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Original Article

Synthesis, Hematological, Biochemical, and Neurotoxicity Screening of Some Mannich Base Hydrochlorides

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ABSTRACT

Background: Mannich bases are an important class of compounds in medicinal chemistry with a wide spectrum of biological activities, however, knowledge on their toxicity is limited. **Materials and Methods:** Two Mannich base hydrochlorides 1a (2-thienyl-β-dimethylaminoethyl ketone hydrochloride) and 1b (β-dimethylaminopropiophenone hydrochloride) were synthesized and characterized on the basis of their infrared and nuclear magnetic resonance spectral data. The potential effects of the synthesized compounds (5 mg/kg, i.p, during 30 days) on relative weight, hematological parameters, biochemical parameters, and neurotoxicity were tested using male Wistar rat. **Results:** The results showed that compound 1b alters body weight on the first 10 days (182%, $P < 0.01$) and on the last 10 days (107%, $P < 0.01$) of treatment. The same treatment decreases food intake ($P < 0.01$) and increases water intake ($P < 0.05$). Both compounds induced a deficit on rotarod test manifested by a decrease of grasping time (1a: 65.33%, $P < 0.01$; 1b: 60.55%, $P < 0.01$) and fall time (1a: 59.75%, $P < 0.01$; 1b: 56.81%, $P < 0.01$) only on the last day of training. Moreover, Mannich base 1b decreases the liver relative weight (22.24%, $P < 0.01$). It was also observed that both products decrease the total serum cholesterol (Ch) levels (1a: 52.87%, $P < 0.01$; 1b: 64.70%, $P < 0.01$). Interestingly, compounds 1a and 1b affect hematological parameters manifested by an increase of the number of white blood cells (1a: 32.29%, $P < 0.05$; 1b: 20.64%, $P < 0.05$) and red blood cells (RBCs) (1a: 12.57%, $P < 0.05$; 1b: 20.11%, $P < 0.05$), an increase of red cell hemoglobin concentration (1a: 10.48%, $P < 0.05$; 1b: 16.12%, $P < 0.05$) and of the volume occupied by RBCs or hematocrit (1a: 18.28%, $P < 0.05$; 1b: 15.56%, $P < 0.05$), and an increase of the number of platelets (1a: 16.80%, $P < 0.05$; 1b: 39.96%, $P < 0.05$) accompanied by a decrease in hemoglobin level only with the compound 1a (7.41%, $P < 0.05$). **Conclusion:** These results show that both compounds 1a and 1b induced a hypoxia status associated to low level of Ch and liver toxicity. The deficit observed by rotarod could be explained by the myorelaxant effect of the used products.

Key words: Biochemical, hematological, hypoxia, Mannich bases, myorelaxant, neurotoxicity

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INTRODUCTION

A Mannich base is a β-aminoketone, which is formed in the reaction of a primary or secondary amine or ammonia with formaldehyde and an enolizable ketone. This process is known as the Mannich reaction.[1,2]

Mannich bases are an important class of compounds

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in medicinal chemistry with a wide range of biological properties including antimicrobial,[3-6] anticancer,[7-10] anti-inflammatory,[11-14] analgesic,[15] and anticonvulsant[16-18] activities. Some of these compounds are also known for their applications as anti-verus of human imminidéficience (VIH) and antituberculosis,[19-24] antimalaria,[25-28] antifungal,[29,30] antiflaviviridae,[31] and spermicidal[32] agents.

The biological activity of such molecules has been attributed to the α-βunsaturated ketones liberated from Mannich bases by deamination process under physiological conditions.[33-35] These α-, β-unsaturated ketones are very sensitive for Michael addition to alkylate certain cellular constituents especially thiol groups.[33,36] Among these cellular thiols, glutathione (GSH) is the most abundant, and Mannich bases are reported to inhibit one or more of the following enzymes in the GSH metabolism namely GSH S-transferases, GSH reductase, gamma-glutamyl transpeptidase, and GSH peroxidase.[37]

It is important to note here that the disadvantages of traditional alkylating agents used in cancer chemotherapy such as mutagenicity[38] and carcinogenicity[39] may be prevented by using Mannich bases, since interactions with nucleic acids, which are completely devoid of thiol groups, would be avoided.

