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Safety and Efficacy of Caffeine versus Diazepam on Recovery of Suppressed Hypothalamo-Pituitary-Adrenocortical Axis by Prednisolone Therapy.

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ABSTRACT

Prednisolone is a glucocorticoid Therapy which induces suppression of the hypothalamo-Pituitary-Adrenocortical (HPA) axis. The beginning of suppression and rate of recovery of HPA axis depend on the dose and duration of prednisolone intake. However, there is a possibility to stimulate or inhibit the recovery of the HPA axis by the simultaneous administration of certain drugs. We investigate the effect of caffeine or diazepam intake on the recovery of suppressed HPA axis function from prednisolone therapy. We included 45 male subjects with minor diseases were divided into three groups. Group (1) 15 patients received orally prednisolone (10 mg/day) at morning beside a suitable placebo for 14 days. Then prednisolone was stopped while the placebo continued for other 10 days. Group (2) 15 patients received orally prednisolone (10 mg/day) combined with caffeine (300 mg/day orally) at morning for 14 days. Then prednisolone was stopped while caffeine intake continued for other 10 days. Group (3) 15 patients received orally prednisolone (10 mg/day) at morning and diazepam (5 mg/day) at night for 14 days. Then prednisolone was stopped while diazepam continued for other 10 days. The HPA axis function was assessed before therapy (basal), after 14 days of the designed therapy (single prednisolone, prednisolone+caffeine or prednizolone+diazepam) and after prednisolone stoppage and maintenances of the respective group on placebo or caffeine or diazepam only for 10 days. Results: In 1st group at the 1st day of recovery from prednisolone therapy, serum cortisol and plasma ACTH levels were significantly decreased from their basal pretreatment levels ($P<0.01$). After 5 days of prednisolone stoppage, serum cortisol and plasma ACTH concentrations were significantly increased than their 1st day stoppage levels ($P<0.01$) but still significantly lower than the basal value ($P<0.01$). At the 10th day after prednisolone cessation the circulating cortisol and ACTH levels were significantly higher than their 5th day levels ($P<0.01$), the levels almost returned to their basal. In the 2nd group by the end of combined prednisolone+caffeine therapy, endogenous cortisol and ACTH concentrations were significantly lower from their basal values. After 5 days of recovery with maintained caffeine intake only, serum cortisol and plasma ACTH levels increased to their basal levels. Caffeine significantly enhanced the recovery of the prednisolone-suppressed HPA axis. On the other hand, in the 3rd group simultaneous intake of prednisolone with diazepam for 14 days induced significant reduction of both serum cortisol and plasma ACTH concentrations. During the recovery period (10 days) after stopping prednisolone only while diazepam intake was continued, serum cortisol and plasma ACTH concentrations were still significantly decreased than their basal values. Diazepam intake did not significantly enhance the correction of the prednisolone-mediated suppression of the HPA axis. Conclusion: Simultaneous intake of caffeine with prednisolone enhanced recovery of the investigated HPA components (ACTH and cortisol). On the other hand, diazepam intake did not affect significantly prednisolone suppression of the HPA axis and recovery was delayed.

Keywords: Hypothalamo - pituitary - adrenocortical axis, corticotropin releasing factor, adrenocorticotropin, recovery, anxiety disorders, caffeine, diazepam.

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INTRODUCTION

The circulating prednisolone concentration exerts a significant negative feedback effect on both continuous and pulsatile pituitary adrenocorticotropic hormone (ACTH) and also on the secretion of hypothalamic corticotropin releasing factor (CRF). CRF is controlled through the adrenal cortex cortisol, the pituitary ACTH and suprapituitary CNS components including a rapid or intermediate temporal and higher CNS domain (Dorm et al, 1996). The recorded recovery period after chronic prednisolone intake was two weeks after termination of daily administration. At this time there was no significant difference between means in baseline ACTH and cortisol, post CRF stimulation ACTH -cortisol, or post ACTH stimulation cortisol values, compared with values in normal controls. These results indicate complete HPA axis recovery by this time (Moore and Hoenig, 1992). In literature, there is no controversy about recovery after prednisolone administration but the disagreement exists about the date of beginning of recovery of the HPA axis from the prednisolone suppression. Caffeine (1, 3, 7 trimethylxanthine) is a stimulant of CNS, it is consumed daily by about 85% of adults in coffee, tea and soft drinks (Vikas et al., 2018), according to this fact caffeine is one of the most usually used substance (David et al., 2018). Controversy about the effective dose, strength and duration of caffeine activity on HPA axis is obvious. The responsiveness of the HPA axis was particularly obvious in those who consuming moderate doses (300 mg per day) of caffeine but was abolished in those consuming high doses (600 mg/day) of it (Lovallo et al., 2005). Oral caffeine intake by habitual coffee drinkers (300 mg/day) did not affect urinary cortisol excretion (Lane et al., 2002). Alternatively, habitual caffeine consumption can reduce, but not eliminate cortisol secretions in healthy subjects (Lovallo et al., 2005). On the other hand, caffeine intake increased cortisol levels but only during stress (Shepard et al., 2000). Moreover, caffeine in dietary doses (3.3 mg/kg/day) increased adrenocorticotropin and cortisol secretions in humans whether at rest, mental stress or psychomotor task (Michael, 2010). In this respect, caffeine not only activates the stress components and thus elevates glucocorticoid and catecholamine outputs (Lane et at., 2002; al'Absi and Lovallo, 2004), but also lowers the threshold for stress induced cortisol release (Laurent et al., 2000; Eynav, 2017). Benzodiazepines (BZPs) are clinically effective anti-anxiety drugs. The high potency BZPs such as alprazolam suppress the activity of the HPA axis, reducing basal ACTH and cortisol release, following both acute (Curtis et al., 1997; Pomara et al., 2004) and chronic administrations (Abelson et al., 1996; Fukuda et al., 1998; Pomara et al., 2004). This is because BZPs attenuate stress-induced activation of the HPA axis (Judd et al., 1995; Santagostino et al., 1996; Joyner et al., 1998; Pruneti et al., 2002; Maria et al., 2010), In contrast, low potency BZPs, such as diazepam, influence on HPA axis activity showed disagreement. It depresses the axis only in individuals experiencing stress (De Souza, 1990; Vongsavan et al., 1990; Roy-Byrne et al., 1991; Svob et al., 2012). Acute diazepam intake decreased plasma cortisol concentration which is a consequence to decreased activity of the HPA axis, especially in individuals experiencing stress. On the other hand, during chronic diazepam treatment for (> 2 weeks), the elderly subjects' experienced significant reductions in plasma cortisol levels, but the youth did not (Roy-Byrne et al., 1991; Cowley et al., 1995). Moreover, both dosage and duration of diazepam was not related to the drug effects on sedation and tension (Pomara et al., 2005). Otherwise, some studies elucidate an effect of diazepam on the activity of HPA axis in non-stressed individuals (Pomara et al., 2005). Long-term antidepressant treatment in human upregulates the HPA axis, whereas short-term treatment downregulates it (Mason and Pariante, 2006). Normal HPA axis plays a role in the mechanism(s) of action of antidepressants (Papiol et al., 2007). Although the role of diazepam effect on HPA axis in healthy or psychological subjects has been studied, almost none has been studying diazepam effect on individual with HPA axis suppression due to chronic prednisolone administration.

