Effects of Chronic Usage of Tramadol, Acetaminophen and Tramacet on Some Biochemical and Immunological Changes in Male Rats

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ABSTRACT: In recent years, tramadol is a widely used as an effective analgesic opioid agent for the treatment of moderate to severe pains. The study was under taken to evaluate the biochemical changes, potential immunological profiles and antioxidant stress biomarkers after chronic usage of tramadol, acetaminophen (APAP) and Tramacet. Sixty-four male adult albino rats were divided into four groups, control group, tramadol group, acetaminophen group and Tramacet group; half of the animals from each group were sacrificed after 30 days of treatment to determine serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine and blood urea nitrogen (BUN), as well as the pro-inflammatory cytokines. The other half of the animals from each group were kept for another one month later representing withdrawal groups to study changes on the above mentioned parameters after drugs withdrawal. Tramadol, acetaminophen and tramacet, caused a marked liver and kidney damage as noted by significant increase in the activities of serum AST, ALT and LDH as well as the levels of BUN and creatinine. On the other hand, acetaminophen and Tramacet showed significant increases in both interferon-gamma (IFN-γ) and interleukin-1 beta (Il-1β). Moreover, administration of the studied medications resulted in significant decreases in liver glutathione (GSH), catalase (CAT) content and superoxide dismutase (SOD) activities which were parallel to an increase in malondialdehyde (MDA) levels. The data obtained exhibited that the acetaminophen had more potent effects on most studied parameters than tramadol and tramacet. The data recorded in the recovery period could be explained in view of the most potent effect of acetaminophen on all studied parameters than the two other utilized drugs, more time may be needed to recovery.
Keywords: Tramadol - Acetaminophen - Tramacet (tramadol/acetaminophen) - Liver toxicity - Kidney toxicity - Antioxidant system - Interferon-gamma (IFN-γ) - Interleukin-1β (IL-1β).

INTRODUCTION:

The central role of liver and kidney in drug metabolism predisposes them to toxic injury (Atici *et al*., 2005). Every drug has been associated with hepatotoxicity almost due to the pivotal role of the liver in drug metabolism. Hepatic metabolism is a first and foremost mechanism that converts drugs and other compounds into products that are more easily excreted. A metabolite may have higher activity and/or greater toxicity than the original drug (Tolman, 1998). Metabolites of the drugs that are excreted from kidneys may also cause cellular damage leading to kidney dysfunction (Singhal *et al*., 1998; Atici *et al*., 2005).

Tramadol is a centrally acting analgesic drug (McClellan and Scott, 2003; Johnson, 2005) with a partial affinity for the opiate receptor (μ-Mu), having analgesic potency estimated to be one tenth that of morphine (Leo *et al*., 2000). Although tramadol binds weakly to μ-opioid receptors (Raffa, 2001), it exhibits cross-tolerance with morphine in rats (Kayser *et al*., 1991) suggesting an opioid-mediated mechanism of analgesia. Tramadol is usually used in parenteral and oral routs for moderate to severe pain treatment (Johnson, 2005; Gillman, 2005). Tramadol bioavailability is in the range of 70-80%, reaching peak blood level within 2 hours after an oral dose. The drug is converted in the liver to at least one active metabolite (O-dimethyl tramadol; M1), which itself is 2 to 4 times more potent than tramadol (Wu *et al*., 2001; Tao *et al*., 2002). Moreover, its affinity for the μ-opioid receptor is only 1 in 6000 that of morphine, a metabolite of tramadol may account for part of the analgesic effect. Tramadol has a dose-dependent analgesic efficacy that lies between that of codeine and morphine, with a parenteral potency comparable to that of pethidine, i.e. about 10-20% of the standard morphine. Tramadol and its metabolites are mainly excreted *via* the kidneys with the mean elimination half-life of about 5 hours. When taken in overdose, it is known to be associated with significant morbidity and mortality.

Acetaminophen (paracetamol) is a non-opiate, non-salicylate analgesic the terms "paracetamol" and "acetaminophen" reflect only geographical differences: "acetaminophen" is the term used in the USA, Canada, Hong Kong, Iran and certain Latin American countries, such as Colombia, while "paracetamol" is used in Europe, Africa and most of Asia. The drug is sometimes abbreviated to "APAP" in all geographic regions. Acetaminophen, is metabolized mainly by conjugation with sulfate and glucuronide, with about 5% to 10% of the drug oxidized by the cytochrome P450 metabolic pathway to a toxic electrophilic metabolite, which is subsequently detoxified by glutathione and eliminated in the urine or bile. If any residual of this toxic electrophilic metabolite is not

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detoxified in this manner, it may bind to hepatocytes, where it can lead to cellular necrosis (Heard *et al*., 2012), one of the most common causes of acute liver failure (Bower *et al*., 2007). Paracetamol has also been linked to hypertension (Wilson and Poulter, 2006; Forman *et al*., 2007; Sudano *et al*., 2010) which is probably caused by the considerable sodium content present in each paracetamol tablet.

