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EFFECT OF COMBINED PARACETAMOL AND *GLYCYRRHIZA GLABRA* OR *CINNAMOMUM ZEYLANICUM* USE IN RATS

(With 4 Tables)

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تأثير الاستخدام المشترك للباراسيتامول وعرق السوس أو القرفة في الجرذان

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أجريت هذه الدراسة لتسليط الضوء على بعض التأثيرات السميه لإستخدام الباراسيتامول مع أو بدون عرق السوس أوالقرفه في الجرذان. قسمت ذكور الجرذان لسبع مجموعات، احتوت كل مجموعه على ستة جرذان بعمر اثنا عشر إسبوعاً. المجموعتان الأولى والثانيه أعطيتا الباراسيتامول بتركيز ٥٠٠ملج/ل (في ماء الشرب) أو ٥٠٠ملج/كجم (في الطعام) على التوالي. المجموعه الثالثه أطعمت عرق سوس بتركيز ٦%، المجموعه الرابعه أطعمت قرفه بتركيز ٦% والمجموعتين الأخيرتين أطعمتا خليط من الباراسيتامول بتركيز ٥٠٠ملج/كجم مع عرق السوس بتركيز ٦% أو القرفه بتركيز ٦% لمدة إسبوعين وأربعة أسابيع. أما آخر مجموعه فأعطيت طعام وشراب من غير إضافات كمجموعة تحكم. لوحظ تأثير مرضي في الكبد والكلى في الجرذان التي أطعمت باراسيتامول بتركيز ٥٠٠ملج/ل أو ٥٠٠ملج/كجم. أما الجرذان التي أعطيت عرق سوس فقد سجلت بها أنيميا. إنخفاض مستوى الهيموغلوبين مصحوباً بزيادة تركيز نشاط إنزيم AST وزيادة تركيز اليوريا سُجلت في الجرزان التي أعطيت القرفه. أنيميا وزيادة تركيز نشاط إنزيمAST وزيادة تركيز الألبيومين لوحظت في الجرزان التي أطعمت باراسيتامول مع عرق السوس. والجرذان التي أطعمت باراسيتامول مع القرفه فقد سُجل بها إرتفاع في تركيز اليوريا.

SUMMARY

In this study we decided to throw light on some toxicological aspects of combined usage of Paracetamol with or without *Glycyrrhiza glabra* or *Cinnamomum zeylanicum* in rats. Seven groups each of six male Wistar rats (12 weeks-old) were used in this study. Paracetamol was provided to the first 2 groups at dose level of 500mg/L (in drinking water) or 500mg/kg

(in feed), respectively. The third group received a diet containing 6% w/w *G. glabra*, a fourth group received a diet containing 6% w/w *C. zeylanicum*, and additional 2 groups received mixture of paracetamol at 500mg/kg and *G. glabra* or *C. zeylanicum* for 2 and 4 weeks. The last group remained without treatment as control group. Hepato-nephropathy was observed in the rats fed the diet containing paracetamol at 500mg/L and 500 mg/kg. Anaemia was recorded in rats supplemented with *G. glabra*. Decrease in haemoglobin level, increase in serum aspartate amino transferase (AST) activity and urea concentration were appearing in rats fed *C. zeylanicum*. Anaemia, increase activity of (AST) and increase in albumin level were observed in rats fed paracetamol and *G. glabra*. Increase in urea concentration in rats fed paracetamol and *C. zeylanicum*.

Key words: *Paracetamol, G. glabra, C. zeylanicum, Toxicity, Hepatoprotective activity.*

INTRODUCTION

Glycyrrhiza glabra, member of the family Leguminosea, locally known as "*Liquorice*" or *Licorice* is useful for many diseases as well as cough but it also helps to increase the appetite for facilitating proper evacuation of stools. It is mainly used as expectorant, anti- inflammatory, antipyretic and laxative for children and for treatment of gastric and duodenal ulcers and chronic fatigue syndrome (Foster, 1993; Leung and Foster, 1996).

