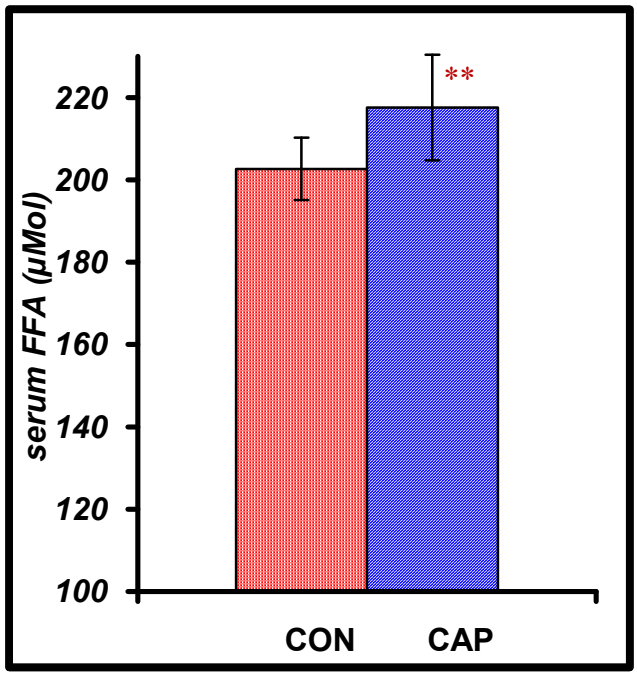

Fig (4b): Comparison between FFA concentration (µMol) in the studied groups at day 3 of experimental period

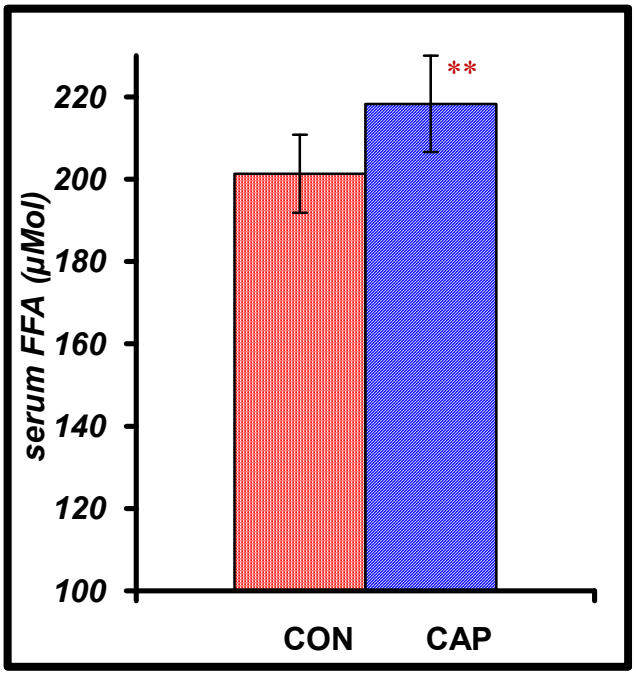

Fig (4c): Comparison between FFA concentration (µMol) in the studied groups at day 7 of experimental period

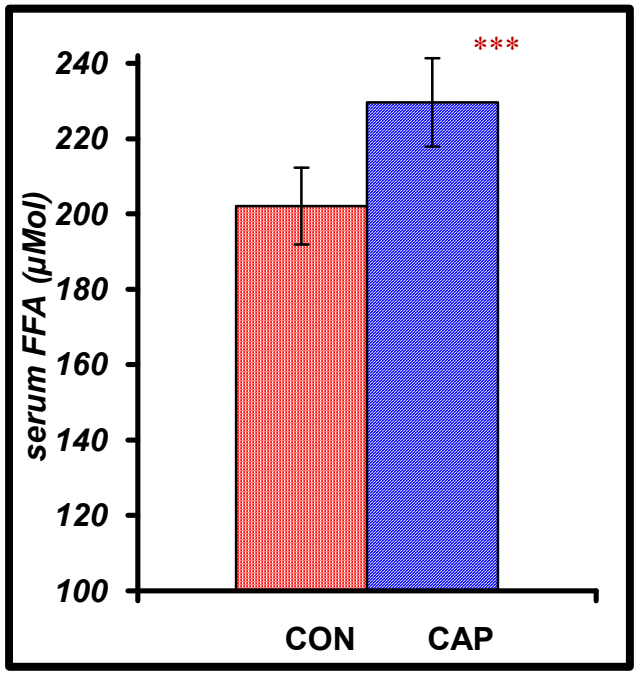

Fig (4d): Comparison between FFA concentration (µMol) in the studied groups at day 10 of experimental period

(**) significant when compared with control group (P<0.01).
(***) significant when compared with control group (P<0.001).

Figure (5): Effects of CAP (3 mg/ kg body weight) on serum glycerol concentrations (mg/dl) in rats throughout the experimental period.