Despite the wide spectrum of biological activities associated to Mannich bases, knowledge on their toxicity is limited. Therefore, we report in the present study, the synthesis of 2-thienyl-β-dimethylaminoethyl ketone hydrochloride (1a) and β-dimethylaminopropiophenone hydrochloride (1b) and the evaluation of their potential effect on relative weight, hematological parameters, biochemical parameters, and neurotoxicity in rats at the dose of 5 mg/kg considered like active dose.[40]

MATERIALS AND METHODS

Chemistry

Mannich base hydrochlorides 1 were prepared according to the reported Mannich synthetic procedure which involves a three-component condensation of an enolizable ketone with formaldehyde and a secondary amine.[41] Experimentally, the reaction of the enolizable ketone with equimolar amounts of paraformaldehyde and dimethylamine hydrochloride, performed in refluxing 95% ethanol, for 3 h, in the presence of a catalytic amount of concentrated HCl, led to Mannich base hydrochlorides 1 [Figure 1].

The structures of compounds 1 were confirmed by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopies. ^{1}H and ^{13}C NMR spectra were recorded with a mixture of $CDCl_3$ and DMSO-d_6 as the solvent, on a Bruker-300 spectrometer. The chemical shifts are reported in ppm relative to tetramethylsilane (internal reference). The coupling constants are reported in Hz. For the ^{1}H NMR, the multiplicities of signals are indicated by the following abbreviations: s: Singlet, d: Doublet, t: Triplet, q: Quartet, and m: multiplet. IR spectra were recorded on a Nicolet IR200 spectrometer.

General procedure for the synthesis of Mannich base hydrochlorides 1

A mixture of the methylketone (0.20 mol), dimethylamine hydrochloride (0.20 mol), paraformaldehyde (0.25 mol), and concentrated HCl (0.50 mL), in 30 mL of 95% ethanol, was refluxed for 3 h. After cooling, acetone (150 mL) was added and the mixture was left overnight in the refrigerator. The crystals formed were filtered and recrystallized from a mixture of acetone and 95% ethanol.

Spectral data for compounds 1

2-thienyl-β-dimethylaminoethyl ketone hydrochloride (1a). White solid, mp = 194°C; yield (%) = 58; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ = 2,04 (s, 6H, $C\underline{H}_3$– NH– $C\underline{H}_3$); 2.48 (t, 3H, $^{3}J_{HH}$ = 6,0 Hz, O=C–$C\underline{H}_2$–CH_2); 3.56 (t, 3H, $^{3}J_{HH}$ = 6,0 Hz, CH_2–$C\underline{H}_2$–NH $(CH_3)_2$); 6,68-7,27 (m, 3H, arom-H); 9,01 (broads, 1H, $\underline{H}$–N–$(CH_3)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$): 34.0 (s, $\underline{C}H_3$–NH–$\underline{C}H_3$); 42.2 (s, O=C–$\underline{C}H_2$–CH_2); 51.5 (s, CH_2–$\underline{C}H_2$–NH $(CH_3)_2$); 189.2 (s, $\underline{C}$=O); thienyl carbons: δ =128.5, 133.3, 135.1, 142.6; IR (neat): $\nu_{C=O}$ = 1662 cm^{-1}; ν_{N-H} = 3399 cm^{-1}.

β-dimethylaminopropiophenone hydrochloride (1b).