Tyler (1994) and Blumenthal (1996) have shown that the active constituent, glycyrrhizin, stimulates the secretion of hormone by the adrenal cortex and that glycyrrhizin has similar chemical structure to corticosteroids released by the adrenals. The anti-bacterial activity of methanol extract of *Glycyrrhiza glabra* against *Streptococcus mutants* was described by Hwang *et al.* (2003).

Cinnamumum zeylanicum, a member of the family Lauraceae, is locally known as "*Gerfa*" and its bark is used in Sudan and other countries as carminative, antiseptic, stimulant, antiemetic, antidiarrhoeic and astringent in cosmetics often as skin lotion for the treatment of blotchy skin (Patrick *et al.*, 2004). The therapeutic value of Cinnamon depends on the volatile oil, the most important ingredient is cinnnamic aldehyde, and the bark was found to contain some gum, a coloring matter and tannin (Chang *et al.*, 2001).

Paracetamol (Acetaminophen, *N*-acetyl-P-aminophenol) is a potent analgesic and anti-pyretic agent but is relatively devoid of anti-

inflammatory activity (Al-Swayeh *et al.*, 2000). Unlike aspirin and related non-steroidal anti-inflammatory drugs (NSAID), it is the most widely used pharmaceutical analgesic and antipyretic agent in the world (Anker and Smilkstein, 1994; Farrell, 2002). Indeed, paracetamol pretreatment has actually been reported to protect against aspirin and ethanol-induced gastric mucosal damage in man (Stern *et al.*, 1984). The high potency and lack of gastrointestinal side effects of this drug have led to the widespread use of paracetamol which is regarded as a safer alternative to NSAID for mild to moderate analgesia. However, high doses of paracetamol do cause severe and often fatal acute liver in man (Plevris *et al.*, 1998).

Hepatic dysfunction due to ingestion or inhalation of hepatotoxins such as acetaminophen, cadmium chloride, ethanol, carbon tetrachloride (CCl4) and alkyl alcohols are increasing worldwide (Wolf, 1999). Paracetamol is a common antipyretic agent which can produce fatal hepatic necrosis by formation of metabolite *N*-acetyl -*p*-benzoquinoneimine in the liver (Wong, *et al.*, 1981; Savides and Oehme, 1983).

The main objective of this study is to evaluate the hepatoprotective effect of combined usage of paracetamol with *Glycyrrhiza glabra* or *Cinnamomum zeylanicum* in rats with emphasis on hepato-renal function.

MATERIALS and METHODS

Plant materials: Cinnamomum zeylancium and *Glycyrrhiza glabra* was purchased from a local market, ground separately to a fine powder and then mixed into a normal rat diet.

Drug: Paracetamol produced by Amipharma laboratories, Sudan, was purchased from a local pharmacy in Khartoum and used in this study.

Experimental animals: Forty two 12 –week old- male Wistar rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute (MARPI), National Centre for Research (NCR), Khartoum, under light /dark cycles with feed and water provided *ad libitum.*

The rat basal diet: The rats were given a basal diet which fulfilled their requirement. The composition was as follows:

Wheat flour	657g
Dry meat	220g
Sodium chloride	3g
Oil	120g

Experimental design: the rats were allotted randomly into 7 groups, each of 6 rats. Rats in group 1 served as control and continued to be fed the normal diet and water. Groups 2 and 3 received drinking water containing paracetamol at 500mg/L or diet containing paracetamol at 500 mg/kg, respectively. Groups 4 and 5 received diet containing 6% (w/w) of *Cinnamomum zeylanicum* or 6% (w/w) of *Glycyrrhiza glabra*, respectively. Groups 6 and 7 received a diet that contained a mixture of paracetamol at 500mg/kg and 6% (w/w) of *Cinnamomum zeylanicum* or paracetamol at 500mg/kg and 6% (w/w) *Glycyrrhiza glabra*, respectively.

Blood sampling: Blood was collected from the cervical blood vessels of rats during slaughter (1.5 ml) in test tubes; allowed to clot, and then blood was centrifuged at 3000 r.p.m. for 5 minutes. T serum was harvested into plane containers and used immediately.