Aim of the study

Clarifying the effect of intake of either caffeine (300 mg/day at morning) or diazepam (5.0 mg/day at night) on the recovery of hypothalamo-Pituitary-Adrenocortical (HPA) axis function which suppressed by chronic prednisolone therapy (10 mg/day at morning for two weeks).

SUBJECTS AND METHODS

Forty five male patients were recruited from the Outpatient Clinics of Mansoura University Hospitals. All of them were not yet receiving any anti-inflammatory glucocorticoid (cortisol, prednisolone, prednisol, and dexamethasone ..... etc.) for different diseases. Each patient gave informed consent to participate in this study after the purpose, nature and potential risks of the study were explained to him. All volunteers were nondiabetics, normotensives (caffeine increases blood pressure) and nonsmokers (nicotine decreases caffeine

half-life). All participants were free from significant medical (cardiac, CNS, respiratory, hepatic or renal) disease as determined by medical history, physical examination, electrocardiogram, and routine laboratory tests. None of the subjects gave a history of receiving medications that interfere with the study (e.g. sedatives, and hypnotics as barbiturates, morphine, phenothiazine, meprobamate, CNS depressants or stimulants, drugs of abuse, broad spectrum antibiotics, spironolactone, quinidine, reserpine and hormonal therapy) for at least one week prior to participation in the study. These subjects were classified into; Group (1): 15 male patients suffering from postoperative uveitis (Ophthalmic Center Mansoura University). These patients received at morning 10 mg prednisolone (prednisolone 21-sodium succinate) orally for 14 days as an essential therapeutic measure in their medication maintenance dosage (10 mg/day for 13 days) and withdrawal regimen (5 mg/day for 2 days). In addition, a placebo (fruit juices) was taken during therapy and for 10 days after prednisolone stoppage. Group (2): 15 male patients were suffering from rheumatoid arthritis (Internal Medicine Outpatient Clinic, Mansoura University) who received oral prednisolone (10 mg/day) together with oral caffeine (300 mg/day) at morning for 14 days. After prednisolone stoppage, the patients continued only caffeine consumption for other 10 days. To avoid significant caffeine withdrawal effects, all subjects ended the protocol within 2 days tapering caffeine doses (200 mg and 100 mg/daily). Group (3): 15 male patients with anxiety disorders (AD) due to skin disease obtained from Outpatient Clinic, Mansoura University Hospital. These subjects had urine screens for drugs of abuse, which proved negative. The participants received a single oral dose of diazepam (5.0 mg) per nightly (at 10 p.m.) in addition to prednisolone treatment (10 mg daily for 13 days and 5 mg daily for 2 days) for two weeks.

Blood sampling

Four blood samples were obtained from every patient included in the study as follow: (1) Basal blood sample (5.0 ml) was obtained from every patient before the designed therapy course between (9.00 and 10.00 A.M) to minimize variation due to the circadian rhythm of ACTH and cortisol secretions. (2) A second blood sample was obtained from every patient at the day of cessation of prednisolone therapy (2 weeks). (3) A blood sample obtained at the mid-recovery period in the 5^{th} days after prednisolone cessation. (4) Blood sample at the end of recovery period i.e. immediately after placebo, caffeine or diazepam discontinuation.

Of the 5.0 ml blood, 2.5 ml were added into an iced EDTA tube that was immediately immersed in an ice bath. Then plasma was separated by refrigerated centrifuge and aliquots were added directly into 2 clean plastic tubes, each contained about 250µl. Both tubes were kept at -80°C deep freeze till used. Before biochemical assay, samples were thawed in an ice bath and kept at 4.0°C all the times. Plasma ACTH concentrations were determined in duplicate by solid phase-two sites sequential chemiluminescent immunometric assay (Irvine et al., 2016). The remaining 2.5 ml blood were left in a water bath at Results 37°C for about half an hour and serum was separated by centrifugation at 3500 r.p.m. The obtained serum was added into two Eppendorf tubes, each contained about 250µl serum and kept in a deep freeze -80°C till analysis. Serum cortisol concentrations were estimated in duplicate by the solid phase competitive chemiluminescent enzyme immunoassay method (Damian et al., 2012). Both plasma ACTH and serum cortisol were estimated using the Immulite apparatus (Diagnostic Products Corporation, DPC; USA). Normal A.M range of plasma ACTH concentration is up to 46.0 pg/m); while normal A.M. range of serum cortisol concentration is 5.0 – 25.0 ug/dl. Lipemic or hemodialyzed plasma or serum was excluded from the study. The exogenous prednisolone therapy does not interfere with these specific method used for cortisol estimation.

