Hepatic DNA Damage and Abnormality in Serum Protein Pattern Due to Long Term Use of Tramadol in Rats

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Abstract

Background:

Tramadol is a widely used analgesic that stimulates the μ opioid receptor and inhibits serotonin and noradrenalin reuptake. In this study, we investigate the effect of chronic administration of 200 and 400mg of tramal for 15,30 ,45 days followed by withdrawal periods(w15) on protein activity in male rats as manifested by changes in electrophoretic serum protein patterns and gene expression manifested by DNA damage, measured by Comet Assay .

Materials and methods:

100 male Wistar rats (100-150 g) were included and divided into three groups, control group (n = 20), Tramal(I) group (n = 40), received the drug orally at doses of 200mg/kg/day for 15,30,45 days of the study, respectively(10 rats for each subgroup).TR45 group was followed by 15 withdrawal period(W15) ,Tramal(II) group (n = 40), received the drug orally at doses of400mg/kg/day for 15,30,45 days of the study, respectively. TR45 group was followed by 15 withdrawal period (W15).

Results:

Results exhibited major changes in the protein pattern which included changes in the molecular weight of the control bands and the relative percentage of protein fraction as well as the total number of bands, as a result of disappearance of some original bands and appearance of other new one. Serum protein fraction revealed an increase in total number of protein fractions being in Tr200mg (14 and 16 bands), whileTR400mg revealed a decrease in total number of bands (except TR15 which exhibited an increase in bands (14). The changes were observed all over the treated groups as well as in the withdrawal groups In this study, the alkaline version of the comet assay has been used to determine the effect of tramal administration (200 and 400mg/Kg) on peroxide-initiated free radical-mediated DNA damage in rat liver cells. Indeed, levels of strand breaks in rat liver cell exposed to tramal400mg/Kg were significantly higher than in cells exposed to 200mg/Kg, especially after a long administration period (TR45 days). The intensity of the comet tail relative to the head reflects the number of DNA breaks. The rates of tailed cells detected by the comet assay increased significantly when the rats were exposed to 200 and 400mg/kg of tramal compared with control (however, the tail length did not differ significantly between the same groups). The intensity of the comet tail relative to the head reflects the number of DNA breaks.

Conclusions:

Our findings pointed out the risk of increased lipid peroxidation, hepatic DNA damage and abnormality in serum protein pattern due to long term use of tramadol, although opioids are reported to be effective in pain management, their toxic effects should be kept in mind.

Keywords: Tramadol, protein electrophoresis, DNA damage, Comet assay, Albino rat.

Introduction:

Tramadol is a synthetic, opioid like, centrally acting analgesic that is neither an opiate-derived nor a non steroidal anti-inflammatory drug. It is a member of the amino cyclohexanol group used to control moderate to severe pain (Cherubino *et al*., 2012). Used in therapy as a racemic mixture, the (+)-enantiomer weakly binds to the mu-opioid receptor, and both enantiomers inhibit serotonin and norepinephrine reuptake. Tramadol's major active metabolite, O-desmethyltramadol (ODT), shows higher affinity for the mu-opioid receptor and has twice the analgesic potency of the parent drug. The synergism of these effects contributes to tramadol's analgesic properties with the (+)-enantiomer exhibiting 10-fold higher analgesic activity than the (-)-enantiomer, reported a large number of adverse events attributed to tramadol including abuse by opioid-dependent patients, allergic reactions, and seizures are reported (Overholser and Foster, 2011and Stewart, *et al*., 2011)

Tramadol is a widely used synthetic opioid analgesic that commonly prescribed for the treatment of moderate to severe pain (Boshra,2011and Oyama *et al.*,2012). Used in therapy as a racemic mixture of (±) *cis*-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride. For the treatment of painful conditions tramadol 50-100 mg can be administered as needed for relief every 4-6 hours. For moderate pain tramadol 50 mg may be adequate as the initial dose, and for more severe pain, tramadol 100 up to400 mg is usually more effective as the initial dose (Ortner *et al.*,2012).

Racemic tramadol is rapidly and almost completely absorbed after oral administration. The mean absolute bioavailability of a 100 mg oral dose is approximately 75%. The mean peak plasma concentration of racemic tramadol and M1 occurs at 2 and 3 hours, respectively, after administration in healthy adults. In general, both enantiomers of tramadol and M1 follow a parallel time course in the body following single and multiple doses although small differences (~10%) exist in the absolute amount of each enantiomer each of which is biotransformed to an active metabolite: (+)-O-desmethyltramadol or (−)-O-desmethyltramadol (Valle *et al*, 2000) These metabolites are believed to be responsible for most of tramadol's mu-agonist properties (Gillen *et al*.,2000)

Kinetics and OP (oxidation products) identification confirmed that the lone electron pair of the amine-N is the predominant site of oxidant attack. An oxygen transfer mechanism can explain the formation of N-oxide-TRA, while a one-electron transfer may result in the formation of N-centered radical cation intermediates, which could lead to the observed N-dealkylation and to the identified

formamide and aldehyde derivatives via several intermediate steps. The proposed radical intermediate mechanism is favored for Fe (VI) leading predominantly to N-desmethyl-TRA (ca. 40%), whereas the proposed oxygen transfer prevails for O (3) attack resulting in N-oxide-TRA as the main OP (ca. 90%) (Zimmermann *et al.*, 2012).