Blood Analyses: serum samples were analyzed for the activity of aspartate aminotransferase (AST), and alanine aminotransferase (ALT) according to Reitman and Frankel (1957); and alkaline phosphatase (ALP) (Tietz, 1995), and for concentration of total protein (King and Wooton, 1956), albumin (Bartholomew and Delany, 1966), globulin (globulins (g/l) were determined by deduction of the values of serum albumin from total serum protein values), bilirubin (Heinemann and Vogt, 1988), cholesterol (Allain *et al.*, 1974); and urea (Fawcett and Scott, 1960).

Haemoglobin (Hb) concentration, red blood cells (RBC) count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cells (WBC) count were determined using standard methods (Schalm *et al.*, 1975).

Statistical Analysis: the significance of the differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochran, 1989).

RESULTS

Table 1: Haematological changes in Wister rats fed normal diet (group 1), paracetamol 500 mg/L (group 2), paracetamol 500mg/kg (group 3), *G. glabra* 6% w/w (group 4), *C. zeylanicum* 6% w/w (group 5), paracetamol 500mg/kg and *G. glabra* 6%w/w (group 6), paracetamol 500mg/kg and *C. zeylanicum* 6%w/w(group 7) for2 weeks.

Parameters	Treatment groups						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Hb (g/dl)	17.3±0.05	10.8±0.06*	12.4±0.21*	11.2±0.41*	11.4±0.44*	10.6±1.36*	10.8±2.22
RBC ($X10^6mm^3$)	3.7±0.05	6.0±0.08*	7.1±0.20*	6.2±0.33*	6.4±0.21*	6.0±0.72	6.0±1.30
PCV (%)	36.0±0.09	34.7±0.24*	40.3±1.10	36.2±1.5	37.2±1.04	33.8±4.15	35.2±7.07
MCV (%)	98.6±0.05	57.2±0.41*	57.1±0.63*	8.3±..80*	57.7±0.85*	55.9±0.37*	58.4±1.46*
MCH(pg)	47.4±0.05	17.9±0.31*	17.6±0.24*	18.1±0.29*	17.7±0.38*	17.6±0.25*	17.9±0.24*
MCHC (%)	48.1±0.3	31.3±0.32*	30.9±0.32*	31.1±0.23*	36.6±0.36*	31.4±0.26*	30.7±0.48*
WBC($X10^3mm^3$)	3.4±0.05	6.9±0.72*	5.2±0.27	6.1±0.37*	5.7±1.70	3.9±1.35	7.3±3.13
Neutrophils (%)	10.0±0.57	50.٢±8.70*	53.7±3.20*	46.4±10.11	51.8±2.05*	49.9±10.69	4٧.٦±2.18*
Lymphocytes (%)	90.0±0.57	49.8±8.70*	46.٢±3.2*	53.٦±10.11	48.٢±2.05*	50.1±10.69	52.4±1.81*

Values are means ± SE.

* Means in the same row with asterisk are significantly different ($p<0.05$) with control group (group 1)

Table 2: Haematological changes in Wister rats fed normal diet (group 1), paracetamol 500 mg/L (group 2), paracetamol 500mg/kg (group 3), *G. glabra* 6% w/w (group 4), *C. zeylanicum* 6% w/w (group 5), paracetamol 500mg/kg and *G. glabra* 6%w/w (group 6), paracetamol 500mg/kg and *C. zeylanicum* 6%w/w(group 7) for 4weeks.

Parameters	Treatment groups						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Hb(g/dl)	12.3±0.	12.6±0.4	14.5±0.0	12.6±0.5	13.6±0.2	9.9±2.9	12.0±0.4
RBC ($X10^6mm^3$)	5.1±0.0	6.5±0.05	7.7±0.12	6.5±0.59	6.8±0..0	6.3±0.26	6.2±0.21
PCV (%)	24.1±0.	40.4±0.7	47.6±0.0	40.9±3.1	43.3±0.2	39.7±1.9	38.4±1.2
MCV (%)	47.1±0.	62.0±0.6	58.7±0.3	62.4±0.8	63.4±0.5	62.5±0.2	61.2±1.1
MCH (pg)	24.1±0.	19.3±0.6	18.1±0.3	19.4±0.8	20.0±0.0	15.4±4.2	19.2±0.2
MCHC (%)	51.3±0.	31.2±1.0	30.1±0.2	31.0±1.0	31.5±0.4	24.6±6.7	31.4±0.4
WBC ($X10^3mm^3$)	3.1±0.0	6.5±1.16	4.5±0.23	5.2±0.95	6.8±0.87	4.2±0.88	4.7±1.13
Neutrophilils (%)	59.7±0.	38.٦±0.4	61.9±0.5	44.3±6.1	40.1±1.4	43.٥±7.6	43.٢±1.0
Lymphocytes (%)	40.2±0.	61.4±0.8	38.١±0.5	55.7±6.1	59.9±1.4	56.5±7.6	56.8±1.0