The data were presented as (means±SD). Significant effects were obtained by student t-test. Differences were considered significant when reaching $P<0.01$ or less. Statistical analysis was done using a personal computer SPSS-version 24 (SPSS, Armonk, NY: IBM Corp).

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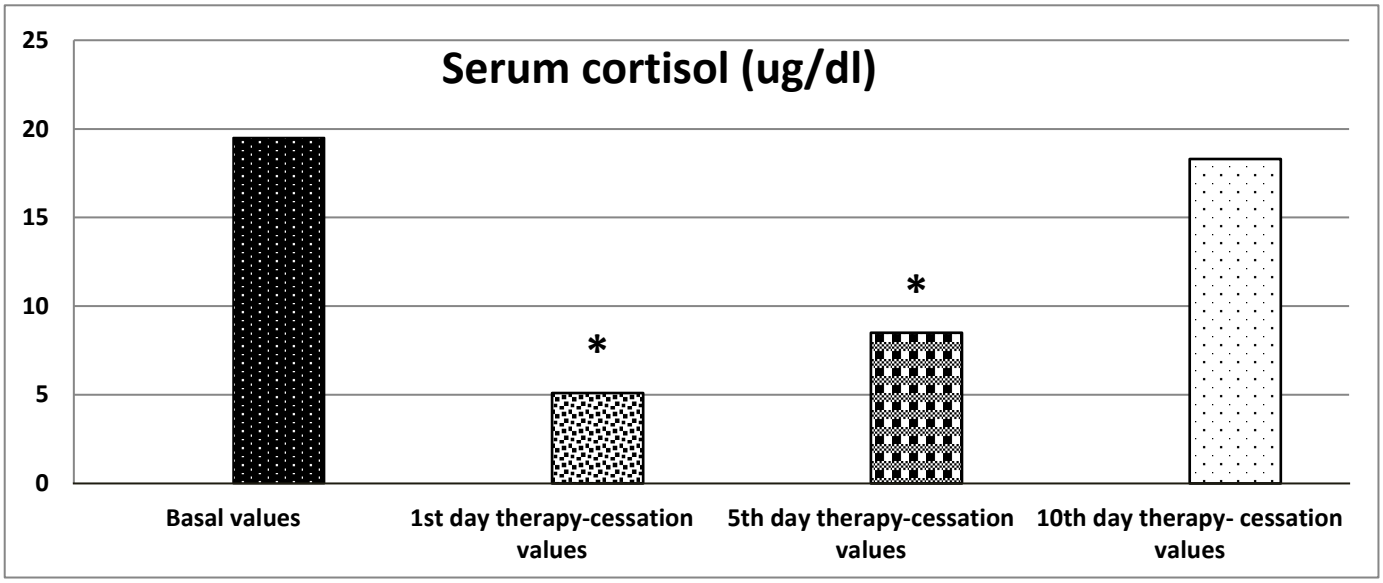
RESULTS

Results are presented in the following figures:

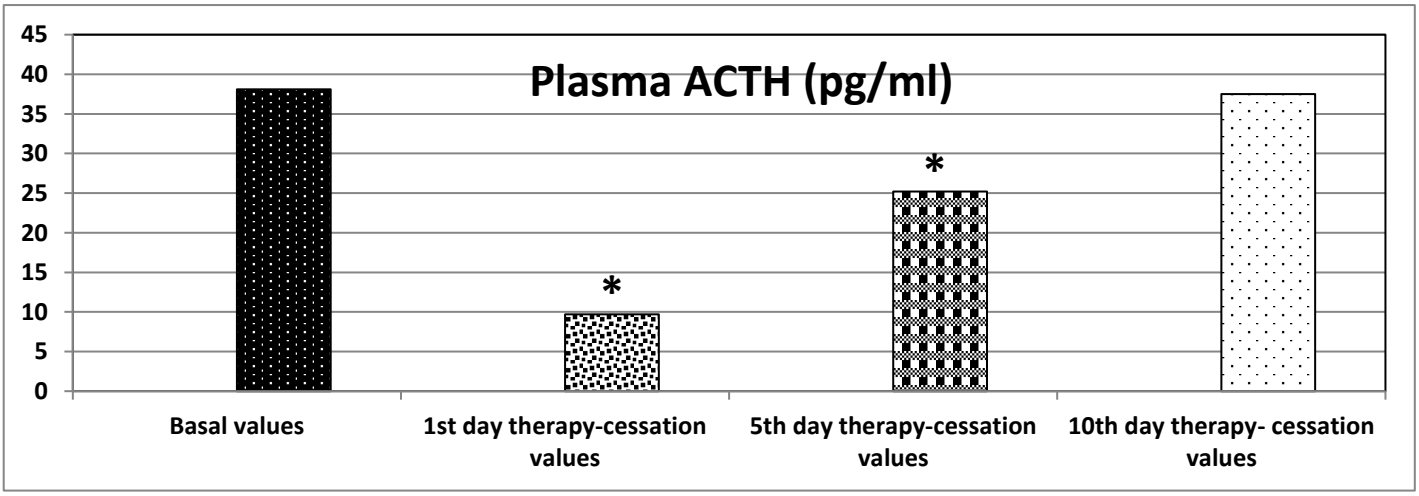
Figure (1): Serum cortisol (ug/dl) levels by (mean±SD) basal, after prednisolone therapy and in the 5th and 10th days of the recovery period.



