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Short and Long Term Effect of Caffeine on Liver, Kidney as well as Glucose, Insulin, Triglycerides and Cholesterol on Normal Rats.

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Abstract: The present study investigated the long and short term effect of caffeine on some blood parameters in rats. In short term the rats administered caffeine at either (10, 40, 160 mg/kg body weight) for 1 month. In long term the caffeine was administered orally each day at either (5, 20, 80 mg/kg body weight) for 2 months and all analysis of long term were done after one and two months of treatment. The levels of glucose insulin, urea, creatinin, uric acid, ALT, AST, albumin, total protein, globulin, albumin/globulin ratio, total cholesterol and triglycerides were determined in serum of these rats. The results showed that there is no significant differences between control and groups given caffeine in both short and long term except in the case of glucose, there is a significant reduction in glucose level at 5, 20 and 80 mg/kg body weight/day (after 2 months of the long term), and 160 mg/kg body weight/day (short term) caffeine in the treated groups. It was observed that there is a significant reduction in insulin level at 80 mg caffeine/kg body weight/day in the treated group (after 2 months). The concentration of urea was increased after treatment at 5 and 20 mg caffeine/kg body weight/day. The results showed that low dose of caffeine did not affect on blood parameters in both short and long term, but the high dose (80 mg caffeine/kg body weight/day) decreased serum glucose and insulin levels.

Key words: Caffeine, kidney function, liver function, lipids, glucose and insulin levels

INTRODUCTION

Coffee and tea have been the most popular beverages in western society for several-hundred years. Caffeine additive to beverages. Thus, the consequences of caffeine ingestion on the public health are potentially profound. Caffeine has many significant effects on metabolism in humans (Avogaro *et al*. 1973). such as reduction of glucose and insulin levels in rats (Tofovic *et al* 2002).

The long term consumption of caffeine help alleviate diabetic symptoms by enhancing insulin sensitivity and beta cells function through improved insulin / IGF-1 signaling through induction of insulin receptor substrate 2 in mildly diabetic rats *(Park et al*. 2007).

Also it was reported that caffeine increased net hepatic lactate, and net hepatic glucose uptake during a glucose load (Pencek *et al.* 2004) , insulin (Robinson *et al.* 2004; Battram *et al.* 2006) , creatinin, urea (Portolés *et al*. 1985*,* Tofovic *et al*. 2007), AST, ALT in serum of rats *(*Cheul Do *et al*. 1997). It was reported that the caffeine consumption reduce the risk of elevated serum ALT *(*Ruhl, and Everhart, 2005, Klatsky *et al* 2006, Cadden *et al* 2007). Caffeine has been linked to cardiovascular diseases for a long time (Tverdal *et al.* 1990 ; Grobbee *et al.* 1990; Chou and Benowitz 1994) based on the presumption that it may increase levels of total cholesterol in serum (Kokjohn *et al.* 1993; Jee *et al*. 2001) and homocysteine (Urgert *et al.* 2000 ; Olthof *et al*. 2001). There is another study shows that caffeine reduce serum cholesterol and triglycerides levels (Inoue *et al.* 2006). There was an inverse association between coffee drinking and serum uric acid concentration (*Kiyohara et al* 1999). There were no significant differences in the concentration of total protein, albumin, albumin/globulin ratio, (Cheul Do *et al.* 1997), plasma triglycerides (Tofovic *et al* 2007; Poornahavandi and Zamiri, 2008)

It was noted that Caffeine significantly reduced body weight, had no effect on plasma cholesterol after 9 weeks of treatment with caffeine *(*Du *et al* 2005; Tofovic *et al.* 2007)

The aim of this study was to investigate the effect of short and long term intake of caffeine on liver, kidney functions and glucose, insulin levels in serum of rats given different concentrations of caffeine.

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MATERIALS AND METHODS

Chemicals:

Caffeine was purchased from Sigma Chemical Co. All other chemicals and kits were of analytical grade, Biocon kit (Germany), Biosource INS-EASIA kit (Belgium), Diamond Diagnostics kit (Egypt), Cinnagen Inc. kit (Iran).