Values are means ± SE.

* Means in the same row with asterisk are significantly different ($p<0.05$) with control group (group 1)

Table 3: Serum biochemical tests in Wister rats fed normal diet (group 1), paracetamol 500 mg/L (group 2), paracetamol 500mg/kg (group 3), *G. glabra* 6% w/w (group 4), *C. zeylanicum* 6% w/w (group 5), paracetamol 500mg/kg and *G. glabra* 6%w/w (group 6), paracetamol 500mg/kg and *C. zeylanicum* 6%w/w (group 7) for 2 weeks.

Parameters	Treatment groups						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
AST (i.u)	41.00±0.57	152.6±5.45	193.0±14.29*	181.0±31.0**0150	181.0±23.15*	195.6±16.82*	166.3±14.62*
ALT (i.u)	15.0±0.57	25.3±3.17	49.3±5.04*	40.5±2.50*	56.6±21.05	26.6±4.05	36.6±3.18*
ALP (i.u)	318.0±0.57	256.3±23.20	273.6±17.23	229.0±23.0	245.0±49.89	236.0±46.70	208.0±39.15
Total protein g/dl)	8.0±0.57	6.6±0.33	7.3±0.66	6.5±..50	6.6±0.33	7.3±0.33	7.0±0.21
Albumin (g/dl)	3.0±0.57	4.0±0.10	4.3±0.33*	3.5±0.50	3.6±0.33	3.3±0.33	3.6±0.33
Globulins (g/dl)	5.0±0.57	3.3±0.33	3.6±0.33*	4.0±0.14*	2.6±0.33*	3.6±0.33*	3.6±0.33*
Bilirubin (mg/dl)	0.7±0.05	0.5±0.05	0.43±0.03*	0.50±0.180	0.46±0.033*	0.50±0.05	0.46±0.06*
Cholestero (mg/dl)	82.0±0.57	92.0±5.03	75.0±13.31	61.5±7.50	69.0±11.59	66.3±4.33	67.6±14.51
Urea (mg/dl)	60.0±0.57	60.3±2.33	47.6±4.66	60.0±3.00	58.6±9.28	53.6±1.45*	63.0±4.04

Values are means ± SE.

* Means in the same row with asterisk are significantly different ($p<0.05$) with control group (group 1)

Table 4: Serum biochemical tests in Wister rats fed normal diet (group 1), paracetamol 500 mg/L (group 2), paracetamol 500mg/kg (group 3), *G. glabra* 6% w/w (group 4), *C. zeylanicum* 6% w/w (group 5), paracetamol 500mg/kg and *G. glabra* 6%w/w (group 6), paracetamol 500mg/kg and *C. zeylanicum* 6%w/w (group 7) for 4weeks.