The term Tramacet is commonly used in Canada, while Ultracet is the term used in U.S. Tramacet is a fixed combination analgesic, containing 37.5 mg tramadol plus 325 mg acetaminophen. Opioid analgesics are effective but are associated with adverse events as well as concerns over tolerance and addiction, finding an analgesic product that offers both effective pain relief and a good safety profile has led to increasing interest in combination products. Combining analgesics may allow for lower doses of the individual agents, with doses possibly low enough to significantly reduce potential adverse events. While the theory of combination analgesic products holds promise, combination products require rigorous scrutiny and testing since not all combinations are ideal. Combining two or more agents may result in an additive or synergistic analgesic effect (Raffa *et al*., 2010).

Recently, new combination analgesic products based on scientifically reasonable design have been introduced to offer effective analgesia with a good/benefit ratio. The combination product tramadol/ acetaminophen may be an important aid for the treatment of acute and chronic pain syndromes. Pain involving multiple mechanisms, can be safely and effectively treated with combination analgesics, for example, tramadol/acetaminophen (Freeman *et al*., 2007; Ko *et al*., 2010). In comparative study, the current investigation throws the light on the effect of the chronic usage of these medications on liver and kidney functions, potential immunological changes determining, interferon-gamma (IFN-γ) and interleukin-1 beta (IL-1β) as well as the antioxidant stress biomarkers after 30 days treatment and 30 days recovery period after last dose.

MATERIALS AND METHODS:

Ethics statement

All animal experiments were approved and carried out according to the Guide for the Care and Use of Animals, Animal Care Committee of Tanta University.

- Drugs

Tramadol: Tramadol hydrochloride (Tamol-X tablets, 225 mg, Royal Hamburg, Germany).

Acetaminophen: **(**Paracetamol, 325 mg, Tylenol capsule (APAP), McNeil Healthcare, LLC, USA).

Tramacet (tramadol/acetaminophen): It's a combination of tramadol and acetaminophen. Tramacet was prepared by dissolving the equivalent therapeutic doses of each tramadol hydrochloride (37.5 mg/kg) and acetaminophen (325 mg/kg) in saline

- Experimental protocol

Sixty-four adult male albino rats, with initial body weight ranging between 180-200 g were used. They were obtained from Breeding Unit of the Egyptian Organization for Vaccine and Biological preparation. As a standard protocol, all the rats were housed in a quiet non-stressful environment of acclimatization period for one week before the study. Rats were divided into four groups. The first group (control group, n = 16) received physiological saline by oral gavage, the second group (tramadol group, n = 16) received tramadol hydrochloride (37.5 mg/kg body wt.), the third group, (acetaminophen group, n = 16) received acetaminophen (325 mg/kg body wt.). The fourth group, (Tramacet group, n = 16) received tramadol/ acetaminophen preparation. All treatments were given orally, three times daily for one month. These daily doses were calculated for rats according to Paget and Barnes (1964) conversion Tables.

Half of the animals from each control and treatment groups were sacrificed after 30 days of treatment to determine serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine and blood urea nitrogen (BUN) activities, as well as the pro-inflammatory cytokines. The other half of the animals from each group were kept for another one month later without treatment representing withdrawal groups to study the changes on the above mentioned parameters after drugs withdrawal. Blood samples were drawn by cardiac puncture.

-Biochemical analysis

-Blood samples were collected in glass tubes with rubber caps, labelled and centrifuged at 4000 *g* for 10 min. Serum enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel method (1957) and lactate dehydrogenase (LDH) were measured with a colorimetric test according to the manufacturer's instructions (MaxDiscovery Lactate Dehydrogenase Enzymatic Assay kit, BIOO Scientific Corp).

-Serum creatinine and blood urea nitrogen (BUN) levels were determined using commercial kits according to van der Heiden *et al*., (1968).

- Determination of anti-oxidant enzyme activities

Hepatic levels of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) as byproducts of lipid peroxidation were measured by using commercial kits according to Beutler *et al*. (1963), Satoh (1978) and Aebi (1984). Tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4) containing ethylene diamine tetra acetic acid (EDTA).