The major metabolic pathways of tramadol appear to be *N*- and *O*-demethylation and glucuronidation or sulfation in the liver. One metabolite (*O*-desmethyltramadol, denoted M1) is pharmacologically active in animal models. Production of M1 is dependent on the CYP2D6 isoenzyme of cytochrome P-450 and as such is subject to both metabolic induction and inhibition which may affect the therapeutic response. CYP2D6 catabolizes O-demethylation of tramadol to more potent metabolites. (Meyer and Maurer ,2011andHendrickson and McKeown ,2012). Based on analgesic studies, tramadol is thought to be approximately one-tenth as potent as morphine when each is administered parenterally, and approximately one-third as potent when each is administered orally (Gutstein & Akil, 2001).

Tramadol HCl has a potential to cause psychic and physical dependence of the morphine-type (μ-opioid). The drug has been associated with craving, drug-seeking behavior and tolerance development. Cases of abuse and dependence on tramadol HCl have been reported. Tramadol HCl should not be used in opioid-dependent patients. Tramadol HCl can reinitiate physical dependence in patients that have been previously dependent or chronically using other opioids. In patients with a tendency to drug abuse, a history of drug dependence, or are chronically using opioids, treatment with tramadol HCl is not recommended. Cases of overdose with tramadol have been reported. Tramadol overdose has been one of the most frequent causes of drug poisoning in the country in the recent years, especially in male young adults with history of substance abuse and mental disorders. Nausea, vomiting, Central Nervous System (CNS) depression, tachycardia, and seizure are the most common findings in this kind of poisoning. Cardiopulmonary arrest was found as the cause of death in cases who had ingested more than 5000 mg tramadol (Shadnia *et al.*, 2008) .Estimates of ingested dose in foreign fatalities has been in the range of 3-5 g. A 3 g intentional overdose by a patient in the clinical studies produced emesis and no sequelae. The lowest dose reported to be associated with fatality was possibly between 500 and 1000 mg in a 40 kg woman, but details of the case are not completely known. Serious potential consequences of over dosage are respiratory depression and seizure (Rehni *et al.*, 2010). In treating an overdose, primary attention should be given to maintaining adequate ventilation along with general supportive treatment. While naloxone will reverse some, but not all, symptoms caused by over dosage with tramadol HCl the risk of seizures is also increased with naloxone administration. In animals convulsions following the administration of toxic doses of tramadol could be suppressed with barbiturates or benzodiazepines but were increased with naloxone. Naloxone administration did not change the lethality of an overdose in mice.

Hemodialysis is not expected to be helpful in an overdose because it removes less than 7% of the administered dose in a 4 hour dialysis period. Severe serotonin toxicity and manic switch induced by combined use of tramadol and paroxetine (John andKoloth., 2007).

Increased lipid peroxidation, hepatic and renal damage due to long term use of tramadol (Atici *et al*., 2005). Chronic use of tramadol in increasing doses is found to cause red neuron degeneration in the rat brain, which probably contributes to cerebral dysfunction (Atici *et al*., 2004). A possible cytochrome P450-based interaction between tramadol and benzodiazepine is considered (Clarot *et al*., 2003). 34 mg L (-1) tramadol administration for 21-day significantly reduced the expression level of the Vtg gene, a biomarker of the reproduction ability in an oviparous animal (Le *et al.*, 2011). The use of tramadol should be carefully monitored for drug-related problems because of their individual drug metabolism and multiple drug-drug interactions. Tramadol toxicity-induced rhabdomyolysis results from skeletal muscle injury with release of muscle cell contents into the plasma. (Yousef Khan., 2010). Tramadol exerts a seizurogenic effect on mice via an H (1) receptor activation-linked pathway possibly through an opioid receptor-dependent release of histamine from the mast cells (Rehni et al., 2010). Neither carisoprodol (Soma) nor tramadol (Ultram) is a controlled substance at the federal level. However, evidence indicates that these medications may have abuse potential, particularly in patients with a history of substance abuse. We report three cases in which a combination of carisoprodol and tramadol was used illicitly to obtain psychotropic effects. Carisoprodol or tramadol should be prescribed with caution for patients at risk for substance abuse, and extreme caution should be used when prescribing both drugs simultaneously for any patient.(Reeves and Liberto .,2001). The mechanism of tramadol intoxication death could be caused by respiratory depression induced by over-expression of GABA (A)alpha1 and GABA(B)1 in medulla oblongata solitary nucleus and ambiguous nucleus. (Stamer *et al.,* 2008and Zhang *et al*., 2011). Tramadol caused a concentration-dependent inhibition of ACh-induced detrusor contraction that was reversed by raising the concentration of ACh. Propranolol, but not naloxone, reversed the tramadol-induced inhibition of contractions to ACh in the detrusor. These results suggest that tramadol inhibits ACh-induced contractility of the isolated detrusor. They also suggest that tramadol does so by an indirect anticholinergic mechanism involving the stimulation of β-adrenoceptors. Tramadol may be useful in managing clinical conditions requiring relaxation of the detrusor muscle (Kumar *et al*., 2012). Serotonin syndrome can occur in children intoxicated with tramadol, induced neurologic and cardiovascular effects (Maréchal *et al.,* 2011 and Sanaei, 2012).