Parameters	Treatment groups						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
AST(i.u)	32.0±0.57	48.6±10.17	29.3±0.64	43.5±10.50	77.0±47.50	138.6±8.19*	129.3±1.66*
ALT(i.u)	15.0±057	48.6±10.17	29.3±6.43	34.5±10.50	33.3±3.84*	25.6±3.84	25.6±2.02*
ALP(i.u)	410±0.57	239.3±70.33	258.6±48.3	267.0±106.0	238.3±39.25*	151.0±34.22*	202.0±43.01*
Total protein (g/dl)	8.0±0.57	7.3±0.33	7.0±0.57	7.0±0.12	6.6±0.33	6.6±0.33	7.0±0.21
Albumin (g/dl)	2.6±0.33	3.6±0.33	3.6±0.33	4.0±0.15*	2.0±1.00	3.6±0.33*	4.0±0.41*
Globulin (g/dl)	4.6±0.33	4.0±0.32	3.3±0.33*	3.5±0.50	3.0±0.13*	3.0±.08*	3.0±0.33*
Bilirubin (mg/dl)	0.66±0.03	0.53±0.53*	0.53±0.33*	0.55±0.05	0.53±0.03*	0.46±0.88	0.46±0.66
Cholesterol (mg/dl)	77.3±0.33	56.3±9.87	73.0±12.5	85.5±25.5	56.6±6.33	40.6±2.84*	56.6±19.33
Urea (mg/dl)	36.3±0.33	66.0±6.08*	56.3±4.97*	66±10.5	67.0±2.51*	49.6±4.66	58.0±4.04*

*Values are means ± SE.

* Means in the same row with asterisk are significantly different ($p<0.05$) with control group (group 1)

DISCUSSION

In the Sudanese system of medicine, certain herbs are claimed to have medicinal properties against several disorders. The claimed therapeutic reputation has to be verified in a scientific manner. This study was conducted to investigate if "Gerfa" and "Liquorice" could provide some sort of hepato protection against Paracetamol hepatotoxicity.

Haematological changes: are summarized in Tables (1 and 2). After 2 weeks of treatment as we could observe in Table (1), the values of Hb (except in group7), MCV, MCH and MCHC were significantly lower ($P<0.05$) in all groups when compared with control group. The values of RBCs were significantly higher ($P<0.05$) in groups 2, 3, 4 and 5. The value of PCV was significantly lower ($P<0.05$) in group 2 than control and other groups. WBCs were significantly higher ($p<0.05$) in groups 2 and 4. Neutrophils were higher and lymphocytes were lower ($p<0.05$) in groups 2, 3, 5 and 7.

Based on above data, the lower level of MCV, MCH, and MCHC in groups 2 and 3 following 2 weeks of treatment, as we could observe in Table (1) may be influenced by the higher value of Hb reported in control group. But results obtained by the end of the study ensure that the higher value of Hb following two weeks of feeding in the control group may be attributed to machinery fault, as the value of Hb in the different groups is in close relation with the control.

As shown in Table (2), after 4 weeks of treatment, the values of Hb were higher ($P<0.05$) in groups 3 and 5. PCV and MCV were significantly higher ($p<0.05$) in all groups than control group. MCH and MCHC were lower ($p<0.05$) in all groups than control group. The values of RBCs were higher ($p<0.05$) in groups 2, 3, 5, 6 and 7. Neutrophils were lower and lymphocytes were higher ($p<0.05$) in groups 2, 5 and 7 than control and other groups.

The decrease in the level of MCV, MCH and MCHC in group 4 (6% *G. glabra*) and in group 6 (500 mg/kg paracetamol and 6% *G. glabra*), as we could observe in Table (1) may indicate microcytic hypochromic anaemia. There was significant decrease in haemoglobin level with rats in group 5 (6% *Cinnamomum zeylanicum*) as shown in Table (1), and this was also described by Shah *et al.* (2004).

Serum biochemical changes: the obtained data are presented in Tables (3 and 4). Following 2 weeks of treatment, as we could observe in Table (3), the activity of serum AST was significantly higher ($p<0.05$) in groups 2, 3, 4, 5, 6 and 7. The activity of serum ALT in groups 3, 4, and 7 was higher ($p<0.05$) than control group. Globulins concentration was lower ($p<0.05$)

in groups 2–7 than control. Bilirubin was significantly lower ($p<0.05$) in groups 3, 5 and 7. Urea concentrations was lower ($p<0.05$) in group 6 than other groups as recorded in Table (3).