- Measurement of serum IFN-*γ* and IL-1β

To investigate the effect of tramadol, acetaminophen (APAP) and Tramacet administration on cytokine production (IFN-*γ* and IL-1β) were estimated. Immunoassay kits (rat ELISA KITS), life technologies were used to determine serum concentration of IFN-*γ* (Rubinstein *et al*., 1981) and IL-1β (Liu *et al*., 2011).

- Statistical analyses

Data regarding evaluation of the interactions between the utilized drugs were presented as mean±SE and were assessed by analysis of variance (one-way ANOVA) using the computer program of SPSS. If this analysis revealed a significant difference among the group means, then significances among groups were assessed

by post hock LSD test for multiple comparisons. *P* values less than 0.05 were considered to be significant

RESULTS:

The current study has been conducted to give information on the biochemical toxicity profiles, antioxidant (stress) biomarkers, as well as the potential immunological effects after 30 days use of tramadol, acetaminophen and Tramacet in a mammalian experimental animal.

Effects on serum ALT, AST and LDH activities

The results revealed that all treatments, tramadol, acetaminophen and Tramacet induced significant (p<0.001) increase in ALT activity by 47%, 123% and 95%, respectively (Fig. 1) and in AST activity by 99%, 188% and 138.5%, respectively (Fig. 2) as compared to control. Concerning the effects of the utilized drugs on LDH activity, it was observed that tramadol, acetaminophen and Tramacet induced significant (p<0.001) increase in LDH activity by 78%, 171% and 115%, respectively (Fig.3).

After one month recovery, serum of acetaminophen-treated rats exhibited significant increase (p<0.05) by 29%, 54% and 44% in ALT, AST and LDH activities, respectively (Figs. 1, 2&3).

Effects on serum BUN and creatinine levels

Regarding the effects of treatment on kidney function, daily tramadol, acetaminophen and Tramacet treatment for 30 days induced increase in BUN level by 11, 32% and 24%, respectively (Fig. 4) as well as in serum creatinine level by 50%, 111% and 95.5%, respectively (Fig.5). After one month of stopping treatment, serum of acetaminophen-treated rats exhibited significant increase (p<0.05) by 28% and 42% in BUN and creatinine levels, respectively (Figs. 4&5).

Effects on serum pro-inflammatory cytokines (IFN-γ and IL-1β)

The statistical evaluation of the effects of the drugs on the pro-inflammatory cytokines, the results revealed that acetaminophen and Tramacet caused increases in both pro-inflammatory cytokines, IFN-γ and IL-1β by 17% and 15% for IFN- γ (Fig.6) and 127% and 112% for IL-1β, respectively (Fig.7). After the recovery period, the two tested pro-inflammatory cytokines returned back to their normal ranges after stopping Tramacet treatment. However, serum IFN-γ and IL-1β levels exhibited significant ($p\leq0.05$) rise by 11% and 38%, respectively after stopping acetaminophen treatment.

Effects on anti-oxidant system

The effects of the studied medications on the antioxidant system were illustrated in the Figs. 8, 9, 10&11). The statistical analysis, showed that the effect of acetaminophen was more potent on the studied anti-oxidant system causing a significant decrease (p<0.001) in GSH, CAT and SOD activities by 59%, 59% and 47%, respectively (Figs. 8, 9&10), with parallel increase in MDA levels by 121% (Fig. 11). On the other hand, tramadol induced significant decreases in GSH, CAT and SOD activities by 23%, 21% and 21%, respectively (Figs. 8, 9&10), paralleling to an increase in MDA level by 62% (Fig. 11). Regarding the effect of Tramacet treatment, it induced significant decrease in GSH, CAT and SOD activities by 42%, 43% and 27%, respectively (Figs. 8, 9&10), paralleling to an increase in MDA level by 85% (Fig. 11).

Although all the tested anti-oxidant parameters returned back to their normal values as has been shown in Figs. (8, 9, 10&11) after 30 days of stopping tramadol and Tramacet medication, the studied parameters of acetaminophen-treated group exhibited significant decreases by 15%, 15%, and 32% in GSH, CAT and SOD (Figs. 8, 9&10), respectively and an increase in MDA level by 39% (Fig. 11).

DISCUSSION

The present study focuses on the effect of tramadol, acetaminophen and Tramacet (tramadol/ acetaminophen) on both liver and kidney function activities and antioxidant stress biomarkers, as well as the potential pro-inflammatory cytokines level by determining IFN-γ, IL-1β and antioxidant stress biomarkers. In the current study, the daily oral administration of tramadol, acetaminophen and Tramacet (tramadol/acetaminophen) three times/day for 30 consecutive days, induced significant increases in the levels of ALT, AST and LDH production as well as lipid peroxidation (MDA) among all treated groups.