*** Significant different from basal**.

Figure (1) shows that serum mean cortisol level was significantly decreased by prednisolone therapy than the mean basal pretreatment level (5.1±0.3 vs 19.5±2.3 ug/dl; p<0.01). After 5 days of prednisolone stoppage but maintaining the additive placebo, serum mean cortisol concentration was increased than the 1st day recovery mean level (8.5±1.1 vs 5.1±0.3 ug/dl; P<0.01). Moreover, the cortisol level after 10 days of prednisolone stoppage was significantly higher than that noted after the 5 days (18.3±2.0 vs 8.5±1.1 ug/dl; p<0.01).

Figure (2): Plasma ACTH (pg/ml) levels by (mean±SD) basal, after 14 days of prednisolone therapy and in the 5th and 10th days of the recovery period.



*** Significant different from basal**.

Figure (2) shows that plasma ACTH mean level was significantly suppressed at the end of prednisolone therapy period than the mean basal pretreatment level (38.1±5.8 vs 9.7±2.6 pg/ml; p<0.01). Thereafter, suppression of plasma ACTH mean level was still significantly decreased at 5th day of recovery than its basal value (25.2±4.5 vs

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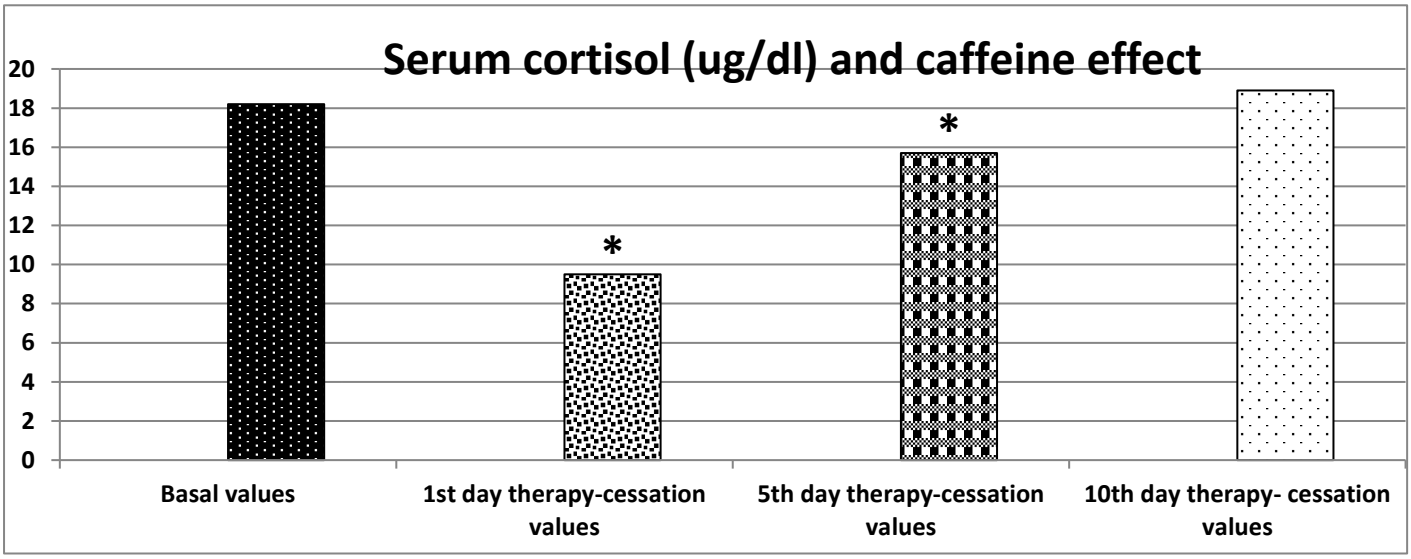
38.1±5.8 pg/ml; P<0.01), but significantly higher than the first day recovery (25.2±4.5 vs 9.7±2.6 pg/ml; P<0.01), while it had been almost normalized at the 10th day of stopping prednisolone (37.5±4.9 vs 38.1±5.8 pg/ml).