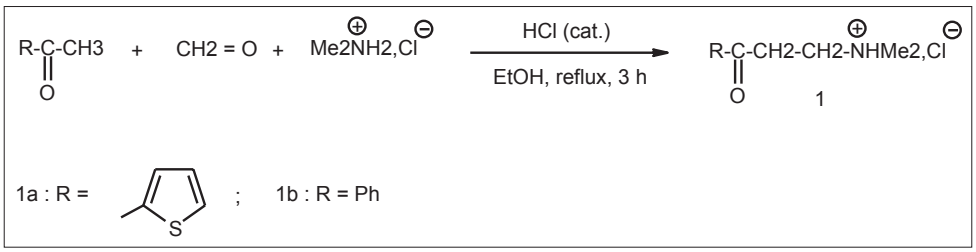


Figure 1: Synthesis of Mannich bases hydrochloride 1

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White solid, mp = 166°C; yield (%) =53; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ =2,82 (s, 6H, $C\underline{H}_3$–NH–$C\underline{H}_3$); 2.82 (t, 3H, $^{3}J_{HH}$ = 6,0 Hz, O=C–$C\underline{H}_2$–CH_2); 3.63 (t, 3H, $^{3}J_{HH}$ = 6,0 Hz, CH_2–$C\underline{H}_2$–NH $(CH_3)_2$); 7.38-7.93 (m, 5H, arom-H); 11,40 (broad s, 1H, $\underline{H}$–N $(CH_3)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$): 33.1 (s, $\underline{C}H_3$–NH–$\underline{C}H_3$); 42.6 (s, O=C–$\underline{C}H_2$–CH_2); 52.1 (s, CH_2–$\underline{C}H_2$–NH $(CH_3)_2$); 195.8 (s, $\underline{C}$ = O); phenyl carbons: δ = 127.5, 128.0, 133.3, 135.3; IR (neat): $\nu_{C=O}$ = 1687 cm^{-1}; ν_{N-H} = 3402 cm^{-1}.

Biological investigation

A total of 18 male Wistar rats were used in this study, in conformity with the local ethic committee of Tunis University and in agreement with the NIH guidelines.[42] They were maintained in animal facility at controlled temperature (22 ± 2_C), a 12-h light/dark cycle and divided into three groups of six animals each fed either a standard diet (SD) for 4 weeks, the SD consisted of 5% fat, 70% carbohydrate, and 25% protein (ALMAS, Bizerte, Tunisia), as control rats (C, n = 6), treated with compound (1a, n = 6) and treated with compound (1b, n = 6). Initially both of the compounds were suspended in NaCl 0.9% and administered intraperitoneally (i.p.) in a volume of 1 mL/kg for body weight (bw) of rats and at the doses of 5 mg/kg bw of rats. The control group received physiological serum NaCl 0.9%.

Study of bw

The evaluation of bw was done at the first day and every 10 days of the experiment for the three groups. The study of food intake and water is determined every 24 h with 500 g for food and 750 g for water, during 30 days.

Neurotoxicity screening

Minimal motor impairment was measured in rats by the rotarod test. The rats were trained to stay on an accelerating rotarod that rotates at 30 rpm. The rod diameter was 3.20 cm.[43-46] Trained animals were given i.p. injection of the tested compounds at the dose of 5 mg/kg.

Neurotoxicity was indicated by the inability of animal to maintain equilibrium on the rod in each of the four trials during 3 days. The full time, the walking, and grasping time were recorded in second (s) and compared to control to evaluate behavior deficits.

Blood sampling protocol

Blood samples (approximately 0.50 mL/sample) were collected in vials containing ethylenediaminetetraacetic acid for hematological investigation or heparin for biochemical studies. Hematological parameters were assayed by Medonic precision instruments for hematology research (CDS Medonic Hematology Analyzer Series, CA 620/530).

