Enhanced DNA repair, immune function and reduced toxicity of C-MED-100™, a novel aqueous extract from *Uncaria tomentosa*

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Abstract

Female W/Fu rats were gavaged daily with a water-soluble extract (C-MED-100™) of *Uncaria tomentosa* supplied commercially by CampaMed at the doses of 0, 5, 10, 20, 40 and 80 mg/kg for 8 consecutive weeks. Phytohemagglutinin (PHA) stimulated lymphocyte proliferation was significantly increased in splenocytes of rats treated at the doses of 40 and 80 mg/kg. White blood cells (WBC) from the C-MED-100™ treatment groups of 40 and 80 mg/kg for 8 weeks or 160 mg/kg for 4 weeks were significantly elevated compared with controls ($P < 0.05$). In a human volunteer study, C-MED-100™ was given daily at 5 mg/kg for 6 consecutive weeks to four healthy adult males. No toxicity was observed and again, WBC were significantly elevated ($P < 0.05$) after supplement. Repair of DNA single strand breaks (SSB) and double strand breaks (DSB) 3 h after 12 Gy whole body irradiation of rats were also significantly improved in C-MED-100™ treated animals ($P < 0.05$). The LD_{50} and MTD of a single oral dose of C-MED-100™ in the rat were observed to be greater than 8 g/kg. Although the rats were treated daily with *U. tomentosa* extracts at the doses of 10–80 mg/kg for 8 weeks or 160 mg/kg for 4 weeks, no acute or chronic toxicity signs were observed symptomatically. In addition, no body weight, food consumption, organ weight and kidney, liver, spleen, and heart pathological changes were found to be associated with C-MED-100™ treatment. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Uncaria tomentosa; Rat; Immune response; DNA repair; Toxicity

1. Introduction

DNA repair is important in maintaining both cell viability and genomic stability. A cell responds to DNA damage in one of three ways: (1) by tolerating the damage; (2) by repairing the damage; and (3) by undergoing apoptosis. The latter two responses represent defenses against genomic instability and tumorigenesis resulting from unrepaired damage (ap-Rhys and Bohr, 1996). Several signal transduction pathways including p53-dependent signal transduction (Wang and Ohnishi, 1997; Evan and Littlewood, 1998) and cyclic ADP-ribose signaling (Willmott et al., 1996) are activated by DNA damage resulting in cell-cycle arrest. Cell-cycle arrest increases the time available for DNA repair before DNA replication and mutation fixation. Loss of cell cycle control and the inability of the cell to repair DNA at cell cycle checkpoints results in the propagation of genetic lesions, which ultimately leads to cancer (Hong and Sporn, 1997). Enhancing DNA repair, reducing DNA damage and stimulating immune response by blocking growth signal transduction, leading to induction of apoptosis and differentiation will have great potential to prevent the development of invasive cancer either (1) by blocking the DNA damage that initiates carcinogenesis or (2) by arresting or reversing progression of premalignant cells into malignant cells.

Uncaria tomentosa (Willd.) DC (Rubiaceae), commonly known as uña de gato or cat's claw, is used in South American folk medicine (Jones, 1995). The extracts from various components of this plant have been shown to have cytostatic, contraceptive, anti-inflammatory, antiviral, and antimutagenic activities as well as to have an enhancement of phagocytosis (Keplinger, 1982; Wagner et al., 1985; Cerri et al., 1988, Aquino et al., 1989, 1991; Rizzi et al., 1993). Most of these studies were based on organic solvent extraction and attributed to the effects of indole alkaloids separated from the plant parts.

Apoptosis is a naturally occurring form of cell death or suicide of particular significance to maintaining competent homeostatic inflammatory and immune responses necessary as a primary defense against many diseases including cancer, viral infections, AIDS, autoimmune and neurodegenerative disorders. Agents that can induce apoptosis are potential anti-inflammatory and anti-tumor drugs because they may have the ability to induce apoptotic death in malignant or inflammatory macrophages or monocytes, which are in turn known to be particularly sensitive to induction of apoptosis. Likewise, such agents simultaneously stimulate immune cell function by limiting or reducing the production of inflammatory cell TNFα which is a well known naturally occurring agent that is cytotoxic to lymphocytes and thus immunosuppressive (Thompson, 1995). In previous studies we have shown that C-MED-100™, the proprietary aqueous extract from *U. tomentosa*, is effective in induction of apoptosis in HL60 leukemic cells (Sheng et al., 1998a), and thus this preparation may well have important anti-tumor, anti-inflammatory and immune stimulating properties. Our laboratory has also demonstrated that a significant DNA repair enhancement has been observed after supplementation of a combination of carotenoids, nicotinamide and zinc (Sheng et al., 1998b). Applying similar methodologies the present study has shown that supplementation of a unique water-soluble fraction (C-MED-100™) of *U. tomentosa* extract enhanced DNA repair and immune responses in vivo while no toxicity was observed within the dose range tested.

2. Materials and methods

2.1. Materials

C-MED-100™ water extracts were supplied commercially by CampaMed, New York, New York, USA. The product is based on a hot water extraction for 20–24 h at 90–100°C and separation from higher molecular weight conjugated components such as tannins. The pale-yellow extract powder was dissolved directly in sterile tap water to give a homogeneous, clear solution for oral supplementation of animals. C-MED-100™ was given as 350-mg tablets in the human volunteer study provided from the same supplier for the animal studies. Commercial extract A was provided by M.W. International, Hillside, NJ (Lot

: E-40639). This is a water soluble 4:1 cat's claw extract, but not purified from high molecular weight toxic components as is C-MED-100™. Uña de gato bark powder (in capsule) was commercially available from Oscar Schuler Egg (Lima, Peru). Water/ethanol extract of *U. tomentosa* (4% alkaloids) was also provided by MW International (Hillside, NJ).