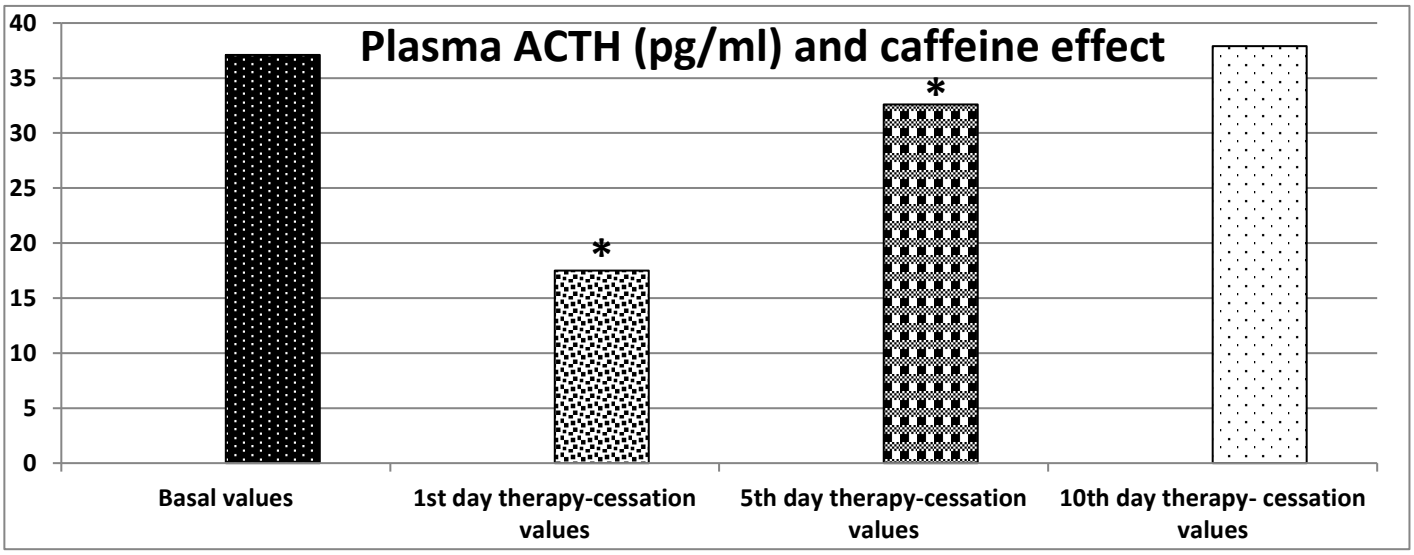
Figure (3): Serum cortisol (ug/dl) levels by (mean±SD) basal, immediately after prednisolone stoppage and in the 5th and 10th days of caffeine intake.



*** Significant difference from basal value**

Figure (3) the results showed that serum cortisol levels immediately after prednisolone cessation were significantly lower than the corresponding basal levels (9.5±2.0 vs 18.2±2.5 ug/dl P<0.01). On the other hand, serum cortisol levels in response to continued caffeine intake for 5-days of recovery were significantly higher than their levels at prednisolone stoppage (15.7±2.6 vs 9.5±2.0 ug/dl P<0.01). Moreover, serum cortisol in the recovery period 10th day showed more increase that may exceed their respective basal levels (18.9±2.4 vs 18.2±2.5 ug/dl P<0.01).

Figure (4): plasma ACTH (pg/ml) levels by (mean±SD) basal, immediately after prednisolone stoppage and in the 5th and 10th days of caffeine intake.



*** Significant difference from basal value**

Figure (4) the results showed that plasma ACTH levels immediately after prednisolone cessation were significantly lower than the corresponding basal levels (17.5±3.2 vs 37.1±4.9 pg/ml P<0.01). On the other hand, plasma ACTH levels in response to continued caffeine intake for 5-days of recovery were significantly higher than their levels at

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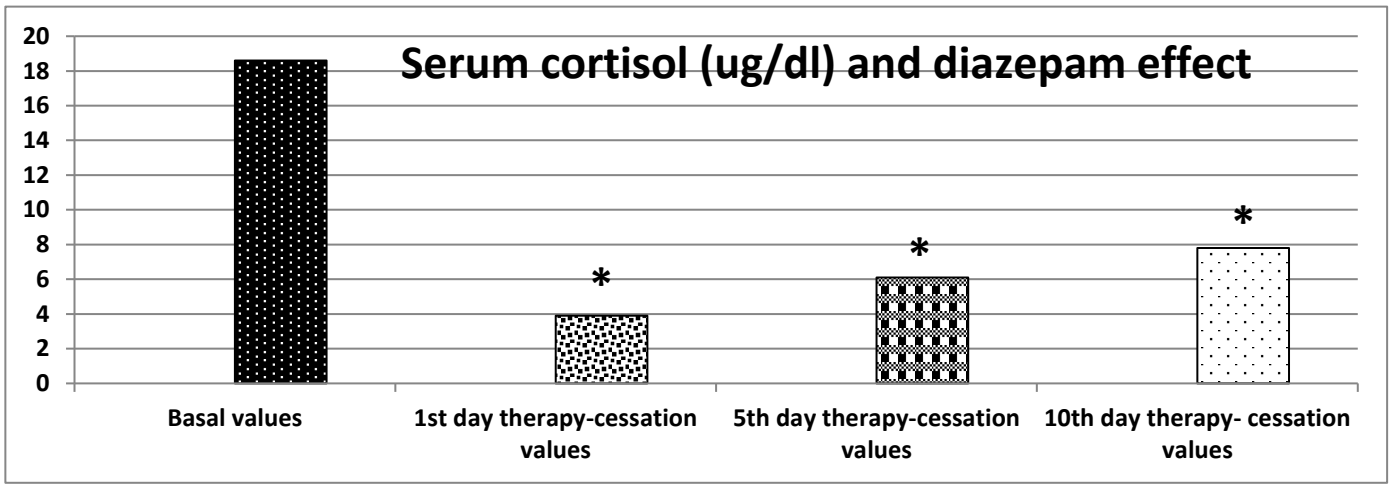
prednisolone stoppage (32.6±5.9 vs 17.5±3.2 pg/ml P<0.01). Moreover, serum cortisol in the recovery period 10th day showed more increase that may exceed their respective basal levels (37.9 ±6.3 vs 37.1±4.9 pg/ml P<0.01).