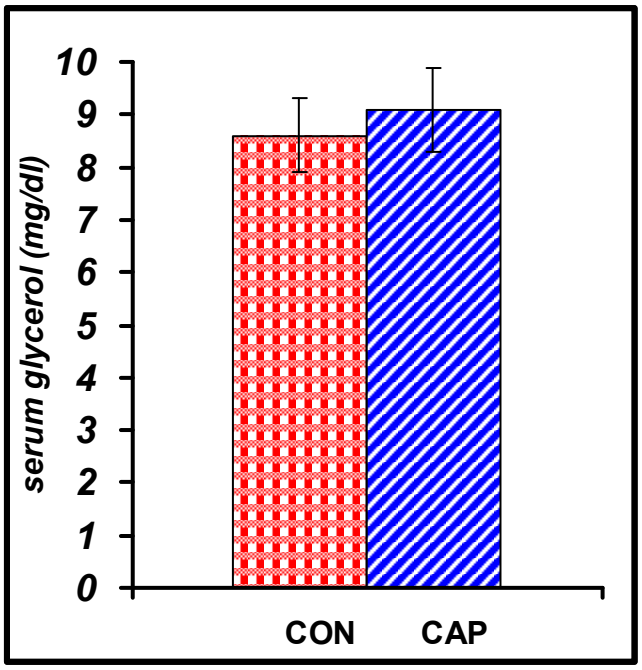

Fig (5a): Comparison between glycerol concentration (mg/dl) in the studied groups at day 1 of experimental period

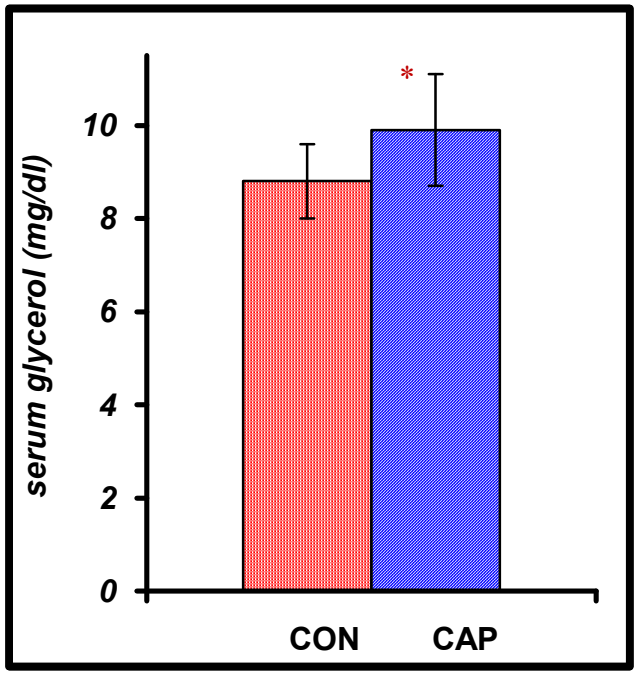

Fig (5b): Comparison between glycerol concentration (mg/dl) in the studied groups at day 3 of experimental period

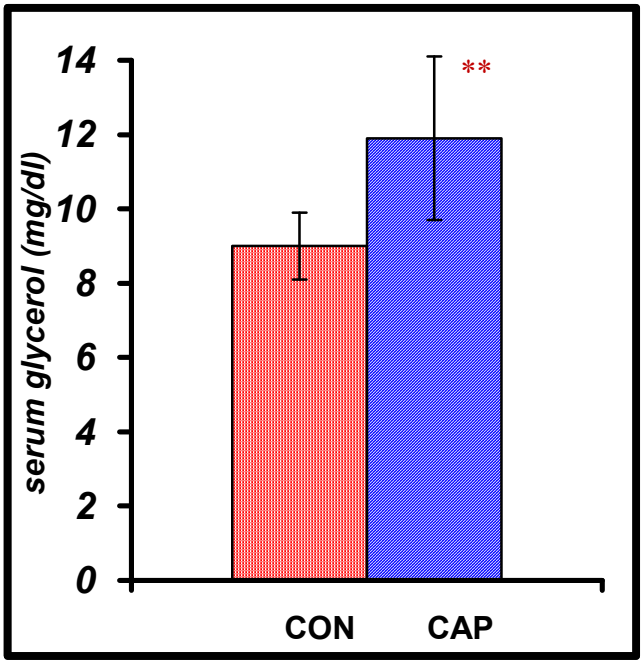

Fig (5c): Comparison between glycerol concentration (mg/dl) in the studied groups at day 7 of experimental period