These findings suggest the possible hepatotoxic effects of these medications in chronic use. This comes in agreement with (Atici *et al*., 2005; Galal *et al*., 2012) who reported that paracetamol caused marked liver damage as noted by a significant increase the activity of serum AST and ALT. In addition, a significant increase in the levels of ALT, AST and LDH production was reported among chronic heroin users (Panchenko *et al*., 1999). Furthermore, many chemical reagents could significantly increase the levels of ALT and AST causing a serious injury to the liver. Borzelleca *et al*. (1994) and Sturgill and Lambert (1997) reported increased levels of ALT, AST and LDH in rats after long-term usage of morphine like agent levo-alpha hepatic and renal damage due to long term use of opioids. However, in the present study Tramacet administration caused a pronounced increase more than tramadol alone. This could be explained in view of the benefit of this drug comes from the complement actions of the constituent having the rapid onset of paracetamol and sustained effect of tramadol (Dhillon, 2010; Pergolizzi *et al*., 2012).

Although opioids are reported to be effective in pain management, their toxic effects should be kept in mind. The liver and kidney are responsible for tramadol metabolism and excretion. It may cause hepatotoxicity and nephrotoxicity during its metabolism (Wu *et al*., 2001). In the current investigation, the recorded data indicated significant increases in both BUN and creatinine levels in rats received the examined drugs. This could be considered as an evidence of renal damage. This is in accordance with John and Koloth (2007) and contradicts with Atici *et al*. (2005) who reported that both BUN and creatinine levels remained unchanged after tramadol administration.

Inflammation is a critical component of the overall pathophysiology, not only as a potential factor that may aggravate cell damage, but more importantly as a vital response to limit cell injury, remove cell debris and promote regeneration (Jaeschke, 2005). Treatment of acetaminophen and Tramacet could activate T cells, natural killer (NK) cells and Kupffer cells. The activated T cells, NK cells and Kupffer cells release numerous signaling molecules including hydrolytic enzymes, nitric oxide and superoxide (Hogaboam *et al*., 1999; Bourdi *et al*., 2002). Nevertheless, Liu *et al*. (2004) demonstrated that NK and T cells produce IFN-γ and play a critical role in APAP-induced liver injury as well as cause apoptosis of hepatocytes (Iwakura *et al*., 2011). IFN-γ is a cytokine produced by activated T lymphocytes and natural killer cells. IFNγ boosts the production of IL-1β. The IFN-γ has important anti-inflammatory effects (Kubera *et al*., 2001). Furthermore, IFN-γ exerts pleiotropic effects including antiviral and bactericidal activities, activation of macrophages, NK cells and up-regulation of major histocompatibility complex class II (MHC class II) expression on macrophages has been reported to be involved in various kinds of liver injury models (Mizuhara *et al*., 1996; Mihm *et al*., 1996). Enhanced IFN-γ expression is presumed to induce inflammatory responses, leading to parenchymal cell damage in the liver. In APAP-induced liver injury, IFN-γ levels in the liver were correlated with severity of cell damage. Clinically, paracetamol hepatotoxicity was markedly enhanced in patients treated with IFN-γ (Kellokumpu-Lehtinen *et al*., 1989). In spite of the beneficial role of these anti-inflammatory drugs, these drugs are incriminated in some body organs (Harrill and Rusyn, 2008). Moreover, several studies showed that opiates and opioids have been shown to interact with the immune system (Kim *et al*., 2013).

The action of the oral administration of the three drugs under investigation: tramadol, acetaminophen and Tramacet showed a discernible effect on the potential inflammatory cytokines. Treating rats with acetaminophen and Tramacet induced a significant increase in IFN-γ and IL-1β. This could be explained in view of enhancement of IFN-γ to induce inflammatory responses leading to cell damage in the liver. It was previously reported that paracetamol-induced hepatotoxicity through the release of pro-inflammatory cytokines and mediators, including IFN-γ and IL-1β (Martin-Murphy *et al*., 2010; Lu *et al*., 2013; Patterson *et al*., 2013; Ye *et al*., 2014). The present data is supported all the previous studies as well as observation that paracetamol treatment resulted in a significant increase in IL-1β (Galal *et al*., 2012). On the other hand, tramadol administration in the present study caused insignificant change in IFN-γ which contradicted those of previous studies (Vallejo *et al*., 2004; Qian *et al*., 2005) who showed that treatment with tramadol caused decrease in IFN-γ level.