Animals:

The experiment was carried out in Biochemistry Depart. Fac., Agric.Cair.Univ. using 28 healthy male Sprague-Dawley rats, 2 months – old , from central farm for experimental animals of Vaccera . The rats were housed in cages in an animal room controlled under the following conditions: temperature, 23 ^{o}C; relative humidity, 50 % and lighting cycle, 12 hours. The rats were divided into 3 groups. The first group of rats was the control which kept on standard diet and drinking water instead of caffeine. The second group (short term experiment) the rats were divided into 3 sub-groups according to the concentration of caffeine which was given to rats by oral administration for 1 month. The first sub-group was given 10 mg caffeine/kg body weight/day. The second sub-group was given 40 mg caffeine/kg body weight/day. The third sub-group was given 160 mg caffeine/kg body weight/day. After 1 month of treatment, rats were fasted 12 h. and then the blood was collecting from eye vein for measurements of glucose, insulin, urea, creatinin, uric acid, AST, ALT, total cholesterol and triglycerides, albumin, globulin, total protein, and albumin / globulin ratio.

The third group (long term experiment) the rats was divided into 3 sub-groups according to the concentration of caffeine. The first sub-group was given 5 mg caffeine/kg body weight/day by oral administration for 2 months. The second sub-group was given 20 mg caffeine/kg body weight/day. The third sub- group was given 80 mg caffeine/kg body weight/day. After 1 and 2 months of treatment, rats were fasted 12 h. and then the blood was collecting from eye vein for measurements of glucose, insulin, urea, creatinin, uric acid, AST, ALT, total cholesterol and triglycerides, albumin, globulin, total protein, and albumin / globulin ratio.

Blood analysis:

The glucose was determined according to Passing and Bablok (1983). The insulin was determined according to Starr *et al.,* (1978). The urea was determined according to Tabacco *et al*. (1979). The creatinin was determined according to Peheim and Colombo (1981). The uric acid was determined according to Praetorius and Poulsen (1953). The AST and ALT activities were determined according to Reitman and Frankel (1957). The triglycerides were determined according to Wahlefeld and Bergmeyer (1974). The total cholesterol was determined according to Allain *et al (*1974). The total protein was determined according to Henry (1946). The albumin was determined according to Doumas *et al.* (1971). The globulin concentration and albumin/globulin ratio were determined using the following equations:

Serum globulin concentration = serum total protein concentration – serum albumin concentration.
Serum albumin and globulin ratio = serum albumin concentration / serum globulin concentration.

Statistical analysis:

Statistical analysis were performed using a statistical software program. Statistical methods included mean , standard deviation .The Kruskal-Wallis analysis of variance (ANOVA) test was used for multiple comparisons of data, followed by a Fisherś LSD test .Comparison of data (single-point data) was performed by t-test. The probability value of $p < 0.05$ was considered statistically significant. All data are presented as mean + S.D.M.

RESULTS AND DISCUSSION

Short Term Effect of Caffeine on Blood Chemistry:

Table 1 shows the short term effects of the administration of caffeine for one month on serum glucose, insulin, urea , creatinin , uric acid , total cholesterol, triglycerides, albumin, globulin, total protein, albumin/globulin ratio and ALT , AST activities. The results show that, Caffeine consumption for 1 month significantly decrease the glucose and insulin levels at 160 mg caffeine/kg body weight/day which the glucose level reached 61.5 ± 7.77 mg/dl compared to control 98.7 ± 2.2 mg/dl. The level of insulin was significantly decreased at 160 mg/dl.

The results showed that caffeine concentration negatively correlated to serum glucose and insulin levels in both short and long term intake of caffeine. Chronic caffeine consumption reduced fasting glucose and insulin levels. This is not surprising in view of the significant and tissue specific effects of adenosine on insulin sensitivity. Adenosine via activation of A1 receptors decreases the sensitivity to insulin in skeletal muscle, a tissue that is considered the most important site for glucose disposal in response to insulin. Furthermore, adenosine A1 receptor antagonists have been shown to increase sensitivity to insulin in skeletal muscle from lean (Budohosk *et al.* 1984) and obese rats, and to improve glucose tolerance in obese Zucker rats in vivo (Crist *et al.* 1998; Xu *et al.* 1998)

Caffeine consumption improved glucose homeostasis. This effect due to the inhibition of adenosine receptors, because adenosine stimulates hepatic glucose production through the activation of A2B adenosine receptors, selective A2B receptor antagonists have hypoglycemic effect in diabetic mice and A1 adenosine receptor antagonism improve glucose tolerance in obese rats. Therefore, the balanced A1 and A2B antagonism may explain the positive affects of caffeine on glucose homeostasis. (Tofovic *et al.* 2007)

Caffeine increased net hepatic lactate and net hepatic glucose uptake during a glucose load (Pencek *et al*. 2004*;* Park *et al*. 2007)

The level of urea increased after treatment with 160 mg/kg body weight/day caffeine which reached 94.0 ± 53.7 mg/dl. (Table 1).