After 4 weeks of treatment, as we could observe in Table (4), the activity of serum AST was significantly ($p<0.05$) higher in groups 6 and 7 than control and other groups. The activity of serum ALT in groups 5 and 7 was significantly ($p<0.05$) higher than control group. The activity of ALP was significantly ($p<0.05$) lower in groups 5, 6 and 7. Albumin was significantly ($p<0.05$) higher in groups 4, 6 and 7 than control. Globulins level was significantly ($p<0.05$) lower in groups 3, 5, 6 and 7 than control and other groups. Bilirubin was significantly ($p<0.05$) lower in groups 2, 3 and 5 than control as recorded in Table (4). Cholesterol was significantly ($p<0.05$) lower in group 6 than control and other groups. Urea concentration was significantly ($p<0.05$) higher in groups 2, 3, 5 and 7 than other groups.

Elevated activity of serum AST, ALT combined with decrease in globulin concentration as shown in Table (3), following 2 weeks of treatment, may indicate an effect in the rat liver of groups 2 and 3. That may suggest paracetamol hepatotoxicity in rats. Sever hepatic necrosis was first observed in cats treated with paracetamol (25 mg/kg and then 50 mg/kg) for 22 weeks (Eder, 1964). In a similar study, Tuse and his colleagues (2009), observed hepatic damage produced by paracetamol (3 gm/kg; p.o.), which was reflected by increased levels of SGOT and SGPT as compared to control ($p<0.001$).

The liver was affected as evident by an increase in the activity of AST and ALT, decrease in activity of ALP and increase in albumin concentration, as shown in Table (3), so 6% *G. glabra* does not exhibit hepatic-protection, a similar effect was recorded previously by (Ge Lin *et al.*, 1999), and single dose (35mg/kg) did not show hepatic -protection, but high dose (200mg/kg) can do.

Also we observed neutrophils increase than control in this study, the macrophages and neutrophils are attracted to the damage areas and lead to additional protein thiol (Mitchell, 1988).

Rang and his colleagues (2007), reported that toxic doses (10-15 grams) of paracetamol cause potentially fatal hepatotoxicity. This occurs when the liver enzymes catalyzing the normal conjugation reactions are saturated, causing the drug to be metabolized instead by mixed function oxidases. The resulting toxic metabolite, N-acetyl-p-benzoquinone imine, is inactivated by conjugation with glutathione, but when glutathione is depleted the toxic intermediate accumulates and reacts with nucleophilic

constituents in the cell. This causes necrosis in the liver and also in the kidney tubules. The initial symptoms of acute paracetamol poisoning are nausea and vomiting, the hepatotoxicity being a delayed manifestation that occurs 24-48 hours later.

The data obtained in this study revealed significant increase in the activity of serum AST, ALT which was increased after 4 weeks and ALP was decreased. Also there was significant elevation in blood urea concentration after 4 weeks (from 36.3 to 67.0) as shown in Table (4). A similar effect was recorded previously (Gawronska- Szklarz, *et al.*, 2003) which may indicate an impairment of kidney function.

In group 7 (500 mg/kg paracetamol and 6% *C. zeylanicum*), there was increase in urea concentration after 4 weeks (Table 4), which indicated a renal abnormality. Increase in albumin concentration, increased activity of serum AST and ALT and decrease activity of ALP as appearing in Table (4), which may be related to liver lesion.

Katzung (2006), reported that paracetamol in therapeutic doses, a mild increase in hepatic enzymes may occasionally occur in the absence of jaundice; this is reversible when the drug is withdrawn. With larger doses, dizziness, excitement, and disorientation are seen. Ingestion of 15 g of acetaminophen may be fatal, death being caused by severe hepatotoxicity with centrilobular necrosis, sometimes associated with acute renal tubular necrosis. Early symptoms of hepatic damage include nausea, vomiting, diarrhea, and abdominal pain. Cases of renal damage without hepatic damage have occurred, even after usual doses of acetaminophen.

Conclusion:

It is to be concluded that, Paracetamol was found to exhibit some degree of hepatotoxicity to rats at 500mg/L and 500mg/kg, excessive usage of paracetamol may lead to hepatotoxicity, nephrotoxicity. Consumption of *G. glabra* may cause fall in haemoglobin level. Usage of *C. zeylanicum* with paracetamol lead to an increase in the level of blood urea, and that may suggest impairment in kidney function.

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