Blood chemistry

Control and treated rats were sacrificed 24 h after the last exposure. Blood was collected in heparinized chilled tubes and immediately centrifuged. Aliquots of plasma were frozen and stored at –80°C prior to biochemical analysis. Plasma triglycerides and total cholesterol (Ch) were measured using the enzymatic methods according to manufacturer instructions (Chrono Labo, France).[47,48]

Statistics

Each group consisted of six animals; data are reported as the mean ± standard error of the mean. Differences between means were evaluated by one-way analysis of variance and the post-hoc test was: Honestly significant difference de Tukey. All analyses were performed using Statistica version 5.00 for Windows. Statistical significance of the differences between the means was assessed by Student's t-test. The level of significance was set at $P < 0.05$.

RESULTS

Effects of compounds 1a and 1b on body and relative weight

We observed that the injection of compound 1b induced a decrease of bw on the first 10 days ($P < 0.01$) and on the last 10 days ($P < 0.01$) of treatment [Figure 2], a decrease of food intake ($P < 0.01$), and an increase of water intake ($P < 0.05$) [Figure 3a, 3b]. Compound 1a, in contrast, failed to alter these parameters. Interestingly, we noted that only Mannich base hydrochloride 1b induced a significant decrease in liver relative weight (3.25 g/100 g bw ± 0.13 vs. 4.18 g/100 g bw ± 0.13, $P < 0.05$). The kidneys and brain relative weights remained unaffected by 1a and 1b. (kidneys relative weight: 0.47 g/100 g bw ± 0.02 vs. 0.43 g/100 g

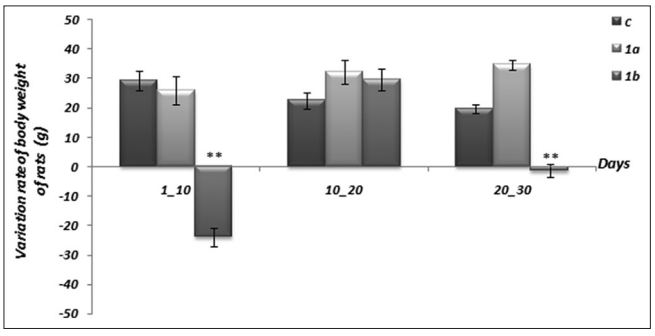


Figure 2: Effects of subchronic treatment of Mannich bases on body weight. C: Control rats; 1a: Treated rats by 1a product, 1b: Treated rats by 1b product. Data represent the means ± standard error of the mean (n = 6). (1a vs. C; 1b vs. C; **$P < 0.01$)

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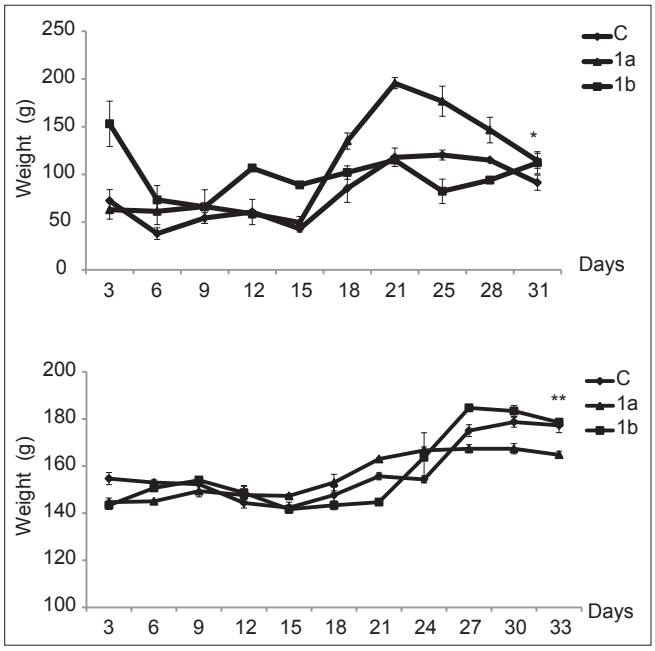