There is no significant difference in creatinin concentration at different concentration of caffeine compared to control. These results are in agreement with Cheul Do, *et al.* (1997) who reported that there is no relationship between caffeine and the concentration of creatinin in serum of rats. In the other hand, these results are disagreement with Tofovic *et al*. *(2002)* who reported that the administration of caffeine lead to increase in serum creatinin.

There is a slightly decrease in uric acid at 10, 40, 160 mg caffeine/kg body weight/day (1.74 ± 0.29, 2.22 ± 0.39, 2.17 ± 1.49 mg/dl , respectively).

There is no significant difference in ALT, AST activities, total cholesterol, triglycerides, total protein, albumin, globulin and albumin/globulin ratio levels after treatment with caffeine. We could conclude that the short term administration of caffeine had no effect on liver or kidney function.

Long Term Effect of Caffeine on Blood Chemistry:

Table 2 shows the effect of caffeine on glucose, insulin, urea, creatinin, uric acid, total cholesterol and triglycerides ALT, AST activities, total protein, albumin, globulin and albumin/globulin ratio levels after treatment with caffeine after 1 month of treatment with caffeine.

It was observed that, the level of glucose not affected after 1 month compared to untreated group (86.3 ± 11.06 mg/dl). It was observed that, There is no significant difference in insulin level after 1 month compared to control (4.96 ± 0.49 µIU/ml).

The results show that, no significant change in urea, creatinin, uric acid, total cholesterol and triglycerides ALT, AST activities, total protein, albumin, globulin and albumin/globulin ratio after treatment with caffeine (5, 20, 80 mg/kg body weight/day) after 1 month of treatment with caffeine.

Table 3 shows the effect of caffeine on glucose, insulin, urea, creatinin, uric acid, total cholesterol and triglycerides ALT, AST activities, total protein, albumin, globulin and albumin/globulin ratio after 2 months of treatment with caffeine.

The level of glucose decreased after treatments at 5, 20, 80 mg caffeine/kg body weight/day (77.0 ± 4.93, 51.25 ± 1.5, 59.25 ± 6.55 mg/dl, respectively) after 2 months of the long term treatment compared to control (86.3 ± 11.06 mg/dl).

It was observed that, the level of insulin decreased after treatment at 80 mg caffeine/kg body weight/day for 2 months (3.61 ± 0.28 µIU/ml) compared to control (4.96± 0.49µIU/ml). In long term treatment it was found that 80 mg caffeine/kg body weight/day lead to a highly reduction in both serum glucose and insulin (59.25 ± 6.55, 3.61± 0.28, respectively) (Table 3).

The results show that, there is an increase in urea concentration at 20 mg which reached 111.5 ± 32.8 mg/dl compared to control 29.06 ± 10.27 mg/dl.

Many researchers showed that caffeine can increase the serum urea and creatinin concentration (Portolés *et al*., 1985, Tofovic *et al.* 2002) and this due to that caffeine through inhibition of A2A adenosine receptors, accelerated the development of interstitial inflammation, for example, activation of A2A receptors was shown to inhibit polymorphonuclear cells infiltration and protect kidneys from ischemic reperfusion injury in rats. It is possible that caffeine by interacting with A2A receptors, accelerates the development of interstitial inflammation and augments proteinuria and the late changes in renal function and structure.

A lot of studies indicate that there is increased sympathetic activity in human chronic renal failure (Tofovic, *et al.* 2002). While others showed that there is no relationship between caffeine and the concentration of urea in serum of rats (Cheul Do *et al.*1997). In the other hand, Birkner *et al.* (2006) reported that the administration of caffeine lead to slightly reduction in serum urea.

Also the results show that, no significant change in creatinin level after treatment with caffeine (5, 20, 80 mg/kg body weight/day) after 2 months of treatment with caffeine.