Glutathione (GSH) is an enzyme which prevents the generation of hydrogen peroxide and alkyl hydro peroxides in association with GSH and GSH reductase, as well as the generation of more harmful metabolites such as the hydroxyl radical (Parodi, 2007). Superoxide dismutase (SOD) is an exceedingly effective defense enzyme that converts superoxide anions into hydrogen peroxide (H_2O_2) (Reiter *et al*., 2000). Catalase (CAT) is a haemeprotein in all aerobic cells that metabolize H_2O_2 to oxygen and water. These antioxidant enzymes are inactivated by lipid peroxides or ROS. Lipid peroxidation has been used as an indirect marker of oxidant-induced cell injury, when liver is damaged by some chemical toxin; hepatocytes generate a large number of free radicals, causing lipid peroxidation of the cytomembrane to produce MDA. Malondialdehyde levels indirectly reflect the extent of cellular damage by free radicals and are widely used as an index of free radical mediated lipid peroxidation (Mansour, 2000).

Several compounds, such as acetaminophen have been implicated in the etiology of liver diseases (Hegde and Joshi, 2009; Adesanoye and Farombi, 2010). In the present investigation, the significant decrease in liver GSH, CAT content and SOD activities which paralleled with an increase in MDA level may be attributed to the enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism. This assumption is supported by the work of (William *et al*., 1991) who reported a marked decrease in glutathione (GSH) level when incubated with isolated rat hepatocytes with varies concentrations of morphine and resulted in cell death. As well as, lipid oxidations were reported among chronic heroin users (Panchenko *et al*., 1999). Similarly, toxic effects of tramadol as opioid drug at cellular level may explained by lipid peroxidation. Moreover, lipid peroxidation was reported among rats receiving an acute dose of cocaine (Masini *et al*., 1997). However, in the present study, the data recorded were potent when comparing the effect of tested dose of acetaminophen with those of tramadol and Tramacet when comparing each with control group.

The data obtained in the recovery period could be explained in view the more potent effect of acetaminophen in all studied parameters than either tramadol or Tramacet after the thirty days treatment, so it needs more time for recovery.

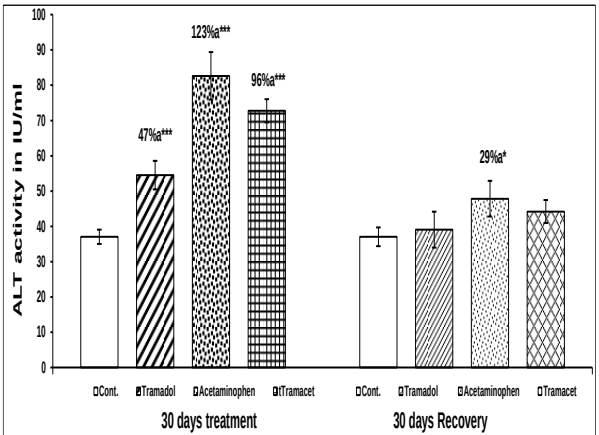

Fig (1): Effect of Tramadol, Acetaminophen and Tramacet treatment for 30 days and after rcovery period of 30 days on ALT activity.

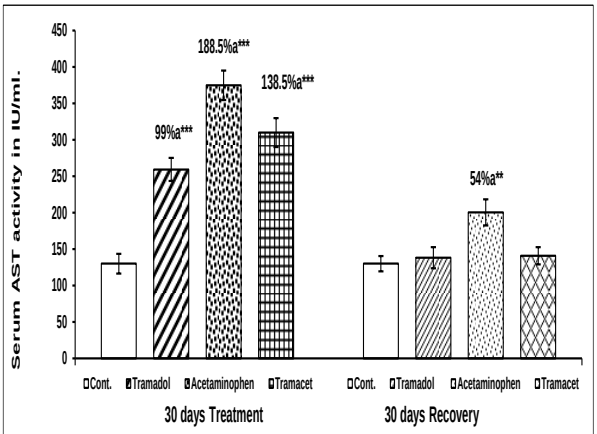

Fig (2): Effect of Tramadol, Acetaminophen and Tramect after 30 days tratment and 30 days Recovery on Serum AST Activity.