Methods:

Electrophoretic separation of serum proteins:

SDS-PAGE electrophoresis was carried out according to the method of Laemmli (1970), the wide range SDS-PPAGE molecular weight standard mixture (Biorad) was applied to the first well. Scanning was applied using gel pro software (ver.3, USA, 1998), for Media Sci Image densitometry 700 Biorad.

DNA damage evaluated by Comet Assay:

Comet assay was performed by the method of Speit and Hartmann, (2005). The comet assay is a simple method for measuring deoxyribonucleic acid (DNA) strand breaks in liver cells. Cells embedded in agarose on a microscope slide are lysed with detergent and high salt to form nucleotides containing super coiled loops of DNA linked to the nuclear matrix. Electrophoresis at high pH results in structures resembling comets, observed by fluorescence microscopy; the intensity of the comet tail relative to the head reflects the number of DNA breaks (Collins, 2004).

Statistical Analysis:

Data were statistically analyzed using one-way analysis of variance (ANOVA) according to Viv *et al*., (2004) using the SPSS statistical software package version 10.

Results:

In the present study an attempt has been made to demonstrate the changes induced in electrophoretic serum protein pattern of male rats by daily administration of 200and 400mg/kg of tramal for 15, 30, 45 days followed by withdrawal periods. The results of electrophoretic separation of serum proteins by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) are shown in tables1, 2, 3, 4 and figures (1). The results exhibited major changes in the protein pattern which included changes in the molecular weight of the control bands and the relative percentage of protein fraction as well as the total number of bands, as a result of disappearance of some original bands and appearance of other new ones.

As seen in table (1), (2) and figure 1

the electrophoretic separation of serum protein showed a wide molecular weight range in the different groups with a maximum of 188.98 KDa and a minimum of 7.85 KDa .The banding pattern showed 13 distinct bands in control group ,14 distinct pattern in TR15 group ,16 for TR30,14 for TR45 and 13 distinct bands in w 45 group. Only 2 bands of 29.376 and 40.385 were expressed in all experimental groups (highlighted in table 1).They seem resistant to the effect of tramal drug. . The 29.376 KDa band was similarly expressed in TR15, 45 and withdrawal groups (around 5.67%) but more expressed in TRC andTR30 groups (around 11.6%) . The 40.385 KDa band was similarly expressed in all groups (around 4.3%). The band of 188.98 KDa was expressed in TR15 group only, while that of 123.8KDa was expressed in withdrawal group only. There were 11 missing bands in TR15 treated group. These were(109.28,98.54,95.345,56.778,20.909,17.204,15.723,14.712,11.741,8.911and 7.85 KDa) versus the control group. On the other hand , 8 missing bands in TR30(109.28, 20.909,17.204,15.723,14.712,11.741,8.911and 7.85 KDa) versus the control group, 8 missing bands in TR45(95.34520.909,17.204,15.723,14.712,11.741,8.911and 7.85 KDa). On the other hand, 11bands were missing in tramal/ withdrawal group versus thecontrolgroup.Thesewere(109.28,98.54,95.345, 56.778,20.909,17.204,15.723,14.712,11.741,8.91and 7.85 KDa). The bands of 20.909, 17.204, 15.723, 14.712, 11.741, 8.911and 7.85 KDa were not expressed in all treated groups.

Table(1): Effect of chronic administration of tramal (200mg) on serum protein electrophoretic patterns in adult male rats.

Lane 6		Lane 5		Lane 4		Lane 3		Lane 2		Lane 1		Lanes Bands
%	KD	%	KD	%	KD	%	KD	%	KD	%	KD	
6.43	123.8	4.78	121.8	5.17	115.73	2.44	188.98	25.699	109.28	7.012	116.3	1
4.05	107.05	3.42	113.56	2.71	114.1	4.54	115.73	3.831	98.538	9.1641	97.4	2
3.55	101.63	4.77	108.58	6.06	107.05	2.79	112.76	2.948	95.348	10.343	66.2	3
8.54	86.014	3.72	101.87	5.15	100.91	8.22	106.29	1.1638	56.778	11.239	45	4
5.1	81.93	3.22	98.324	3.88	98.093	4.12	101.39	2.5803	40.385	8.6157	31	5
3.89	75.549	7.21	87.42	2.47	94.292	5.59	97.63	11.546	29.376	12.509	21.5	6
10.4	66.717	6.95	81.93	5.89	83.72	22.2	79.315	5.2775	20.909	16.32	14.4	7
2.48	60.048	13.6	65.347	6.75	81.049	12.5	65.347	14.466	17.204	21.412	6.5	8
15.7	51.465	7.08	56.851	4.73	73.138	4.55	58.865	9.4634	15.723			9
29.8	43.768	18.1	47.531	11.1	66	16.3	46.133	8.3059	14.712			10
2.15	39.902	11.8	44.175	1.88	59.454	2.7	39.534	5.2623	11.741			11
3.63	33.009	4.26	39.173	2.91	57.42	2.05	37.573	6.6266	8.9113			12
4.35	29.816	5.95	34.732	22.1	47.295	6.36	33.938	2.4486	7.8464			13
		4.89	31.81	4.15	39.352	5.67	30.373					14
				6.54	35.71							15
				8.45	29.816							16
100		100		100				100		100		Sum
100		100		100				100		100		In lane