2.2. Animals

Female Wistar-Furth rats, weighing 150–200 g in experiments I and II and 100–150 g in experiment III at the beginning of the experiment, were housed two to three per cage at ambient temperature of 21–23°C. The lights were on from 6:00 to 18:00 h. and the rats had free access to fresh tap water and standard pellet food. In experiment I, 30 rats were randomly assigned into three groups with ten animals in each. Controls were gavaged with sterile tap water while treatment groups were gavaged with C-MED-100™ at the doses of 40 or 80 mg/kg (1 or 2 ml) per day Monday through Sunday for 8 consecutive weeks. At the termination of the experiment, half of the rats from each group were whole body irradiated with 12 Gy in a ^{137}Cs source (Scanditronix, 1.56 Gy/min) and allowed to repair for 3 h in vivo. The animals were then sacrificed and spleen single cell suspensions were prepared in ice-cold homogenizing buffer (15 mM Tris, 60 mM NaCl, 0.34 M sucrose, 10 mM 2-mercaptoethanol and 10 mM EDTA, pH 7.4) (Romagna et al., 1985). Splenocytes were either immediately used for the phytohemagglutinin (PHA) stimulating response assay or frozen at −80°C after addition of 10% dimethyl sulphoxide (DMSO) for alkaline elution assay. In experiment II, 40 female W/Fu rats were randomly assigned into five groups with eight rats in each group and gavaged with C-MED-100™ doses of 0, 5, 10, 20 and 40 mg/kg. PHA induced lymphocyte proliferation and leukocyte counts were the major biological endpoint indices for this experiment. In experiment III, C-MED-100™ was tested by weight gain growth curves and food consumption at a higher dose of 160 mg/kg daily for 4 consecutive weeks alone and compared to a commercial extract A product at the same dose. The rats were

randomly assigned into different groups and there was no statistically significant difference among the groups in body weight and white blood cells at the beginning of each experiment. The body weights of each rat and the food consumption of each cage (two to three rats) were recorded every week. The rats were treated according to the Swedish guidelines for humane treatment of laboratory animals and the experiments were approved by the Ethical Committee at the University Hospital in Lund, Sweden.

2.3. Acute toxicity study

For LD_{50} (the dose to cause 50% death) and MTD (maximum tolerable dose) 60 W/Fu rats were used in one experiment with five rats in each group for three preparations from the *U. tomentosa*. C-Med-100™ was administered at the doses of 0, 1, 2, 4 and 8 g/kg by a single dose of gavage. Because of the poor solubility, two other commercial products, uña de gato bark powder from Oscar Schuler Egg (Lima, Peru) and water/ethanol extracts of *U. tomentosa* (4% alkaloids, sample provided by MW International, Hillside, NJ) were only dosed up to 0.25, 0.5, 1 and 2 g/kg and to 0.625, 1.25, 2.5 and 5.0 g/kg respectively. The rats were observed for mortality and acute toxicity up to 2 weeks after the drug administration.

2.4. Human volunteer study

A volunteer supplement study of four apparently healthy, adult males was carried out over a 9-week period. The average age was 46 ± 12 (32–58) years. The volunteers were base-lined for 3 consecutive weeks using standard differential blood cell count analysis. After establishing baseline, each subject took one 350 mg C-MED-100™ tablet daily for 6 additional consecutive weeks. No changes in food intake pattern, life style, disease or medication had occurred during the supplementation. The side effects were judged by hematological analysis, body weight loss, work attendance and symptoms including diarrhea/constipation, headache, nausea/vomiting, rash/edema and pain. Total blood cell counts were also used

to monitor the efficacy and toxicity. The volunteer study was carried out in accordance with Helsinki Declaration of 1975.

2.5. Alkaline elution

Frozen spleen single cell suspensions were rapidly thawed at 37°C and layered directly onto polycarbonate filters with 25 mm diameter and 2 μm pore size (Millipore). DNA single strand breaks (SSB) were measured by alkaline elution as described by Kohn et al., (1981) with modifications to measure the unlabeled DNA by microfluorometry (Cesarone et al., 1979; Olsson et al., 1995).

2.6. Phytohemagglutinin (PHA) induced lymphocyte mitogenic response

Freshly prepared splenocytes in single cell suspensions were cultured in microtiter plates at 25 000 cells/well in 200 μl RPMI 1640-10% FCS-10 μl PHA at 37°C and 5% CO_2 for 5 days then pulsed for 6 h with [^{3}H]-thymidine (2 Ci/mmole, final concentration 0.5 μCi/ml). Labeled nuclear material was collected on glass fiber filters in a microtiter plate cell harvester, dried and counted in scintillation fluid.

2.7. Hemotologic parameter

The blood samples were collected into heparin-containing tubes (40 μl heparin at 2500 IE/ml) and then analyzed within 1 h by an automated hematology analyzer (Sysmex, K-1000). A control blood sample (ECN-13, Sysmex™) was routinely checked as a quality control.

2.8. Histopathological examination

Liver, kidney, spleen and heart tissues were fixed in phosphate buffered formalin, embedded in paraffin, sectioned by 5–6 μm, and stained using hematoxylin-eosin. All slides were viewed under light microscope and blinded to the examiner.

2.9. Statistics

Comparison of mean differences between two groups was made by *t*-test. In some case, the raw data were log-transformed (PHA induced lymphocyte proliferation data) to get a nearer normal distribution before the parametric statistics were carried out.

3. Results

3.1. Toxicity

No acute toxic signs or symptoms had been observed in rats in the repeated daily supplement experiments. All groups of the rats gained weight during supplementation while no body weight differences were found among the groups at any time point for C-MED-100™ at 40 and 80 mg/kg for 8 weeks (Fig. 1, panel A, B) and 160 mg/kg for 4 weeks (Fig. 2). No differences were observed in food consumption between the control group and any of C-MED-100™ treatment groups ($P > 0.05$, data not shown). While no evidence of toxicity was observed with C-MED-100™, there was a statistically significant reduction in body weight ($P < 0.05$) and food consumption ($P < 0.01$) in rats receiving the same dose of an another commercial water extract (Fig. 2). Major organ weight and organ weight coefficients are presented in Table 1. There were no significant differences in organ weight coefficients between the control and any of the C-MED-100™ treated groups. The histopathological examination revealed no increased pathological changes such as necrosis, fibrosis, increased mitotic activity and proliferation when compared with control rats.