The results show that, there is a slightly decrease in uric acid level after treatment with caffeine (5, 20, 80mg /kg body weight/day) (1.38 ± 0.47, 2.64 ± 0.98, 2.39 ± 0.97 mg/dl, respectively) after 2 months of long term treatment (Table 3). These results are in agreement with Cheul Do *et al.* (1997) who reported that there is no relationship between caffeine and the concentration of uric acid in serum of rats.

Kiyohara *et al.* (1999) reported that the administration of caffeine lead to decrease in serum uric acid and this due to the diuretic action of caffeine which might affect serum uric acid concentration

The results show that there is no significant difference in both AST, ALT activities after treatment with different concentrations of caffeine. (Table 3)

These results are in disagreement with Cheul Do *et al.* (1997) who reported that there is an increase in the concentration of AST, ALT in serum of rats after treatment with caffeine while Ruhl and Everhart (2005), Klatsky *et al.* (2006); Cadden *et al.* (2007) reported that the administration of caffeine lead to decrease in serum ALT.

The results show that, there is a slight reduction in total cholesterol and triglycerides at different concentrations of caffeine compared to control. These results are in agreement with Inoue *et al (*2006) who showed that the administration of caffeine lead to low in serum total cholesterol and triglycerides levels.

It was found that, there is no significant difference in total protein, albumin, globulin, albumin/globulin ratio levels after treatment with caffeine in both short and long term treatment. (Table 3). These results are in agreement with Cheul Do *et al.*(1997) who showed that there is no relationship between caffeine and the concentration of serum total protein, albumin, globulin and albumin/globulin ratio in serum of rats. In the other hand Birkner *et al.* (2006) showed that the level of total protein increased after treatment with caffeine.

Table 1: Short term effect of caffeine on blood parameters

Parameter	*Concentration of Caffeine			
	Control	10	40	160
** Glucose	98.7 ± 2.2 a	107 ± 3.5a	102 ± 14.1a	61.5± 7.77b
***Insulin	4.61± 0.37 a	5.0 ± 0.43a	4.29± 0.91a	4.05± 0.08a
**Urea	29.0±10.2 a	35.8± 4.25a	34.1± 0.83a	94.0± 53.7b
**Uric acid	3.6 ± 1.49a	1.74± 0.29a	2.22± 0.39a	2.17± 1.49a
*Creatinin *	0.89± 0.23a	1.22 ± 0.1a	0.74± 0.31a	0.94± 0.15a
**Total cholesterol	98.0± 13.6a	78.7± 6.14a	94.6± 10.5a	78.4± 28.8a
* Triglycerides*	56.8± 8.6a	57.5± 16.3a	51.4± 9.35a	44.6± 28.7a
**** AST	66.0± 8.75a	53.3± 12.7a	72.0± 15.1a	64.5± 16.2a
**** ALT	23.5± 6.24a	17.3 ± 2.5a	15.0± 5.29a	32.0 ± 9.9a
Albumin*****	3.25±0.1a	3.36±0.1a	3.13±0.3a	3.3± 0.1a
Globulin *****	1.87±0.2a	1.56±0.5a	2.5±0.5a	1.4±0.2a
Total protein *****	5.12±0.29a	4.95±0.49a	5.63±0.25a	4.75±0.21a
Albumin/ Globulin ratio %	1.74±0.1a	2.32±0.8a	1.29±0.3a	2.2±0.3a

* mg/kg body weight/day ,** mg/dl, *** µIU/ml, **** U/l, ***** g/dl, Means within each row with different letters differ significantly at P ≤ 0.05 according to Duncan's multiple range test,

Table 2: long term effect of caffeine on blood parameters (one month)

Parameter	*Concentration of Caffeine			
	Control	5	20	80
** Glucose	86.3 ± 11.06a	76.0 ± 7.93a	91.0 ± 8.0a	88.0 ± 6.24a
***Insulin	4.96 ± 0.49a	4.28 ± 0.28a	4.53 ± 0.25a	4.78 ± 0.77a
** Urea	32.6 ± 3.61a	44.7 ± 7.35a	60.1 ± 6.38a	33.2 ± 8.26a
**Uric acid	3.26 ± 0.68a	2.86 ± 0.59a	3.47 ± 0.82a	3.3 ± 0.63a
*Creatinin *	0.87 ± 0.16a	0.85 ± 0.08a	0.78 ± 0.03a	0.84 ± 0.11a
**Total cholesterol	108 ± 9.89a	95.6 ± 14.9a	84.0 ± 6.0a	111 ± 16.3a
* Triglycerides*	29.0 ± 1.55a	41.9 ± 7.63a	32.7 ± 6.01a	48.9 ± 10.0a
**** AST	74.9 ± 21.7a	83.4 ± 14.2a	101.3 ± 6.0a	65.5 ± 11.7a