Lane 1=Marker , Lane 2 =Control , Lane 3=TR15 , Lane 4=TR30 ,Lane 5=TR45 , Lane6=WG

Table (2) :Effect of chronic administration of tramal (200mg) on serum protein electrophoretic patterns in adult male rats. (Comparison study)

Tramal/W		Tramal/45		Tramal/30		Tramal/15		C		Groups M.wt (KD)
%	+/	%	+/	%	+/-	%	+/-	%	+/-	
						2.44	+			188.98
6.43	+									123.8
		4.78	+							121.8
				5.17	+	4.54	+			115.73
		3.42	+	2.71	+					114.1
						2.79	+			112.76
		4.77	+					25.7	+	109.28
4.05	+			6.06	+	8.22	+			106.29
3.55	+	3.72	+	5.15	+	4.12	+			101.39
		3.22	+	3.88	+			3.83	+	98.54
						5.59	+			97.63
				2.47	+			2.95	+	95.345
										90.084
8.54	+			7.21	+					87.42
				5.89	+					83.72
5.1	+	6.95	+	6.75	+					81.049
						22.2	+			79.315
				4.73	+					73.138
		13.6	+	11.1	+					66.483
						12.5	+			65.347
										62.81
				1.88	+	4.55	+			58.865
		7.08	+	2.91	+			1.16	+	56.778
		18.1	+	22.1	+	16.3	+			46.133
		11.8	+							44.175
2.15	+	4.26	+	4.15	+	2.7	+	2.58	+	40.385
						2.05	+			37.573
				6.54	+					35.71
3.63	+	5.95	+			6.36	+			33.938
										31.81
4.35	+	4.98	+	8.45	+	5.67	+	11.6	+	29.376
								5.3	+	20.909
								14.5	+	17.204
								9.5	+	15.723
								8.3	+	14.712
								5.26	+	11.741
								6.63	+	8.911
								2.45	+	7.85
13		14		16		14		13		Sum in lane

+= Expressed,- =Not expressed

Table (3) : Effect of chronic administration of tramal (400mg) on serum protein electrophoretic patterns in adult male rats

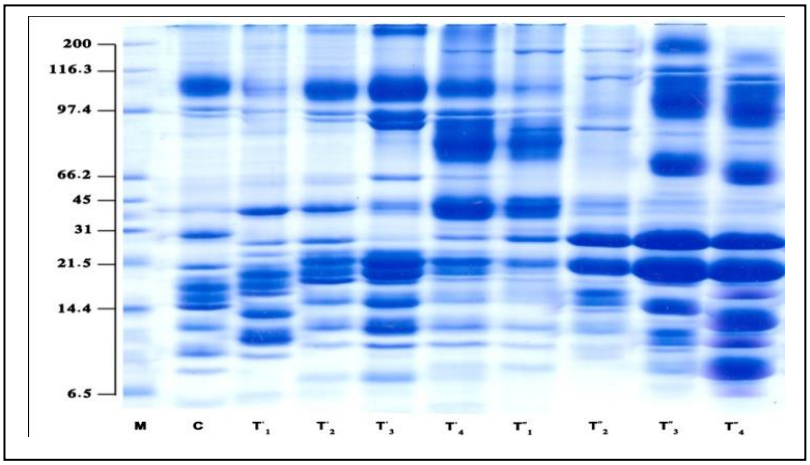
Lane 6		Lane 5		Lane 4		Lane 3		Lane 2		Lane 1		Lanes Bands
%	KD	%	KD	%	KD	%	KD	%	KD	%	KD	
2.336	172.51	23.134	107.91	25.781	107.9	6.588	111.85	25.699	109.28	7.012	116.3	1
10.369	109.16	8.456	95.483	3.1258	97.503	1.465	98.33	3.831	98.538	9.1641	97.4	2
3.405	97.812	5.894	90.084	2.5047	93.604	1.526	87.438	2.948	95.348	10.343	66.2	3
35.810	81.911	3.68	67.242	1.880	65.049	2.258	62.805	1.1638	56.778	11.239	45	4
0.9273	66.483	5.0977	44.462	10.538	40.997	13.454	39.426	2.5803	40.385	8.6157	31	5
21.866	41.368	0.3872	29.061	5.5456	28.138	3.8225	27.465	11.546	29.376	12.509	21.5	6
1.816	29.694	15.653	21.454	15.141	22.446	1.9713	24.137	5.2775	20.909	16.32	14.4	7
8.0861	22.75	12.452	19.398	10.234	20.032	14.808	19.691	14.466	17.204	21.412	6.5	8
4.250	20.422	8.420	15.356	8.345	18.744	9.801	17.728	9.4634	15.723			9
4.187	18.073	10.88	11.741	6.312	16.731	5.175	16.731	8.3059	14.712			10
3.889	13.729	2.5043	10.786	6.5513	11.992	13.773	13.62	5.2623	11.741			11
1.478	10.283	3.104	6.909	3.8411	9.988	21.441	10.338	6.6266	8.9113			12
1.533	8.015					2.596	8.724	2.4486	7.8464			13
						1.3959	5.588					14
												15
												16
100		100		100				100		100		Sum
100		100		100				100		100		In lane