The oral single dose LD_{50} could not be calculated because at the maximum concentration of the various *U. tomentosa* preparations, no lethal effect had been observed. However, it was concluded that the LD_{50} of C-Med-100 was greater than 8 g/kg since no single death in the treatment groups had been observed at this dose or below it. The LD_{50} of two other preparations, uña de gato bark powder (Schuler) and water/ethanol extract (4% alkaloids) of *U. tomentosa* (sample provided

from MW International), were observed to be greater than 2 and 5 g/kg, respectively. The MTD for C-Med-100 was also greater than 8 g/kg, while it was greater than 2 g/kg for uña de gato bark powder (Schuler) and between 2.5 and 5.0 g/kg for 4% alkaloids water/ethanol extracts (Table 2).

In the human volunteer study, there were no toxic side effects observed at a repeated dose of 350 mg/day for 6 consecutive weeks when judged by hematological analysis, body weight loss, work attendance and symptoms (Table 3).

3.2. DNA repair

We have shown in previous studies that DNA repair enhancement could be demonstrated in a rat model by supplementation of nicotinamide, zinc and carotenoids. The principle for this in vivo evaluation of DNA repair is that 3 h after
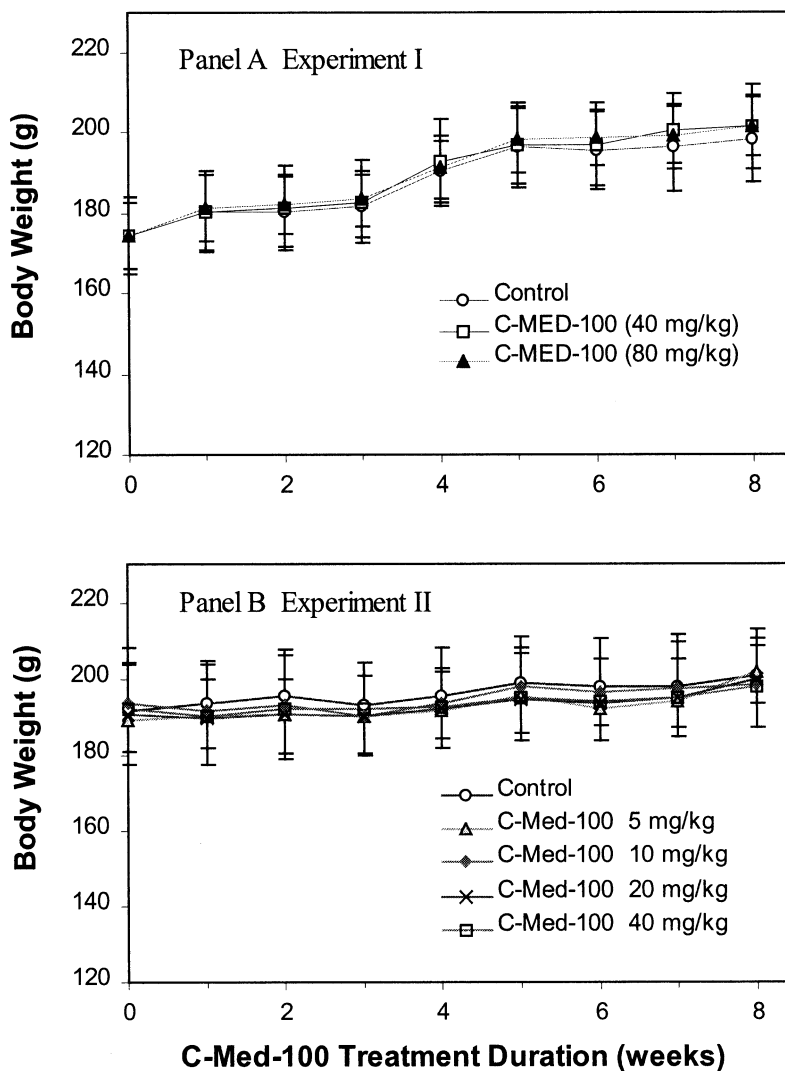

Fig. 1. Body weight growth curves of female W/Fu rats treated with C-MED-100™ oral daily at the doses of 0, 5, 10, 20, 40 and 80 mg/kg for 8 consecutive weeks from two independent experiments. Data are shown as mean ± S.D. ($n = 8$–10). No significant differences were observed by t-test analysis.
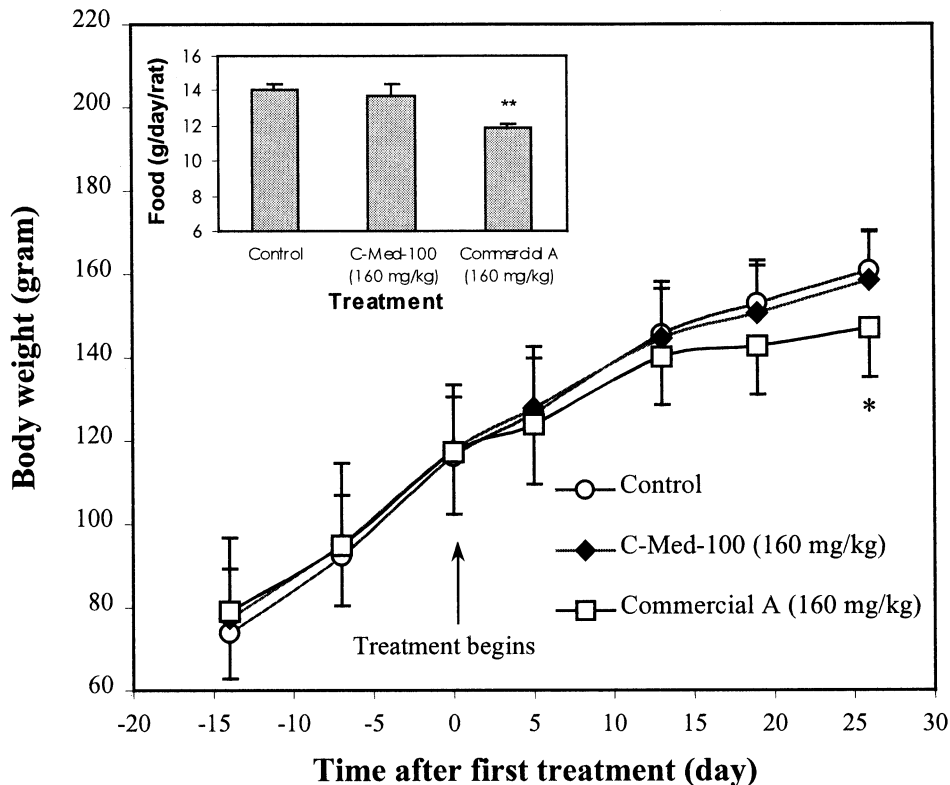
Fig. 2. Comparison of C-MED-100™ and commercial extract A treatment on body weight growth curves and food consumption (inset bars) of W/Fu rats treated for 4 weeks at the same oral daily dose of 160 mg/kg. Data shown are mean $\pm$ S.D. ($n = 7–8$). $*P < 0.05$ and $**P < 0.01$ compared with control by two-tailed t-test.