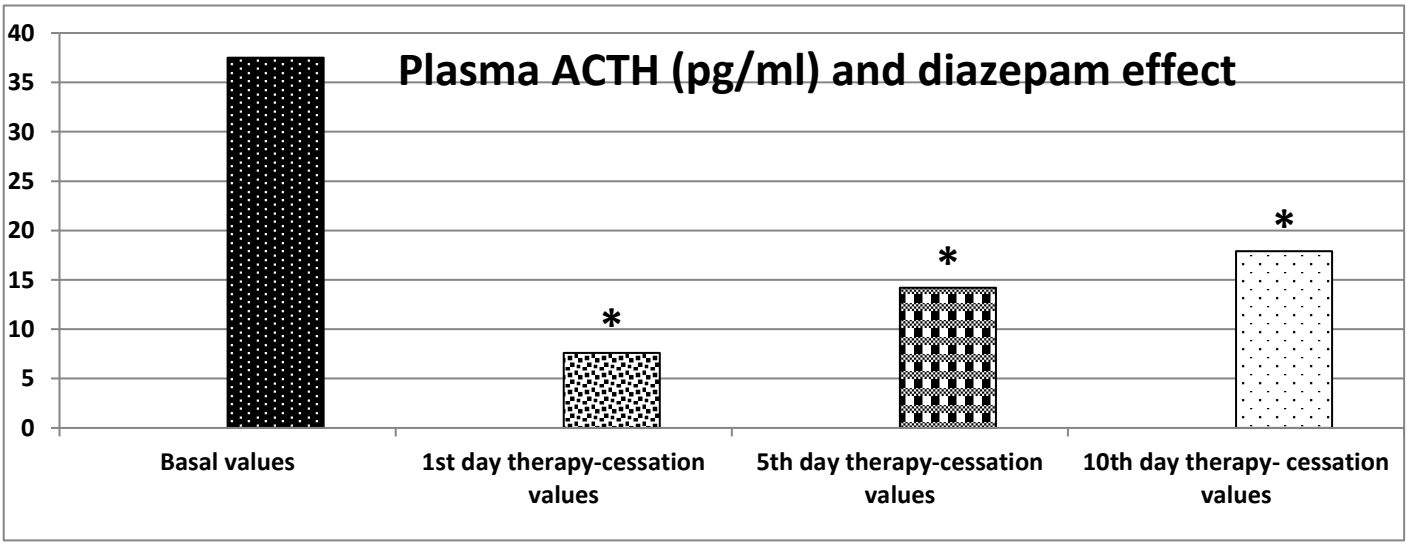
Figure (5): Serum cortisol (ug/dl) levels by (mean±SD) basal, immediately prednisolone stoppage and in the 5th and 10th days of recovery on diazepam intake.



*** Significant difference from basal value**

Figure (5) illustrates the results of the associating intake of prednisolone with diazepam. The intake of both these drugs in combination induced HPA axis inhibition. On prednisolone stoppage (3.9±0.4 vs 18.6±2.0 ug/dl p<0.01), the recovery of HPA axis function was extremely low due to diazepam continuous intake. So, mean serum cortisol levels immediately after prednisolone stoppage were significantly lower than the basal value P<0.01. At the 5th day of the recovery period, the serum cortisol levels were significantly higher than the corresponding value, found immediately after prednisolone stoppage levels (6.1±0.7 vs 3.9±0.4 ug/dl p<0.01). At the 10th recovery day under diazepam only, serum cortisol levels were significantly lower than the initial basal values (7.8±1.5 vs 18.6±2.0 ug/dl p<0.01).

Figure (6): Plasma ACTH (pg/ml) levels by (mean±SD) basal, immediately prednisolone stoppage and in the 5th and 10th days of recovery on diazepam intake.



*** Significant difference from basal value**

Figure (6) illustrates the effect of the associating intake of prednisolone with diazepam on plasma ACTH levels. The intake of both these drugs in combination induced HPA axis inhibition. On prednisolone stoppage (7.6±2.3 vs 37.5±4.7 pg/ml p<0.01), the recovery of HPA axis function was extremely low due to diazepam continuous intake.

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So, mean plasma ACTH levels immediately after prednisolone stoppage were significantly lower than the basal value P<0.01. At the 5th day of the recovery period, the plasma ACTH levels were significantly higher than the corresponding value, found immediately after prednisolone stoppage levels (14.2±2.9 vs 7.6±2.3 pg/ml p<0.01). At the 10th recovery day under diazepam only, plasma ACTH levels were significantly lower than the initial basal values (17.9±2.1 vs 37.5±4.7 pg/ml p<0.01).