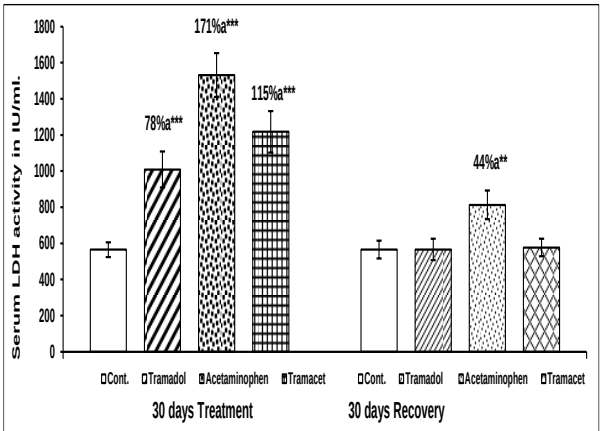

Fig (3): Effect of Tramadol, Acetominophen, Tramacet after 30 days treatment and 30 days recovery.

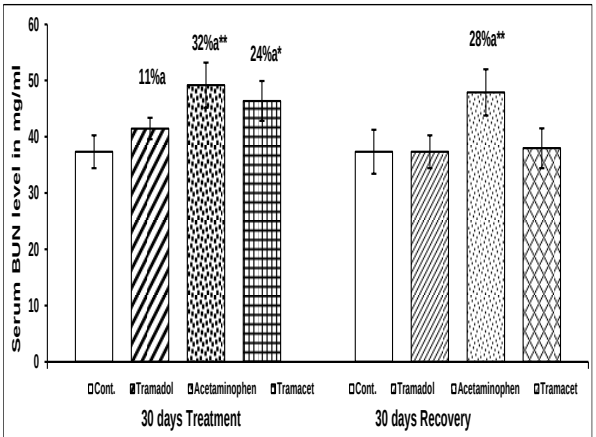

Fig (4): Effect of Tramadol, Acetaminophen and Tramacet on 30 days Treatment and 30 days Recovery.

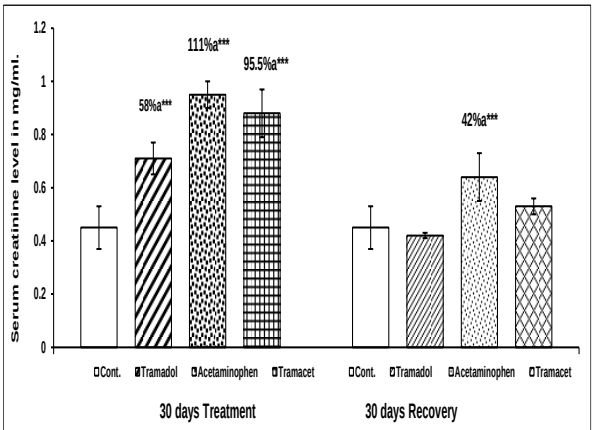

Fig (5): Effect of Tramadol, Acetaminophin and Tramacet after 30 days and 30 days recovery on serum Cretatinin Level.

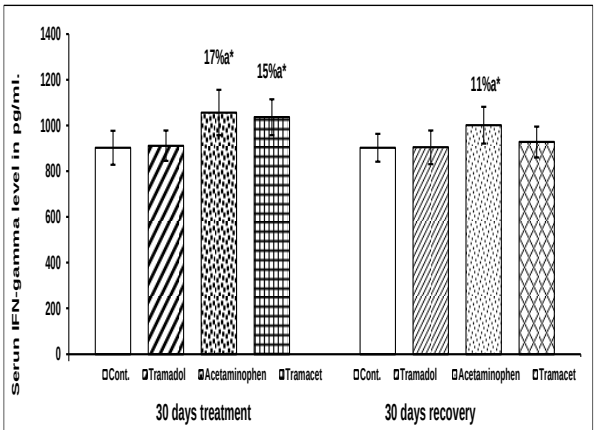

Fig (6): Effect of Tramadol, Acetaminophen and Tramacet after 30 days treatment and 30 days recovery on serum IFN-gamma level

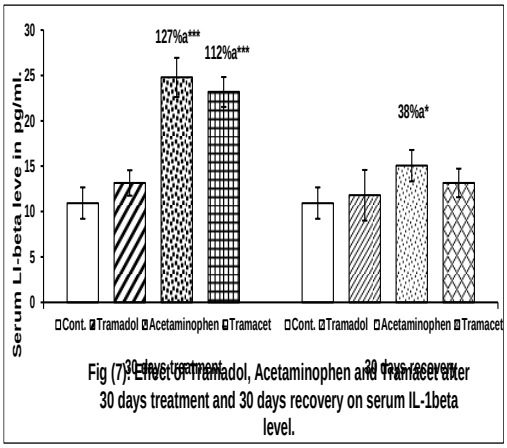

Fig (7): Effect of Tramadol, Acetaminophen and Tramacet after 30 days treatment and 30 days recovery on serum IL-1beta level.