whole body radiation, DNA damage (SSB) is only about 50% repaired in this rat model, and hence inhibition or stimulation can be measured compared to irradiation alone controls (Sheng et al., 1998b). Using the same techniques, we investigated the DNA repair in vivo of spleen cells by C-MED-100™ supplementation. As shown in Fig. 3, when rats were irradiated with 12 Gy and allowed to repair for 3 h in vivo, both DNA SSB and DSB were still significantly higher than those of non-irradiated controls as the DNA retained on filters were significantly lower ($P < 0.01$ and < 0.05, respectively by t-test). However, when the rats were supplemented with C-MED-100™, SSB were almost completely repaired within 3 h (Fig. 3, panel A). DNA repair of DSB was also enhanced in a dose-dependent pattern (Fig. 3, panel B) which is considered more important be-

cause this type of DNA damage is known to be lethal.

3.3. Immune enhancement

PHA stimulated lymphocyte proliferation was performed on splenic single cell suspensions for both experiment I and II and the results are shown in Fig. 4. C-MED-100™ supplemented groups showed an increased tendency of mitogenic response when compared to the control group. When the raw data were log-transformed and the supplemented groups were pooled together, there was a statistically significant increase of lymphocyte proliferation in the C-MED-100™ treatment groups at the doses of 40 and 80 mg/kg when compared with controls ($P < 0.05$ and < 0.01, respectively by two-tailed t-test).

3.4. Hemotologic parameter

When compared with control animals the C-MED-100™ treatment groups showed a significant increase in white blood cells at 40 mg/kg ($P < 0.05$, Fig. 5, experiment II) and 80 mg/kg

Table 1
Organ weight coefficient (%)[a] and pathological change of female W/Fu rats supplemented with oral daily doses of C-MED-100™ for 8 weeks

Group	N	Heart	Liver	Spleen	Kidney	Pathology
Experiment I (8 week)						
Control	5	0.51 ± 0.03	3.64 ± 0.31	0.23 ± 0.01	0.72 ± 0.04	None/trace
C-MED-100™ 40 mg/kg	5	0.52 ± 0.06	3.54 ± 0.25	0.22 ± 0.01	0.72 ± 0.02	None/trace
C-MED-100™ 80 mg/kg	5	0.52 ± 0.02	3.37 ± 0.20	0.23 ± 0.00	0.72 ± 0.02	None/trace
Experiment II (8 week)						
Control	7	0.41 ± 0.04	3.49 ± 0.25	0.20 ± 0.01	0.72 ± 0.04	None/trace
C-MED-100™ 5 mg/kg	7	0.41 ± 0.03	3.32 ± 0.35	0.19 ± 0.01	0.72 ± 0.03	None/trace
C-MED-100™ 10 mg/kg	8	0.41 ± 0.07	3.41 ± 0.23	0.20 ± 0.01	0.73 ± 0.05	None/trace
C-MED-100™ 20 mg/kg	8	0.42 ± 0.04	3.43 ± 0.25	0.20 ± 0.01	0.72 ± 0.04	None/trace
C-MED-100™ 40 mg/kg	8	0.43 ± 0.04	3.43 ± 0.11	0.20 ± 0.02	0.74 ± 0.05	None/trace
Experiment III (4 week)						
Control	9	0.44 ± 0.05	4.54 ± 0.18	0.26 ± 0.01	0.81 ± 0.03	NA[b]
C-MED-100™ 160 mg/kg	9	0.42 ± 0.03	4.28 ± 0.19	0.27 ± 0.01	0.78 ± 0.02	NA
Commercial A 160 mg/kg	9	0.43 ± 0.03	4.44 ± 0.46	0.27 ± 0.02	0.79 ± 0.05	NA

[a] Organ weight coefficient (%) = organ weight/body weight × 100.
[b] NA: data not available or not evaluated.