Lane 1=Marker , Lane 2 =Control , Lane 3=TR15 , Lane 4=TR30 ,Lane 5=TR45 , Lane6=WG

Table (4): Effect of chronic administration of tramal (400mg) on serum protein electrophoretic patterns in adult male rats. (Comparison study)

Tramal/W		Tramal/45		Tramal/30		Tramal/15		C		Groups M.wt (KD)
%	+/	%	+/	%	+/ -	%	+/-	%	+/-	
2.3	+		-		-		-		-	172.51
	_		_		_	6.59	+		-	111.85
10.4	+		_		_		_	25.7	+	109.28
	_	23.1	+	25.8	+		_		_	107.9
	-		-		-	1.47	+	3.83	+	98.54
3.41	+			3.12	+		_		_	97.503
		8.46	+					2.95	+	95.345
				2.51	+		_		_	93.604
		5.98	+							90.084
						1.53	+		-	87.44
35.8	+		-		-		-		-	81.911
0.93	+									66.483
			-	1.88	+	_	_		_	65.049
						2.26	+		-	62.81
							-	1.16	+	56.778
		5.1	+							44.462
21.9	+			10.54	+					41.368
								2.58	+	40.385
						13.45	+		_	39.426
1.82	+	0.39	+					11.6	+	29.376
				5.55	+	_	_		_	28.138
						3.82	+		_	27.47
						1.97	+		_	24.137
				15.1	+		_		_	22.446
		15.7	+							21.454
4.25	+			10.23	+			5.3	+	20.909
		12.5	+			14.81	+		_	19.398
4.19	+			8.35			+		_	18.073
						9.8	+	14.5	+	17.204
				6.313	+	5.17	+		_	16.731
		8.42	+					9.5	+	15.723
								8.3	+	14.712
3.9	+					13.77	+		-	13.62
		10.9	+	6.6	+			5.26	+	11.741
1.48	+	2.51	+	3.84	+	21.44	+		_	10.338
1.5	+	18.1	+	15.36	+	2.59	+	6.63	+	8.911
								2.45	+	7.85
		3.10	+							6.9087
						1.39	+		_	5.58
13		12		12		14		13		Sum in lane

+= Expressed,- =Not expressed



TR(I) **TR(II)**

Fig(1): Electrophoretic pattern of serum protein in adult male rat affected by chronic tramal toxicity for15,30,45 and W15 days. Individual lanes represent individual samples per group. C=Control; T15=Tramal treated group for 15 days;T30=tramal treated group for 30 days;T45= tramal treated group for 45 days and W15= withdrawal group for 15 days.

In table (3), (4) and figure (1):