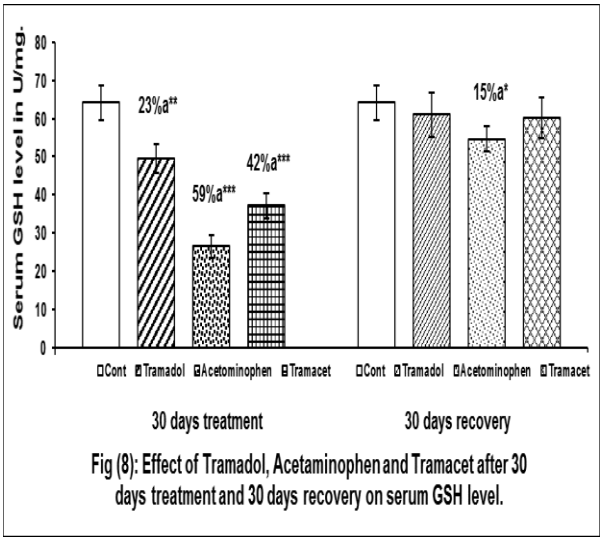

Fig (8): Effect of Tramadol, Acetaminophen and Tramacet after 30 days treatment and 30 days recovery on serum GSH level.

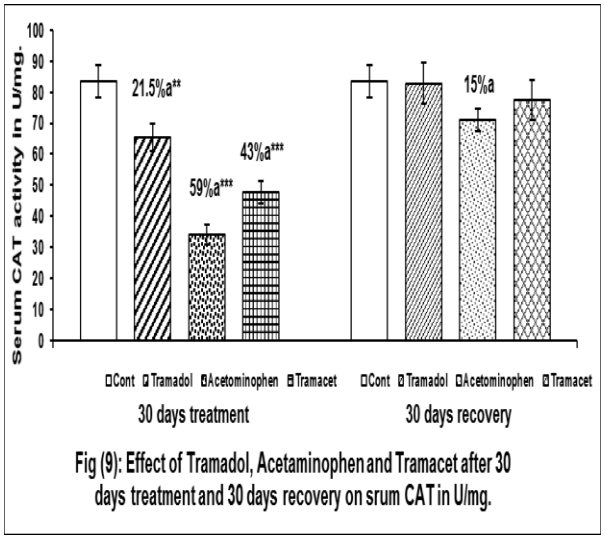

Fig (9): Effect of Tramadol, Acetaminophen and Tramacet after 30 days treatment and 30 days recovery on srum CAT in U/mg.

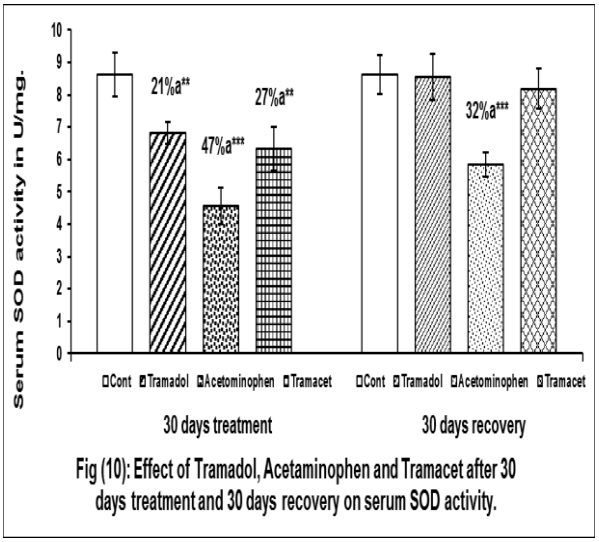

Fig (10): Effect of Tramadol, Acetaminophen and Tramacet after 30 days treatment and 30 days recovery on serum SOD activity.