Table 2
LD_{50} and MTD doses of C-Med-100™ and two Cat's claws products in rats

Drug	Testing dose (g/kg)	N/group	LD_{50} (g/kg)	MTD (g/kg)	Toxic signs
C-Med-100	0, 1, 2, 4, 8	5	>8	>8	None
Commercial supplied bark powder	0, 0.25, 0.5, 1, 2	5	>2	>2	None
Commercial supplied water/ethanol extract	0.63, 1.25, 2.5, 5.0	5	>5	2.5–5	Sedation, diarrhea

Table 3
Toxic side-effects and white blood cells (WBC) before and after C-MED-100™ oral supplement at 350 mg daily for 6 consecutive weeks in a human volunteer study

Subject	Side effect[a]	Before ($n = 3$) WBC(10^9/l)	After ($n = 4$) WBC(10^9/l)[b]
# 1	None	8.28 ± 1.03	9.08 ± 0.15
# 2	None	6.28 ± 0.60	6.00 ± 0.46
# 3	None	5.77 ± 0.32	6.88 ± 0.55
# 4	None	6.10 ± 0.66	6.77 ± 0.84
Total	None	6.60 ± 0.35	7.18 ± 0.50[c]

[a] Side effects were judged by body weight lost, work attendance and symptoms including diarrhea/constipation, headache, nausea/vomiting, rash/edema and pain.
[b] Last 4 weeks data were used for statistics.
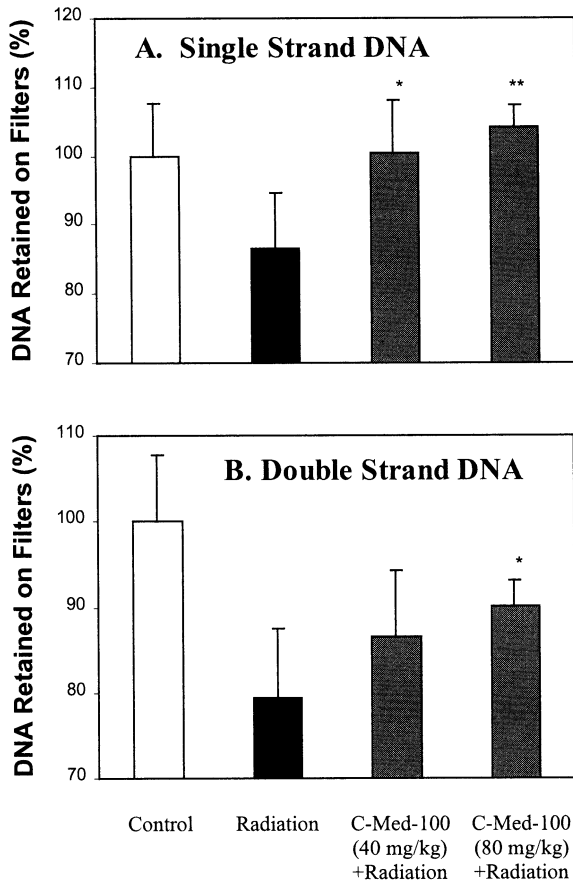[c] $P < 0.05$ by two-tailed t-test comparing before and after supplementation.

Fig. 3. DNA repair enhancement by C-MED-100™ supplementation in a rat model. DNA damage and repair were measured by alkaline elution for SSB (A) and neutral elution for DSB (B) of splenic single cell suspensions from female W/Fu rats. C-MED-100™ oral daily supplemented rats (0, 40 and 80 mg/kg for 8 weeks) were whole body irradiated with 12 Gy and allowed to repair in vivo for 3 h. Data shown are the averages in column and S.D. by error bar ($n = 5$ in each group). * indicates $P < 0.05$ compared with radiation group by two-tailed t-test.

(12.4 ± 0.8 vs 11.3 ± 0.7 of control, $n = 10$ for both groups, $P < 0.05$, experiment I) supplemented for 8 weeks as well as 160 mg/kg (11.7 ± 1.0 vs 10.9 ± 0.9 of control, $n = 9$ for both groups, $P < 0.05$, experiment III) supplemented for 4 weeks. In addition, the human volunteer subjects supplemented with 350 mg C-MED-100™ daily (5 mg/kg) for 6 consecutive weeks also showed a significantly increased level of WBC (Table 3).

4. Discussion

4.1. Dose and toxicity

Phytomedicinal preparations of *U. tomentosa* (also known as uña de gato and cat's claw) have been traditionally used among the native Indians of the Amazon basin of South America. It has also been sold in the United States and other countries for years as an alternative medicine. In spite of its long history of medical use, there is a striking lack of toxicity studies. So far, there has been only one published report evaluating the toxicity of *U. tomentosa* and that was an in vitro study (Santa et al., 1997) which is well known to poorly correlate with in vivo data. The study reported here has shown the toxicity data of a water extract of *U. tomentosa* in both animals and humans. The LD_{50} and MTD of C-MED-100™ were observed to be > 8 g/kg which make it comparable to the LD_{50} of table salt (8–10 g/kg). Because the MTD for commercial water/ethanol extract was 2.5–5 g/kg, C-MED-100™ was shown to be safer than other commercial cat's claw products evaluated (Table 2). C-MED-100™ at doses as high as 80 mg/kg for 8 consecutive weeks or 160 mg/kg for 4 weeks in a rat model receiving daily oral supplement, or at a daily dose of 350 mg (5 mg/kg) for 6 weeks in a pilot human volunteer study, no toxic signs or symptoms have been observed. Although another commercial water extract A of *U. tomentosa* did demonstrate some reduction in body weight gain and food consumption when compared with C-MED-100™ at 160 mg/kg for 4 weeks in rats, both showed the desired effect of increasing WBC. This difference could have been due to the removal of high molecular components in C-MED-100™ extract.

4.2. Immune enhancement

Immune enhancement has long been practiced in traditional medicine and connected with nutrients or trace minerals such as carotenoids (Chew, 1993; Jyonouchi et al., 1994; Hughes et al., 1997), nicotinamide (Weitberg, 1989; Jacobson, 1993; Pero et al., 1995), zinc (Bogden et al., 1988; Sazawal et al., 1996) or the combination of the

three (Sheng et al., 1998b). *U. tomentosa* has been traditionally prepared and used as a tea by the Indians in the rainforest of South America to enhance the immune system and general health (Jones, 1995). Wagner et al. (1985) using organic solvent extraction, showed that four of six alkaloids isolated from *U. tomentosa* (pteropodin, isopteropodin, isomitraphyllin and isorynchophyllin) had a pronounced enhanced effect on phagocytosis determined in two in vitro tests and the in vivo carbon clearance test.