Figure 3: Effects of subchronic treatment of Mannich bases: (a) Effects on water intake, (b) Effects on food intake, C: Control rats; 1a: Treated rats by 1a product. 1b: Treated rats by 1b product. Data represent the means ± standard error of the mean ($n = 6$) (1a vs. C; 1b vs. C, $^{**}P < 0.01$, $^{*}P < 0.05$)

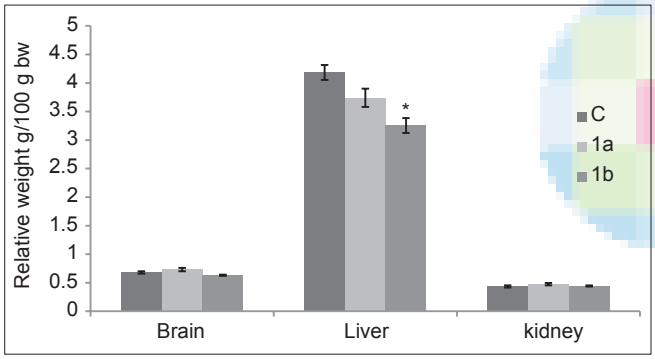


Figure 4: Effects of subchronic treatment of Mannich bases on organs relative weight, C: Control rats; 1a: Treated rats by 1a product. 1b: Treated rats by 1b product. Data represent the means ± standard error of the mean ($n = 6$) (1a vs. C, 1b vs. C, $^{*}P < 0.05$)

bw ± 0.01, $P > 0.05$ for 1a; 0.44 g/100 g bw ± 0.01 vs. 0.43 g/100 g bw ± 0.01, $P > 0.05$ for 1b; brain relative weight: 0.73 g/100 g bw ± 0.03 vs. 0.68 g/100 g bw ± 0.02, $P > 0.05$ for 1a; 0.63 g/100 g bw ± 0.01 vs. 0.68 g/100 g bw ± 0.02, $P > 0.05$ for 1b) [Figure 4].

Neurotoxicity screening

Subchronic exposure of rats to compounds 1 induced a decrease of fall time only on the last day (day 3) of trained on rotarod (4.25 s ± 0.3 vs. 10.56 s ± 2.2, $P < 0.01$ for 1a; 4.56 s ± 0.6 vs. 10.56 s ± 2.2, $P < 0.01$ for 1b). This observation can be explained by the significant effect of both compounds either on the grasping time (3.12 s ± 0.24 vs. 9 s ± 2.2, $P < 0.01$ for 1a; 3.55 s ± 0.6 vs. 9 s ± 2.2, $P < 0.01$ for 1b) or on the walking time (1.12 s ± 0.1 vs.