Table 2: Continue

**** ALT	21.6 ± 5.5a	18.75 ± 3.4a	20.25 ± 2.63a	21.25 ± 2.06a
* Albumin****	2.99 ± 0.11a	3.23 ± 0.21a	3.59 ± 0.27a	3.07 ± 0.38a
Globulin *****	1.73 ± 0.39a	1.71 ± 0.18a	1.9 ± 0.41a	1.99 ± 0.09a
Total protein *****	4.73 ± 0.41a	4.95 ± 0.3a	5.5 ± 0.14a	5.06 ± 0.47a
Albumin/ Globulin ratio %	1.77 ± 0.35a	1.9 ± 0.31a	1.94 ± 0.57a	1.54 ± 0.12a

* mg/kg body weight/day ,** mg/dl, *** µIU/ml, **** U/l, ***** g/dl. Means within each row wit different letters differ significantly at P ≤ 0.05 according to Duncan's multiple range test,

Table 3: long term effect of caffeine on blood parameters (two month)

Parameter	*Concentration of Caffeine			
	Control	5	20	80
* Glucose*	98.75 ± 2.21a	77.0 ± 4.93b	51.24 ± 1.5c	59.25 ± 6.55cd
***Insulin	4.61 ± 0.37a	4.83 ± 0.3a	4.24 ± 0.76a	3.61 ± 0.28b
** Urea	29.06 ± 10.27a	29.66 ± 3.6a	111.5 ± 32.8b	45.3 ± 3.23a
**Uric acid	3.6 ± 1.49a	1.38 ± 0.47a	2.64 ± 0.98a	2.39 ± 0.97a
*Creatinin *	0.89 ± 0.23a	1.11 ± 0.24a	0.72 ± 0.14a	1.06 ± 0.09a
**Total Cholesterol	98.0 ± 13.6a	78.7 ± 7.63a	82.6 ± 18.0a	76.4 ± 4.83a
** Triglycerides	56.8 ± 8.69a	49.7 ± 12.3a	63.0 ± 10.0a	70.0 ± 17.2a
**** AST	66.0 ± 8.75a	76.66 ± 8.5 a	87.0 ± 11.23a	93.3 ± 23.5a
**** ALT	23.5 ± 6.24a	23.3 ± 3.05a	37.7 ± 13.3a	22.6 ± 1.9a
* Albumin****	3.25 ± 0.13a	3.3 ± 0.1a	3.0 ± 0.14a	3.26 ± 0.28a
Globulin *****	1.87 ± 0.19a	1.63 ± 0.23a	2.2 ± 0.37a	2.1 ± 0.26a
Total protein *****	5.12 ± 0.29a	4.93 ± 0.15a	4.53± 0.9a	5.36 ± 0.5a
Albumin/ Globulin ratio %	1.74 ± 0.13a	2.04 ± 0.31a	1.12 ± 0.68a	1.56 ± 0.15a

* mg/kg body weight/day ,** mg/dl, *** µIU/ml, **** U/l, ***** g/dl. Means within each row with different letters differ significantly at P ≤ 0.05 according to Duncan's multiple range test,

Conclusions:

One of the main findings in our study was the association between caffeine concentration with glucose and insulin level. Another interesting finding in our study was that the effect of short term intake of caffeine was different from that of long term of caffeine. Long term intake of caffeine seemed to be surprisingly beneficial to diabetes in decrease in blood glucose level. Also we found that chronic intake of caffeine might increase urea concentration and slight decrease in triglycerides and cholesterol which may be favorable to cardiovascular diseases. The effects of caffeine on the pathogenesis of cardiovascular diseases should be further confirmed. This point especially short and long term effect of caffeine on liver and kidney function in addition to their effects on triglycerides, cholesterol and its relation to cardiovascular, needs further investigation to prove the results.

ABBREVIATIONS

AST: Asparatate transferase; ALT: Alanine transferase; ZSF1: a model of obese rats,

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