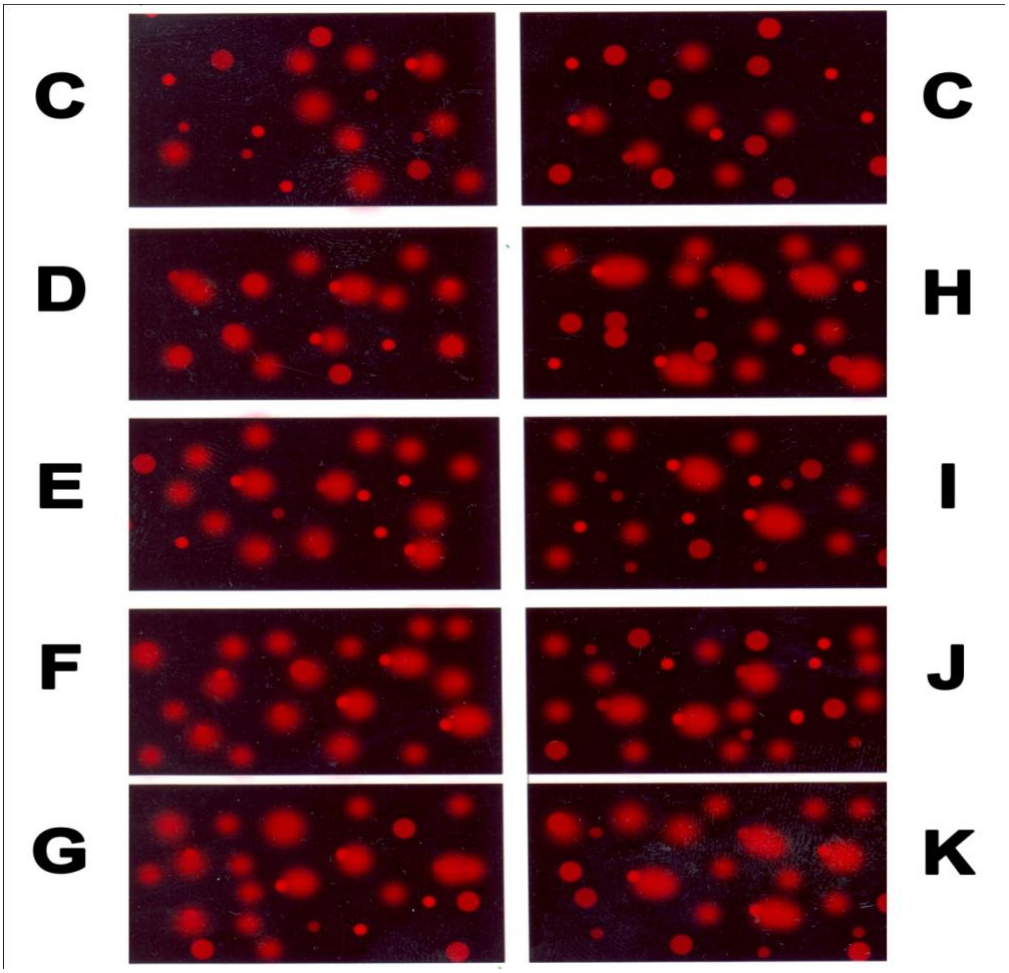
The banding pattern showed 13 distinct bands in control group, 14 distinct pattern in *TR15 group, 12for TR30and TR45 and 13 distinct bands in w 45 group. Only one* band of 8.9113KDa was expressed in all experimental groups (highlighted in table 4).It seem resistant to the effect of tramal400 drug. The 8.9113KDa band was similarly expressed in TR15 and withdrawal groups(around 2.596%) but more expressed in TR30 and TR 45 treated group (15.356 and18.073% respectively). The bands of 56.778, 14.712and7.8464KDa were expressed in control group only, while that of 111.85KDa was expressed inTR15 only and 172.51 KDa in withdrawal group only. There were 10 missing bands in TR15 treated group versus the control group. On the other hand 9 missing bands in TR30versus the control group, 12 missing bands in TR45 treated group and 9 missing bands in w45 group versus the control group.

In the present study an attempt has been made to demonstrate the changes induced in electrophoretic serum protein pattern of male rats by daily administration of 200and 400mg/kg of tramal for 15, 30, 45 days followed by withdrawal periods. The results of electrophoretic separation of serum proteins by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) are shown in tables1,2 and figures(1). The results exhibited major changes in the protein pattern as shown in tables1,2,3,4 and figures (1)which included changes in the molecular weight as compared with the control bands and

the relative percentage of protein fraction as well as the total number of bands ,as a result of disappearance of some original bands and appearance of other new ones. The changes observed by administration of 200 mg tramal and 400 mg of tramal/kg body weight: Serum protein fraction revealed an increase in total number of protein fractions being in Tr15 (14), TR30 (16bands), while Tr 45 group revealed a decrease in total number of bands (14) .The changes were observed all over the treated groups as well as in the withdrawal groups . Except for group Tr 15, 30, 45 which showed an increase in total number of protein fractions (14, 16, 14) bands for 200mg tramal and 14 bands forTR15 400 mg tramal, all the treated groups exhibited a decrease in total number of bands. Our work shows that the change in protein bands should be considered as biomarkers for the investigation of acute liver injury; marked alterations in serum protein pattern and liver DNA damage are in accordance with the hepatic injury.

The obtained results revealed that tramal(200 and 400mg)exerts DNA damage in different groups fig C and table(5) which represented DNA damage pattern obtained by DNA comet assay of rat hepatocytes. The intensity of the comet tail relative to the head reflects the number of DNA breaks. The comet head containing the high molecular weight DNA and the comet tail containing the leading ends of migrating fragments. The results of tramal 200mg, revealed that 1 stgroup(control group),DNA% was found to be 1.25% and DNA tail was2.08,while the 2 nd group after 15 days of tramal treatment recorded that DNA tail was4.39and DNA% was 4.07,while the 3rd group of tramal treatment increases the DNA tail up to 5.51 and DNA% up to 4.29,Meanwhile,4 th group of tramal treatment increases the intensity of the tail up to 6.14 and DNA% up to 5.83.DNAdamage was found in withdrawal group also with DNA tail of (5.89 and DNA% of 5.57).

The results of tramal 400mg, revealed that 1 stgroup(control group), DNA tail was2.08 and DNA% was found to be 1.25%,while the 2 nd group after 15 days of tramal treatment recorded that DNA tail was 5.33and DNA%(4.28),while the 3rd group of tramal treatment increases the DNA tail up to 6.08 and DNA% up to 5.28 ,Meanwhile,4 th group of tramal treatment increases the intensity of the tail up to 6.82 and DNA% up to 6.55.DNAdamage was found in withdrawal group also with DNA tail of (6.21 and DNA% of 6.11).