Recently, Wurm et al. (1998) reported pentacyclic but not tetracyclic oxindole alkaloids from *U. tomentosa* induced EA.hy926 endothelial cells to release some yet to be determined factor(s) into the supernatant; this factor was shown to significantly enhance proliferation of normal human resting or weakly activated B and T lymphocytes. Although the presence of alkaloids in a water extract is not clear or obvious, C-MED-100™

treatment in the present study was observed to dose-dependently enhance PHA stimulated lymphocyte mitogenic response in splenocytes (Fig. 4) and to increase white blood cells (Fig. 5) after supplement in rats. This result suggested there might be some additional active components other than the alkaloids in hot water extracts of cat's claw such as C-MED-100™ that could contribute to immune enhancement.

4.3. DNA repair enhancement

Antioxidant effects have been reported for extracts from Uncaria rhynchophylla (Miq.) (Jacks) (Liu and Mori, 1992), Uncaria hirsuta (Haviland) and Uncaria rhynchophylla (Miquel) (Lin et al., 1995). Rizzi et al. (1993) reported antimutagenic activities of chloroform/methanol extracts and chromatographic fractions of *U. tomentosa* bark. The plant extracts and fractions showed a protec-
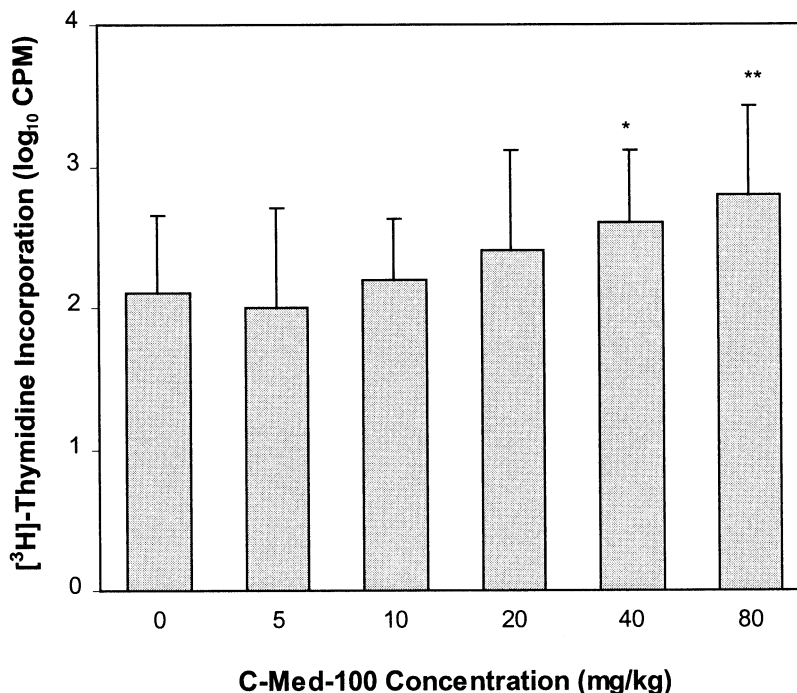

Fig. 4. PHA stimulated lymphocyte proliferation in W/Fu female rats supplemented with C-MED-100™ at the oral daily doses of 0, 5, 10, 20, 40 and 80 mg/kg for 8 consecutive weeks. Splenocytes were prepared as single cell suspensions and cultured on a microtiter plate at 25 000 cells/well in 200 µl RPMI 1640-10% FCS-10 µl PHA at 37°C, 5% CO_2 for 5 days, then pulsed for 6 h with 0.5 µCi ^{3}H-dThd/ml. After that labeled nuclear material was collected and counted in scintillation fluid. Results shown are average in column and S.D. in error bar ($n \geq 5$). *$P < 0.05$, **$P < 0.01$ compared with control by two-tailed *t*-test.
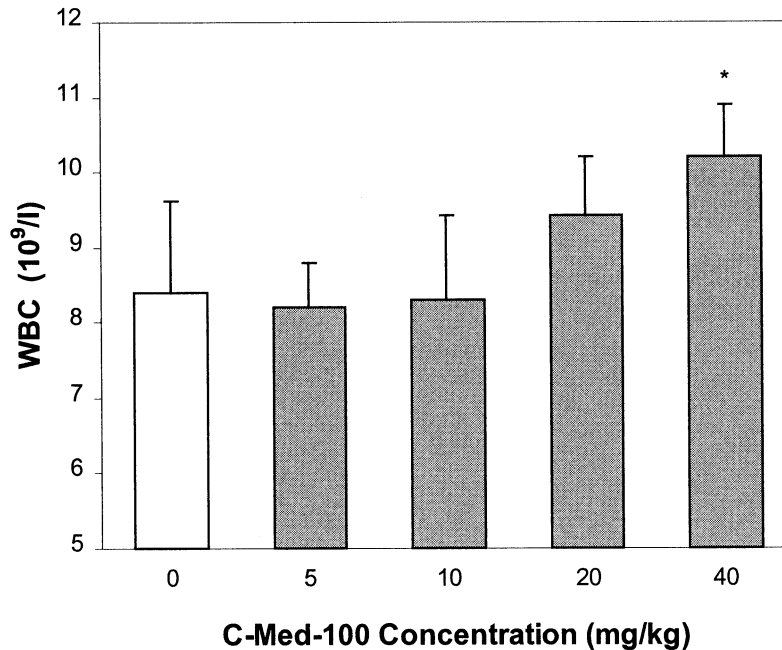
Fig. 5. White blood cell (WBC) counts of rats supplemented with C-MED-100™ at the oral daily doses of 0, 5, 10, 20 and 40 mg/kg for 8 consecutive weeks. Blood samples were collected into 5 ml heparinised tube (BECTON DICKINSON Vacutainer Systems Europe) and then immediately analyzed by an automated hematology analyzer (Sysmex, K-1000). Data shown are the averages in column and S.D. by error bar ($n \geq 7$ in each group). *$P < 0.05$ compared with control (0 mg/kg) group by two-tailed t-test.