Figure (7) Percent of mean change in serum cortisol levels

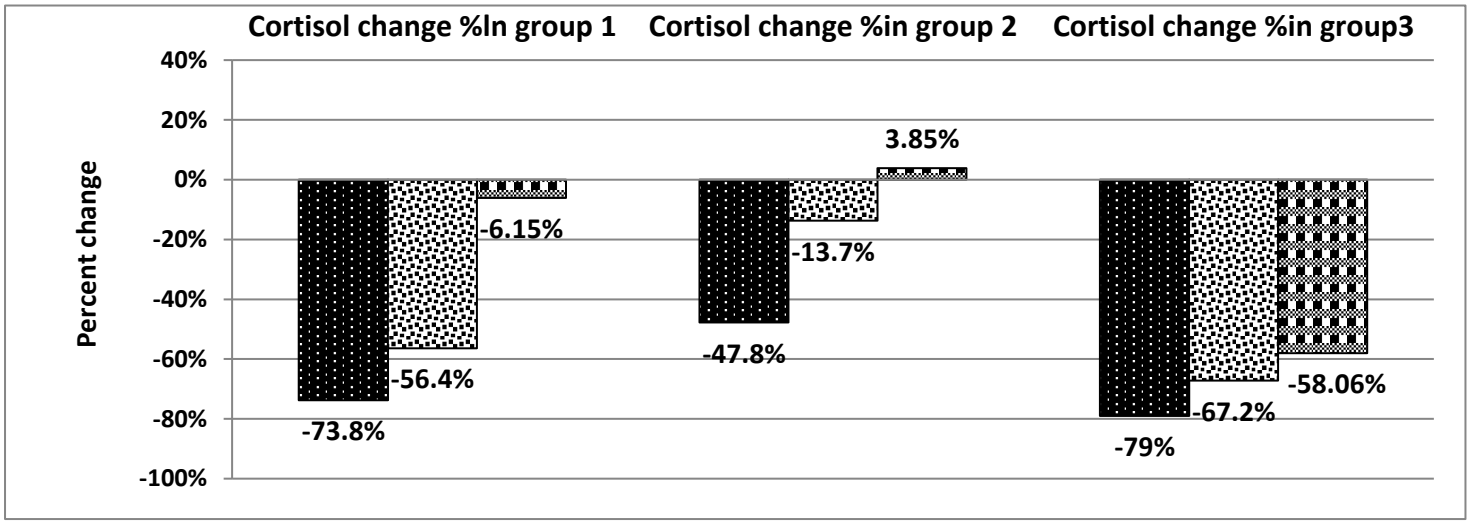

Figure (7): Percent of mean change in serum cortisol levels from baseline in group1 (prednisolone after 1st day, 5th day stoppage, 10th day stoppage), group2 (prednisolone+caffeine, 5th day caffeine only, 10th day caffeine only), and group3 (prednisolone+diazepam, 5th day diazepam only, 10th day diazepam only).

Figure (8) Percent of mean change in plasma ACTH levels

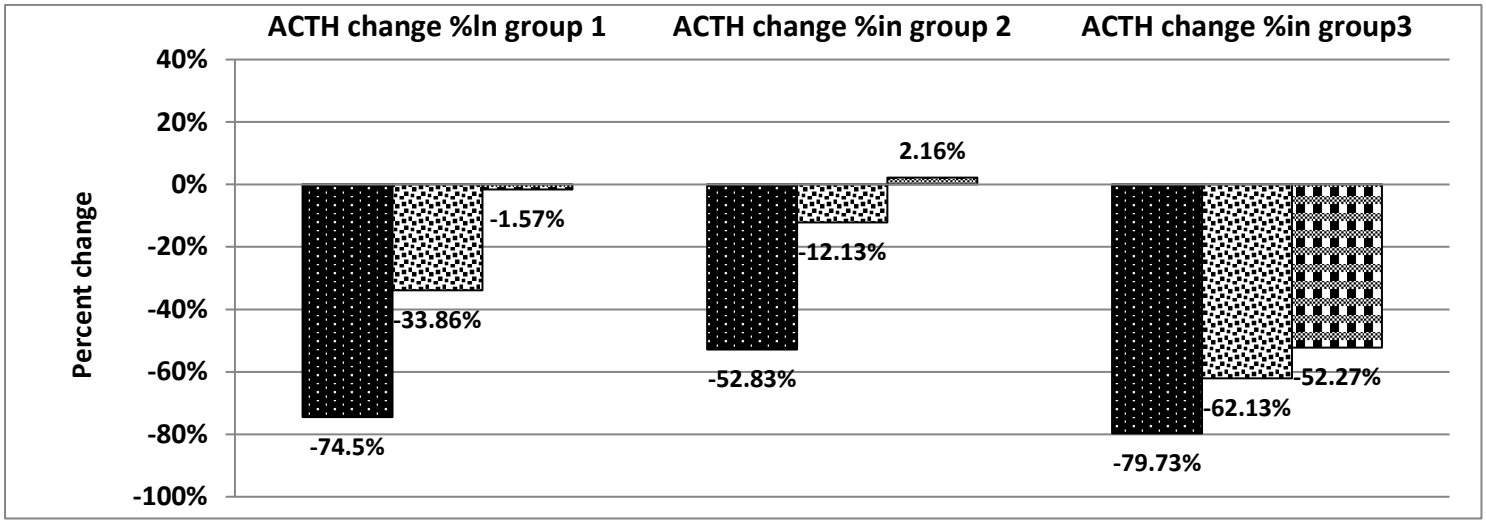

Figure (8): Percent of mean change in plasma ACTH levels from baseline in group1 (prednisolone after 1st day, 5th day stoppage, 10th day stoppage), group2 (prednisolone+caffeine, 5th day caffeine only, 10th day caffeine only), and group3 (prednisolone+diazepam, 5th day diazepam only, 10th day diazepam only).

DISCUSSION

The present investigation was performed using 14-day course of prednisolone with placebo, caffeine or diazepam. Then, placebo, caffeine or diazepam consumption continued for a further 10 days after stoppage of