FIG(2) Representative comet images showing different levels of damage in rat liver DNA In visual scoring :C)score 0(unchangedDNA)and various degree of damage from minor(grade1)to severe (grade 4) ;D) TR15 (200mg);E) TR30(200mg) ;F) TR45 (200mg) ;G) TRW (200mg); H) TR15 (400mg); I) TR30 (400mg); J) TR45 (400mg); K) TRW (400mg).

Table (5)

Effect of chronic administration of tramal (200 and 400mg) on DNA damage in liver of rats using the comet assay.

TR400mg		TR200mg		Parameter groups
DNA%	Tail(um)	DNA%	Tail(um)	
1.25±0.017	2.08±0.02	1.25±0.017	2.08±0.02	Control Mean ±S.D
4.28	5.33±0.11	4.07±0.023	4.39±0.01	TR15 Mean ±S.D
222.4	156.25	225.6	111.1	%change
0.0001	0.0001	0.0001	0.0001	P<
5.28±0.084	6.08±0.065	4.29±0.04	5.51±0.06	TR30 Mean ±S.D
322.4	192.3	243.2	164.9	%change
0.0001	0.0001	0.0001	0.0001	P<
6.55±0.091	6.82±0.071	5.83±0.06	6.14±0.07	TR45 Mean ±S.D
424	227.88	366.4	195.2	%change
0.0001	0.0001	0.0001	0.0001	P<
6.11±0.075	6.21±0.053	5.57±0.02	5.89±0.04	TRW15 Mean ±S.D
388.8	198.558	345.6	183.2	%change
0.0001	0.0001	0.0001	0.0001	P<

P-value <0.05 statistically significant TR_{15}, TR_{30}, TR_{45} and TRW_{15} = duration of time taken 15, 30, 45 and 15.

Discussion:

The effect of tramal administration on serum protein pattern was found to induce severe changes in the protein regions when compared with the control pattern. The decrease of protein bands was reported by many different types of protein oxidative modification which can be induced directly by Reactive Oxygen Species (ROS) or indirectly by reactions of secondary byproducts of oxidative stress (Berlett andStadtman, 1997). The present study aimed to investigate the effect of chronic administration of tramal on protein in male rats as manifested by changes in serum protein patterns and gene expression manifested by DNA damage measured by Comet assay.

The present study has revealed major changes in the electrophoretic mobility of serum protein among the treated groups especially in case of TR400.These changes might reflect a considerable degree of oxidation to protein molecules ,which might in turn results in tertiary structural alteration that promote protein aggregation and amyloidal formation as supported by the study of Shinall *et al*.,(2005).The mechanism involved in the oxidation of protein by ROS has also been described by the work of Stdtmann (2004) in which amino acids ,simple peptides and proteins were exposed to ionizing radiations under conditions where hydroxyl radicals or a mixture of hydroxyl/superoxide radicals are formed The authors' concluded that the side chain of all amino acid residues of proteins ,in particular cysteine and methionine residues of protein are susceptible to oxidation by the action of ROS/RNS. The present data has also reflected a wide range variation in serum protein pattern s due to tramal treatment, which direct reflects a parallel variation in the process of gene expression. Oxygen free radicals produced from tramal have shown to induce DNA breaks and DNA damage .The appearance or disappearance of protein fraction in the present study may be attributed to this damage or may be explained by the report of Bedwell *et al.*, (1989), who claimed that the free radicals promote sulfhydral mediated cross linking of the labile amino acids such as methionine, histidine, cysteine and lysine causing a fragmentation of polypeptide chains in the protein molecule. Tramadol increased lipid per oxidation, due to long term use (Atici *et al*., 2005).In living cells ,when the formation of intracellular reactive oxygen species exceeds the cells antioxidant capacity ,oxidative stress can arise ,resulting in damage to cellular macromolecules such as proteins ,lipids and DNA(Nordberg and Arner,2001andValko *et al*.,2007).DNA is a particularly sensitive cellular target because of the potential to create cumulative mutations that can disrupt cellular homeostasis . in this case ,the reactive oxygen species can lead to the formation of single and double – strand breaks .as well as induce chemical and structural modifications to purine and pyrimidine bases, and also to 2-deoxyribose (Powell *et al*.,2005andHazra *et al.*,2007). Yuan L, Kaplowitz,(2009) stated that acetaminophen overdose is currently the most frequent cause of acute liver failure ,evaluation of the mechanisms of drug-induced liver injury indicates that mitochondria are critical targets for drug toxicity, either directly or indirectly through the formation of reactive metabolites. The consequence of these modifications is generally a mitochondrial oxidant stress and peroxynitrite formation, which leads to

structural alterations of proteins and mitochondrial DNA and, eventually, to the opening of mitochondrial membrane permeability transition (MPT) pores. MPT pore formation results in a collapse of mitochondrial membrane potential and cessation of adenosine triphosphate synthesis. In addition, the release of intermembrane proteins, such as apoptosis-inducing factor and endonuclease G, and their translocation to the nucleus, leads to nuclear DNA fragmentation.

Conclusions:

Our work shows that the change in protein bands should be considered as biomarkers for the investigation of acute liver injury, marked alterations in serum protein pattern associated with liver DNA damage are in accordance with the hepatic injury.