tive antimutagenic effect in vitro against photo-mutagenesis induced by 8-methoxy-psoralen (8-MOP) plus UVA in *S. typhimurium* TA102. A decoction of *U. tomentosa* ingested daily for 15 days by a smoker also decreased the mutagenicity induced in *S. typhimurium* TA98 and TA100 by the subject's urine. Recently, Sandoval-Chacon et al. (1998) showed that an aqueous extract of cat's claw elicited similar beneficial effects as an antioxidant by inhibiting indomethacin induced intestinal inflammation, consistent with previous findings using a bark methanol extract (Desmarchelier et al., 1997). However, to our knowledge, the enhancement of DNA repair in vivo by *U. tomentosa* extract has never been reported. Given orally for 8 weeks, the aqueous extracts of *U. tomentosa* C-MED-100™ showed an enhancement of DNA repair of both SSB and DSB induced by radiation (Fig. 3). DSB are considered a lethal DNA damage to the cell. Although the DSB in the present study are still not completely repaired 3 h after γ-irradiation, the significant

repair improvement ($P < 0.05$) compared to the radiation group is important for the cells to survive. Since a successful DNA repair will reduce the DNA damage, our result is also consistent with the previous antioxidant reports cited above.

4.4. Immune function and DNA repair

Immune dysfunction is linked to oxidative stress and inhibited DNA repair (Pero et al., 1995). The rate of DNA unwinding was significantly increased in cells from patients with rheumatoid arthritis compared with those from healthy controls which supports the hypothesis that DNA damage is one factor contributing to immune dysfunction in this disease (Bhusate et al., 1992). Severe combined immune deficiency (scid) mice fail to produce mature B and T cells and are sensitive to ionizing radiation. Scid cells repair radiation-induced DNA DSB at a reduced rate and lack the ability to undergo repair of sublethal damage (Nevaldine et al., 1997). UV is a

complete carcinogen not only because it can produce the mutagenic DNA photoproducts that lead to activation of skin oncogenes, but also because it can suppress the cellular immune responses that are otherwise able to eliminate highly antigenic skin tumors (Yarosh and Kripke, 1996). Treatment of topical nicotinamide, the active form of vitamin B-3, or niacin, prevented the immunosuppression and skin tumor induction by UVB irradiation in mice (Gensler 1997). On the other hand, the decrease of DNA damage and improved DNA repair is associated with enhanced immune function. One primary mechanism of natural immune enhancers, such as β-carotene (Liebler, 1993), nicotinic acid (Weitberg, 1989; Rawling et al., 1994), zinc (Chiricolo et al., 1993) or the combination of the three (Sheng et al., 1998b), are to scavenge oxygen derived free radicals produced either as by-products of metabolism or from exogenous environmental exposures, protect human lymphocytes from oxidative stress and improve the DNA repair. The simultaneous effects of immune and DNA repair enhancements observed in this study and the antioxidant effects reported by others suggest that natural products such as *U. tomentosa* extracts can be evaluated for development for use in chemoprevention of DNA damage-associated diseases such as cancer, autoimmune and inflammatory diseases.

Acknowledgements

References

ap-Rhys, C.M., Bohr, V.A., 1996. Mammalian DNA repair responses and genomic instability. EXS 77, 289–305.

Aquino, R., De Simone, F., Pizza, C., Conti, C., Stain, M.L., 1989. Plant metabolites. Structure and in vitro antiviral activity of quinovic acid glycosides from *Uncaria tomentosa* and *Guettarda platypoda*. J. Nat. Prod. 52 (4), 679–685.

Aquino, R., De Feo, V., De Simone, F., Pizza, C., Cirino, G., 1991. Plant metabolites. New compounds and anti-inflammatory activity of *Uncaria tomentosa*. J. Nat. Prod. 54 (2), 453–459.

Bhusate, L.L., Herbert, K.E., Scott, D.L., Perrett, D., 1992. Increased DNA strand breaks in mononuclear cells from patients with rheumatoid arthritis. Ann. Rheum. Dis. 51 (1), 8–12.

Bogden, J.D., Oleske, J.M., Lavenhar, M.A., et al., 1988. Zinc and immunocompetence in elderly people: effects of zinc supplementation for 3 months. Am. J. Clin. Nutr. 48 (3), 655–663.

Cerri, R., Aquino, R., De Simone, F., Pizza, C., 1988. New quinovic acid glycosides from *Uncaria tomentosa*. J. Nat. Prod. 51, 257–261.

Cesarone, C.F., Bolognesi, C., Santi, L., 1979. Improved microfluorometric DNA determination in biological material using 33258 Hoechst. Anal. Biochem. 15 (1), 188–197.

Chew, B.P., 1993. Role of carotenoids in the immune response. J. Dairy Sci. 76 (9), 2804–2811.

Chiricolo, M., Musa, A.R., Monti, D., et al., 1993. Enhanced DNA repair in lymphocytes of Down syndrome patients: the influence of zinc nutritional supplementation. Mutat. Res. 295 (3), 105–111.

Desmarchelier, C., Mongelli, E., Coussio, J., Ciccia, G., 1997. Evaluation of the in vitro antioxidant activity in extracts of *Uncaria tomentosa* (Willd.) DC. Phytother. Res. 11, 254–256.

Evan, G., Littlewood, T., 1998. A matter of life and cell death. Science 281, 1317–1322.

Gensler, H.L., 1997. Prevention of photoimmunosuppression and photocarcinogenesis by topical nicotinamide. Nutr. Cancer 29 (2), 157–162.

Hong, W.K., Sporn, M.B., 1997. Recent advances in chemoprevention of cancer. Sciences 278, 1073–1077.

Hughes, D.A., Wright, A.J., Finglas, P.M., Peerless, A.C., Bailey, A.L., Astley, S.B., Pinder, A.C., Southon, S., 1997. The effect of β-carotene supplementation on the immune function of blood monocytes from healthy male nonsmokers. J. Lab. Clin. Med. 129 (3), 309–317.