prednisolone. Serum cortisol and plasma ACTH levels were determined before and after each step. Serum cortisol and plasma ACTH levels remained relatively suppressed up to 5 days after prednisolone withdrawal but they recovered almost their basal value at the10th day of prednisolone stoppage (Figure 1&2). However, the pattern of recovery of the HPA axis from prednisolone inhibition in patients depends on both dose and duration of steroid administration. Chronic elevations of blood prednisolone concentration following its intake may have implications on long-term health (Karcz-Kudlcha et al., 2003; Merih et al., 2016). This drug can alter the immune-system responses, and induce depression (Agnes and Karen, 2011) and some central nervous system untoward effects (Buchanan and Lovallo, 2001; Ciriaco et al., 2013). Caffeine ingestion stimulates the HPA axis (Lane, 1994; Karcz-Kubicha et al., 2003). Physiologically caffeine increases alertness, elevates mood and modulates fatigue perception in addition to its ability to alter energy substrate metabolism by enhancing fat utilization and thereby sparing muscle glycogen store (Spriet et al,, 1992; Laurent et al., 2000; Messina et al., 2015). Alternatively, caffeine intake may exaggerate some deleterious effects particularly on the cardiovascular and neuroendocrine systems. On the other hand, caffeine abstinence is associated with symptoms of caffeine withdrawal e.g. sleep desire, litharge, headache, and reduced socialization (Duncan et al., 2017). In practical terms, tolerance to caffeine's physiological effects is incomplete at the levels of consumption of < 300 mg/day. It is likely that an overnight caffeine abstinence is partially sufficient to overcome tolerance formation (Griffiths and Chausmer, 2000; Watson et al., 2002). Caffeine function on the adenohypophysis -adrenal cortex axis was mirrored by highly significant rise of the circulating cortisol and ACTH (Figure 3&4). The mechanism of the stimulatory effect of caffeine on the HPA axis might involve several steps: (1) an activating action on central neurotransmitter pathways causing increased concentrations and turnovers of serotonin and norepinephrine, which can result in increased CRF activity (Hirsh, 1984; Michael et al., 2006). (2) Direct stimulating effect on ACTH release by the anterior pituitary and on cortisol release by the adrenal cortex (Nicholson et al., 1989; Michael et al., 2006). In the present study consumption of caffeine was able to enhance the recovery of the prednisolone-induced suppression of the HPA axis. Caffeine may increase the speed of recovery of the HPA axis after discontinuation of prednisolone consumption. When 10 days of recovery from prednisolone-uptake were allowed, continuing caffeine intake resulted in normalization of serum cortisol and plasma ACTH concentrations. The present study results suggested that the effect of caffeine consumption during the recovery of the HPA axis from prednisolone intake could be of beneficial value in patients under investigation. In this field, literature showed obvious controversy. So, oral caffeine intake (300 mg/day) did not affect urinary cortisol excretion (Lane, 1994; Lane et al., 2002). Alternatively, cortisol responses to daily caffeine consumption (300 mg/day) were reduced, but not eliminated, in healthy drinkers (Lovallo et al, 2005). However, caffeine intake can increase cortisol levels but only during stress (Shepard et al., 2000). On the other hand, dietary doses of caffeine (3.3 mg/kg/day) elevated plasma ACTH and cortisol levels whether at rest, mental stress or psychomotor state (Michael, 2010), Moreover, an elevation of cortisol may occur in the afternoon hours in those consuming repeated caffeine doses throughout the day (Lovallo et al., 2005). This wide controversy can be observed in the evident responsiveness of the HPA in persons consuming moderate doses (300 mg per day) of caffeine but was abolished by showing almost HPA stimulation in those consuming high doses (Lovallo et al., 2005). Benzodiazepines exert a number of effects, including suppression of HPA axis (Cowley el al., 1995). High potency benzodiazepines (BZPs) as alprazolam suppress the activity of the HPA axis and thus reduce basal ACTH and cortisol release, following their acute or chronic administrations (Arvat et al., 2002; Pomara et al., 2004). This is because BZPs attenuate stress-induced activation of the HPA axis (Pruneti et al., 2002). In contrast, low potency BZPs, such as diazepam attenuates HPA axis activity only in individuals experiencing stress (Maria et al., 2010). However, some recent studies demonstrated an effect of diazepam on HPA axis activity in non-stressed individuals (Pomara et al., 2005). Moreover, early studies have shown short-term stimulatory effect of diazepam on the HPA axis in man (Laakmann et al., 1984; Shimoda et al., 1988; Vargas et al., 2001). The effects of the simultaneous intake of diazepam with prednisolone for 14 days on the pituitary adrenocortical axis were presented in (Figure 5&6). Serum cortisol and plasma ACTH concentrations were significantly decreased at the end of prednisolone together with diazepam therapy course ($P<0.01$). Thereafter, during the 10 days recovery period after stopping prednisolone while diazepam intake continued as a sole therapy, serum cortisol and plasma ACTH levels were still significantly lower than their corresponding basal values but higher than those noted with combined diazepam and prednisolone therapy. During chronic diazepam treatment (>2 weeks), the elderly (> 60 year old) experienced significant reductions in plasma cortisol levels, but the youth (< 35 year old) did not. This effect was not modulated by anxiety disorder (AD) status, diazepam dosage (dose or duration) or drug effects on sedation and tension (Pomara et al., 2005). In this respect, outpatient individuals with AD had comparable baseline cortisol levels as controls (Pomara et al., 2005), so, presence of generalized anxiety disorders (GAD) did not modify cortisol response to diazepam (Cowley et al. 1995; Pomara et al., 2004). Although,

benzodiazepines are much safer than barbiturates, when benzodiazepines are abruptly discontinued, a withdrawal syndrome consisting of a variety of adverse physiological and behavioral reactions ensues (anxiety, HPA axis, and autonomic system activation). These side effects strongly resemble the stress response (Malcolm and Andri, 2016) and the acute phase withdrawal reactions from several drugs that produce dependence as cannabis, cocaine, ethanol, and morphine (Motaghinejad et al., 2016).

Conclusion and Recommendations

- Chronic prednisolone intake decreased ACTH and cortisol concentrations which were maintained for at least 5 days after drug stoppage.
- Simultaneous intake of caffeine with prednisolone and its continued intake after prednisolone stoppage enhanced and accentuated the rate of recovery.
- Simultaneous intake of diazepam with prednisolone and its continuous intake after prednisolone cessation impaired the recovery of blood ACTH - cortisol concentration to normal state.

So while caffeine intake favour good recovery of hypothalamo-Pituitary-Adrenocortical (HPA) axis function from steroid suppression, diazepam intake impaired this process.

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