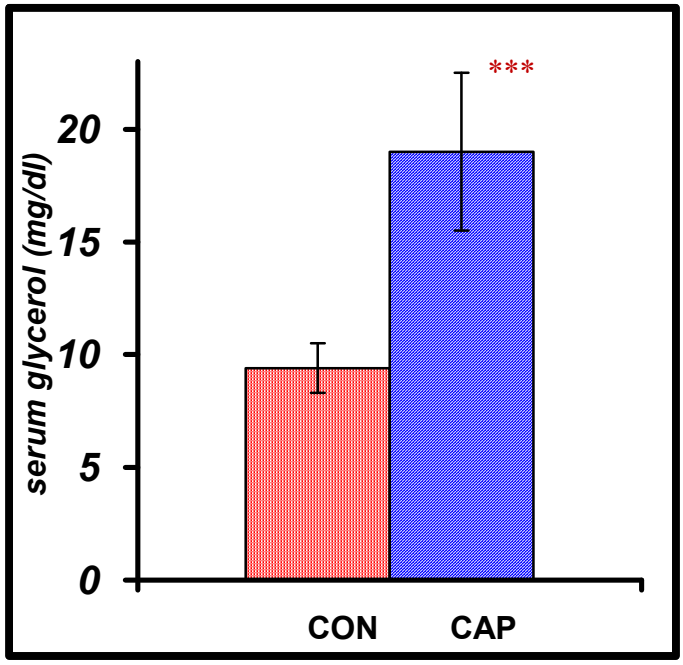

Fig (5d): Comparison between glycerol concentration (mg/dl) in the studied groups at day 10 of experimental period

(*) significant when compared with control group (P<0.05).
(**) significant when compared with control group (P<0.01).
(***) significant when compared with control group (P<0.001).

DISCUTION

The results of the present study clearly showed that capsaicin decreased significantly the body weight and food efficacy as compared with the control group. However, the present study also showed that CAP did not change the food intake, water intake and the weight of white (perirenal and periepidydmal) adipose tissue and brown (interscapular) adipose tissue. Furthermore, CAP increased markedly serum glucose, FFA and glycerol concentrations during the experimental period. These results suggest at least in part that CAP enhances energy metabolism and thermogenesis without inducing lipolytic actions from white and brown adipose tissues in the present conditions. CAP-induced promotive effects on energy metabolism and thermogenesis may be caused by adrenalin secretion from adrenal medulla through activation of sympathetic nervous system [15], [16]. Generally catecholamines are known to increase energy metabolism in liver and thermogenesis in the brown and white adipose tissues, whereas blood glucose and FFA levels are increased [1], [4].

The CAP- induced rise effects of serum glucose, FFA and glycerol levels in the present study could be caused through β-adrenergic enhancement of energy metabolism and thermogenesis by catecholamines. These phenomena may at least in part be caused via the promotive actions of thermogenetic capacity of uncoupler protein (UCP)-1 in brown adipose tissue [11], [17]. This prediction does not contradict with the results of previous studies which showed that capsaicin of hot red pepper increased adrenal sympathetic efferent nerve activities and catecholamine secretion [10], [15], [16].

On the other hand, CAP- induced rises in serum glucose, FFA and glycerol levels may be caused by the activation of glycogenolysis via β-adrenoceptors in liver and lipolytic actions via β-adrenoceptors in adipose tissues and visceral organs through activation of sympathetic system in rats.

Furthermore, CAP- induced response on levels of glucocorticoids may play an important role in the higher levels of serum glucose, FFA and glycerol [13]. However, these possibilities are not fully elucidated. Further studies are indispensable of the subacute effects of capsaicinoids on plasma glucose, FFA and glycerol.

In conclusion, CAP markedly elevates serum glucose, FFA and gylecrol without significant changes in the relative weight of white (perirenal and periepididymal) and brown (interscapular) adipose tissues in rats. So, CAP enhances energy metabolism and thermogenesis without inducing lipolytic actions from white and brown adipose tissues

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التأثيرات تحت الحادة لمنشط مستقبل الجهد المؤقت فانيلويد-١ علي ايض الطاقة في الفئران.

لقد وجد أن نبات الفلفل الاحمر يستخدم كنوع من التوابل لاكساب طعم مميز وان مركبات الكبسياسين مسئولة عن ٩٠% من هذه الخاصية وأن هذه المركبات لها تأثير محفز علي عملية الايض وانتاج الطاقة.

الهدف من البحث

دراسة تأثير الكبسياسين علي مستوي تركيز الجلوكوز والاحماض الدهنية الحرة والجليسرول في دم الفئران.

مواد و طرق البحث

أجريت هذه الدراسة علي ٢٠ فأرا ابيضا من الذكور البالغة و تم تقسيم الفئران الي مجموعتين متساويتين:

- المجموعةالأولي: المجموعة الضابطة.
- المجموعةالثانية: اعطيت كبسياسين تحت الجلد لمدة عشرة ايام متتالية.

نتائج البحث.

وجد أن اعطاء كبسياسين ادى الي زيادة ذات دلالة احصائية في تركيز الجلوكوز من اليوم الاول الي اليوم العاشر من التجربة وزيادة ذات دلالة احصائية في تركيز الاحماض الدهنية الحرة والجليسرول من اليوم الثالث الي اليوم العاشر في حين انه لم يؤثر علي الوزن النسبي للدهون البيضاء والبنية مقارنة بالمجموعة الضابطة في الفئران.

المستخلص من البحث:

- منشط مستقبل الجهد المؤقت فانيلويد-١ (كبسياسين) ادي الي ارتفاع تركيز الجلوكوز والاحماض الدهنية الحرة والجليسرول في دم الفئران دون التاثير علي الوزن النسبي للانسجة الدهنية البيضاء والبنية في الفئران.