Jacobson, E.L., 1993. Niacin deficiency and cancer in women. J. Am. Coll. Nutr. 12 (4), 412–416.

Jyonouchi, H., Zhang, L., Gross, M., et al., 1994. Immunomodulating actions of carotenoids: enhancement of in vivo and in vitro antibody production to T-dependent antigens. Nutr. Cancer 21, 47–58.

Jones, K., 1995. Cat's Claw, Healing Vine of Peru. Sylvan Press, Seattle, WA, pp. 11–55.

Keplinger, K., 1982. Cytostatic, contraceptive and antiinflammatory agents from *U. tomentosa*. PCT Int. Appl. WO 8201, 130.

Kohn, K.W., Regina, A.G.E., Leonard, C.E., et al., 1981. Measurement of strand breaks and cross-links by alkaline elution. In: Friedberg, E.C., Hanawalt, P.C. (Eds.), DNA Repair, a Laboratory Manual of Research Procedures, pp. 379–400.

Liebler, D.C., 1993. Antioxidant reactions of carotenoids. Ann. NewYork Acad. Sci. 691, 20–31.

Lin, J.M., Lin, C.C., Chen, M.F., Ujiie, T., Takada, A., 1995. Studies on Taiwan folk medicine, thang-kau-tin (II): Measurement of active oxygen scavenging activity using an ESR technique. Am. J. Chin. Med. 23 (1), 43–51.

Liu, J., Mori, A., 1992. Antioxidant and free radical scavenging activities of *Gastrodia elata* Bl. and *Uncaria rhynchophylla* (Miq.) Jacks. Neuropharmacology 31 (12), 1287–1298.

Nevaldine, B., Longo, J.A., Hahn, P.J., 1997. The scid defect results in much slower repair of DNA double-strand breaks but not high levels of residual breaks. Radiat. Res. 147 (5), 535–540.

Olsson, A., Sheng, Y., Kjellen, E., Pero, R.W., 1995. In vivo tumor measurement of DNA damage, DNA repair and NAD pool as indicators of radiosensitisation by metoclopramide. Carcinogenesis 16, 1029–1035.

Pero, R.W., Olsson, A., Sheng, Y., et al., 1995. Progress in identifying clinical relevance of inhibition, stimulation and measurements of poly ADP-ribosylation. Biochimie 77 (5), 385–393.

Rawling, J.M., Jackson, T.M., Driscoll, E.R., et al., 1994. Dietary niacin deficiency lowers tissue poly(ADP-ribose) and NAD^+ concentrations in Fischer-344 rats. J. Nutr. 124 (9), 1597–1603.

Rizzi, R., Re, F., Bianchi, A., De Feo, V., de Simone, F., Bianchi, L., Stivala, L.A., 1993. Mutagenic and antimutagenic activities of *Uncaria tomentosa* and its extracts. J. Ethnopharmacol. 38 (1), 63–77.

Romagna, F., Kulkarni, M., Anders, M.W., 1985. Detection of repair of chemical-induced DNA damage in vivo by the nucleoid sedimentation assay. Biochem. Biophys. Res. Commun. 127, 56–62.

Sandoval-Chacon, M., Thompson, J.H., Zhang, X.J., Liu, X., Mannick, E.E., Sadowicka, H., Charbonet, R.M., Clark, D.A., Miller, M.J., 1998. Antiinflammatory actions of cat's claw: the role of NF-kappaB. Aliment Pharmacol. Ther. 12 (12), 1279–1289.

Santa, M.A., Lopez, A., Diaz, M.M., Alban, J., Galan de Mera, A., Vicente Orellana, J.A., Pozuelo, J.M., 1997. Evaluation of the toxicity of *Uncaria tomentosa* by bioassays in vitro. J. Ethnopharmacol. 57 (3), 183–187.

Sazawal, S., Black, R.E., Bhan, M.K., et al., 1996. Zinc supplementation reduces the incidence of persistent diarrhea and dysentery among low socioeconomic children in India. J. Nutr. 126, 443–450.

Sheng, Y., Pero, R.W., Ammir, A., Bryngelsson, C., 1998a. Induction of apoptosis and inhibition of proliferation in human tumor cells treated with extracts of *Uncaria tomentosa*. Anticancer Res. 18 (5A), 3363–3368.

Sheng, Y., Pero, R.W., Olsson, A.R., Bryngelsson, C., Hua, J., 1998b. DNA repair enhancement by a combined supplement of carotenoids, nicotinamide and zinc. Cancer Detect. Prevent. 22 (4), 284–292.

Thompson, C.B., 1995. Apoptosis in the pathogenesis and treatment of disease. Science 267, 1456–1462.

Yarosh, D.B., Kripke, M.L., 1996. DNA repair and cytokines in antimutagenesis and anticarcinogenesis. Mutat. Res. 350 (1), 255–260.

Wagner, H., Kreutzkamp, B., Jurcic, K., 1985. The alkaloids of *Uncaria tomentosa* and their phagocytosis-stimulating action. Planta Med. 5, 419–423.

Wang, X., Ohnishi, T., 1997. p53-dependent signal transduction induced by stress. J. Radiat. Res. 38 (3), 179–194.

Weitberg, A.B., 1989. Effect of nicotinic acid supplementation in vivo on oxygen radical-induced genetic damage in human lymphocytes. Mutat. Res. 216 (4), 197–201.

Willmott, N., Sethi, J.K., Walseth, T.F., Lee, H.C., White, A.M., Galione, A., 1996. Nitric oxide-induced mobilization of intracellular calcium via the cyclic ADP-ribose signaling pathways. J. Biol. Chem. 271 (7), 3699–3705.

Wurm, M., Kacani, L., Laus, G., Keplinger, K., Dierich, M.P., 1998. Pentacyclic oxindole alkaloids from *Uncaria tomentosa* induce human endothelial cells to release a lymphocyte-proliferation-regulating factor. Planta Med. 4 (8), 701–704.