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التغير الجينى للحمض النووي الدى اوكسى ريبوزى والتغير فى أنماط الفصل الكهربى لمصل البروتين فى الكبد لاستخدام الترامادول على المدى الطويل فى الجرذان البيضاء

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المركز القومى للبحوث الا جتماعية و الجنائية/ القاهرة/مصر

الملخص:

الترامادول هو مسكن ألم مركزي له مفعول مقارب للكوديين، وهو نظير هذا الأخير. ويصنف ضمن مسكنات الألم من النوع 2. يؤثر على نفس مستقبلات المورفين، وهو منافس على المستقبلات المورفينية. و لا يحدد مفعول المورفينات الأخرى و يسبب ادمانا ولكن بصفة أقل من باقي المورفينات المنافسة على نفس المستقبلات.
طريقة تأثيره ليست معروفة بشكل جيد، بالإضافة لتأثيره المسكن الناتج عن ارتباطه بالمستقبلات المورفينية، يمنع اعادة استخدام السيروتونين والنورادرينالين وبذلك يزيد نسبة النورادرينالين في الدم والتي تعمل بشكل فعال على تخفيف الالم حسب الجرعات المستعملة.ويستعمل غالبا لتسكين الام مرضى السرطان والاورام الخبيثة.

في هذه الدراسة: تم دراسة التأثير المزمن لعقارالترامادول 200 مج و**400** مج لمدة15و30و 45 يوما، تليها فترت الانسحاب (W15) على الفصل الكهربي لمصل البروتينات (SDS) في ذكورالجرذان والتغير الجيني للحمض النووي الدى أوكسى ريبوزى نتيجة التلف بواسطه مذنب الفحص (comet assay) وقد أجريت هذه الدراسة على 100 من ذكورالجرذان البيضاء من نوع **ويستار** (100-150جم) تم تقسيمهم إلى ثلاث مجموعات متساوية.

المجموعة الأولى: وتسمى المجموعة الضابطة وتشمل 20 جرذا (العدد = 20)
المجموعة الثانية: (ترامال (I): تم حقن هذه المجموعة عن طريق الفم بعقار الترامال 200 مج/كج) (العدد = 40) عن عن طريق الفم و تقسم هذه المجموعة الى مجموعات فرعية تحقن لمدة 15و30و45 يوما متتالية للدراسة، على التوالي (10فئران لكل مجموعة فرعية)و تعقب المجموعه الفرعيه (TR45)على فترة الانسحاب لمدة 15 يوما(W15) .

المجموعة الثالثة: (ترامال (II): تم حقن هذه المجموعة عن طريق الفم بعقار الترامال 400 مج/كج) (العدد = 40) عن طريق الفم و تقسم هذه المجموعة الى مجموعات فرعية تحقن لمدة 15و30و45 يوما متتالية على التوالي (10 جرذان لكل مجموعة فرعية)و تعقب المجموعه الفرعيه (TR45)على فترة الانسحاب لمدة 15 يوما(W15) .

وتظهر نتائج الفصل الكهربي لمصل للبروتينات (SDS) في الجداول1و2و3و4 والشكل (1) :

1- تغيرات كبيرة في نمط البروتين التي شملت تغييرات في الوزن الجزيئي والوزن النسبي لجزىء البروتين وكذلك العدد الإجمالي للشرائح، نتيجة لاختفاء بعض ا لشرائح الأصلية وظهور أخرى جديدة. كم اظهرت النتا ئج انخفاض في العدد الكلي للشرائح (باستثناء TR15 التي عرضت زيادة في عدد الشرائح(14) فى حالة الجرعات المزمنه للترمال 200 مج و 400 مج وTR30 و التي عرضت زيادة في عدد الشرائح(16) فى حالة الجرعات المزمنه للترمال 200 مج . وقد اظهرت النتائج ايضا تغييرات في جميع أنحاء المجموعات المعالجة وكذلك في مجموعة الانسحاب .

2- وقد أظهرت النتائج ايضا تأثير الترامال (200 400مج /كج) على تلف الحمض النووي بواسطة الشوارد الحرة فى خلايا كبد الجرذان وكانت الخلايا المعرضة للترامال 400مج أعلى بكثير منها في الخلايا المعرضة ل200مج/كج، وخصوصا بعد فترة طويلة الإدارة (TR45 يوما). كما ان شدة الذيل المذنب نسبة إلى الراس يعكس عدد فواصل الحمض النووي الدى أوكسى ريبوزى (DNAbreaks). كما ارتفعت معدلات خلايا الذيل (الكشف عنها بواسطة الفحص المذنب) بشكل ملحوظ عندما تعرضت **الجرذان** إلى 200 و400مج/كج من الترامال مقارنة مع المجموعة الضابطة إلا أن طول ذيل المذنب لم تختلف بشكل كبير بين نفس المجموعة. كما ان نتائجنا أشارت إلى زيادة مخاطر الحمض النووي من التلف وخلل في نمط البروتين نظرا لاستخدام الترامال على المدى الطويل ،
ونستخلص من هذه الدراسة انه با لرغم أن المواد الأفيونية تكون فعالة في إدارة الألم، يجب أن تبقى آثارها السامة في الاعتبار.