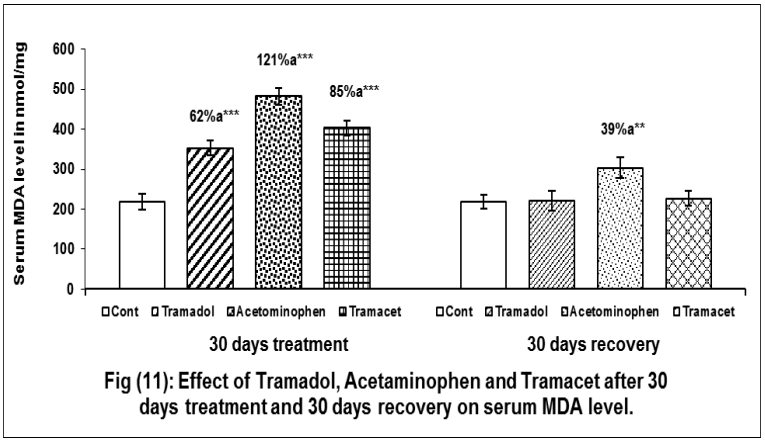

Fig (11): Effect of Tramadol, Acetaminophen and Tramacet after 30 days treatment and 30 days recovery on serum MDA level.

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آثار الإستخدام المزمن للترامادول، أسيتامينوفين والتراماست على بعض التغيرات البيوكيميائية والمناعية في ذكور الجرذان

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هناك دور واضح للكبد والكلية فى أيض الأدوية، ويستخدم مخدر الترامادول على نطاق واسع فى السنوات الأخيرة كمسكن فعال ولعلاج الآلام المتوسطة والشديدة حيث يتحول فى الكبد إلى مشتقات الترامادول وهى أقوى من الترامادول نفسه بأربع مرات، أما عن الأسيتامينوفين (الباراسيتامول) فهو غير مخدر (أفيونى) وليس من السلسلات المسكنة كالأسبرين، ويستخدم كمسكن وخافض للحرارة وهو آمن عند المستويات المحددة له، ولكن عندما يستخدم بكثرة يحدث سمية للكبد، أما فيما يخص التراماست (التراست) فيتكون من 37.5 مجم ترامادول مع 325 مجم أسيتامينوفين (تيلينول) الذي بدأ يستخدم لعلاج الآلام التوسطة والشديدة، وبذلك تقوم هذه الأدوية المزيجة في التخفيف من الآلام بشكل أفضل من الدواء الذى يستخدم على حده.
تهدف الدراسة إلى تقييم التغيرات البيوكيميائية والمناعية المحتملة وكذلك المؤشرات الحيوية لمضادات الأكسدة بعد إستخدام الترامادول وأسيتامينوفين، والتراماست (مزيج من الترامادول والأسيتامينوفين) في دراسة مقارنة والدراسة الحالية تلقي الضوء على تأثير هذه الأدوية على الكبد ووظائف الكلى وكذلك على السيتوكين المسببة للإلتهاب (انترفيرون جاما وانترليوكين-1 بيتا) والمؤشرات الحيوية المضادة للأكسدة بعد علاج شهر مع إلقاء الضوء على سمية هذه الأدوية. 64 جرذ قسمت إلى 4 مجموعات، مجموعة ضابطة، مجموعة الترامادول، مجموعة الأسيتامينوفين، مجموعة التراماست (ترامادول وأسيتامينوفين)، وهذه المجموعات التجريبية كانت تعالج بهذه الادوية لمدة شهر واستمر نصف عدد الجرذان لفترة شهر آخر بدون علاج لدراسة التغيرات الناجمة من سحب هذه الأدوية، وقد تم سحب عينات الدم من القلب لقياس نشاط الإنزيمات فى المصل وهى الـ AST، ALT وLDH، وكذلك الكرياتينين واليوريا والسيتوكين INF-α وIL-1β وكذلك مضادات الأكسدة مثل الـ GSH، CAT، SOD وكذلك MDA. الترامادول والأسيتامينوفين والتراماست تسببوا فى تدمير الكبد والكلى، وذلك بملاحظة زيادة نشاط الإنزيمات ALT، AST وLDH، وكذلك اليوريا والكرياتينن، كما تسبب الأسيتامينوفين والتراماست زيادة ملحوظة فى السيتوكين INF-α و IL-1β بينما العلاج بالترامادول لم يظهر تغيرات ذات دلالة إحصائية عالية فى مستوى السيتوكين، من جهة أخرى تسببت هذه الأدوية فى نقص كل من نشاط الـ GSH، CAT وكذلك SOD، مع زيادة ملحوظة فى الـ MDA. أظهرت النتائج أن الأسيتامينوفين أقوى تأثيرا على كل هذه الدلالات البيوكيميائية من عقارى الترامادول والتراماست، وهذه التغيرات لوحظت بعد 30 يوم من الإنسحاب رغم أنها تحتاج إلى مزيد من وقت لسحب الدواء والشفاء الكامل.