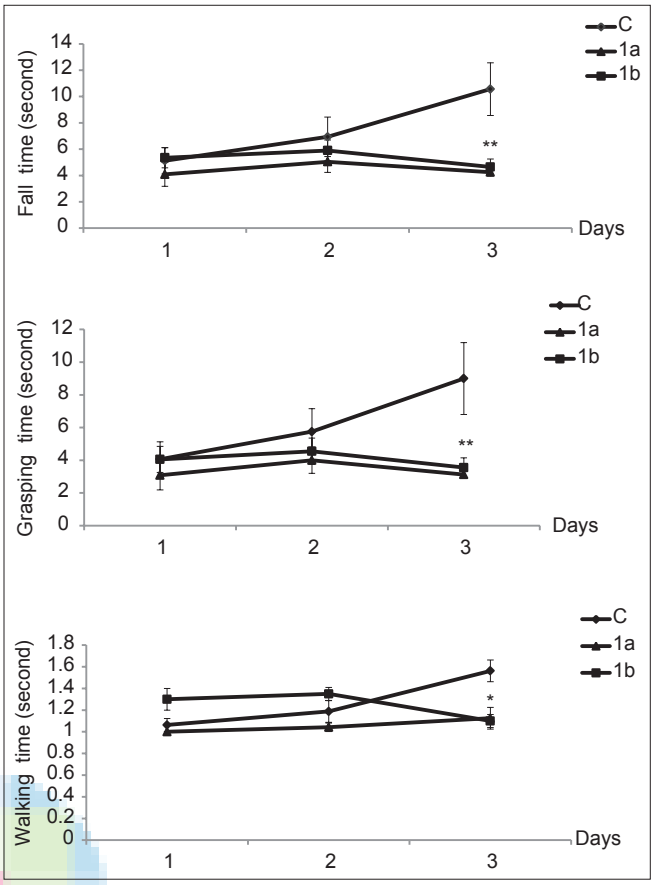


Figure 5: Evaluation of the effect of Mannich bases (1) on rat behavior with rotarod test. C: Control rats. 1a: Treated rats by 1a product. 1b: Treated rats by 1b product. Data represent the means ± standard error of the mean ($n = 6$) of four trials/days/group during 3 days for the grasping, the walking, and the fall times. (1a vs. C; 1b vs. C, $^{**}P < 0.01$, $^{*}P < 0.05$)

1.56 s ± 0.1, $P < 0.05$ for 1a; 1.1 s ± 0.06 vs. 1.56 s ± 0.1, $P < 0.05$ for 1b) [Figure 5].

Hematological parameters

Both compounds 1a and 1b affect hematological parameters manifested by an increase of the number of white blood cells (WBCs) (12.37 10^3/mm^3 ± 0.56 vs. 9.35 10^3/mm^3 ± 0.5, $P < 0.05$ for 1a; 11.28 10^3/mm^3 ± 0.42 vs. 9.35 10^3/mm^3 ± 0.5, $P < 0.05$ for 1b) and red blood cells (RBCs) (7.61 10^3/mm^3 ± 0.24 vs. 6.76 10^3/mm^3 ± 0.28, $P < 0.05$ for 1a; 8.12 10^3/mm^3 ± 0.09 vs. 6.76 10^3/mm^3 ± 0.28, $P < 0.05$ for 1b), an increase of red cell hemoglobin concentration (Hb) (13.7 g/dl ± 0.22 vs. 12.4 g/dl ± 0.42, $P < 0.05$ for 1a; 14.4 g/dl ± 0.2 vs. 12.4 g/dl ± 0.42, $P < 0.05$ for 1b) and of the volume occupied by RBCs or hematocrit (HT) (38.23 % ±0.4 vs. 32.32 % ±0.92, $P < 0.05$ for 1a; 37.35 % ±0.44 vs. 32.32 % ±0.92, $P < 0.05$ for 1b), and an increase of the number of platelets (PLT) (861.67 10^3/mm^3 ± 10.57 vs. 737.67 10^3/mm^3 ± 14.18, $P < 0.05$ for 1a; 1032.5 10^3/mm^3 ± 16.68 vs. 737.67 103/

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mm³ ± 14.18, $P < 0.05$ for 1b) accompanied by a decrease in Hb level only with the compound 1a (HL) (35.83 g/dL ± 0.36 vs. 38.7 g/dL ± 0.39, $P < 0.05$).

It is important to note here that we observed no significant effect on the diameter of red blood cells (50.38 microns ± 1.27 vs. 47.65 microns ± 0.75, $P > 0.05$ for 1a; 45.98 microns ± 0.51 vs. 47.65 microns ± 0.75, $P > 0.05$ for 1b) and on the mean corpuscular erythrocytes (18.05 pg ± 0.43 vs. 18.41 pg ± 0.24 for 1a; 17.73 pg ± 0.26 vs. 18.41 pg ± 0.24, $P > 0.05$ for 1b) [Table 1].

Biochemical parameters

The same treatment with Mannich base hydrochlorides 1 decreased the total Ch level (0.41 g/L ± 0.02 vs. 0.87 g/L ± 0.09, $P < 0.01$ for 1a; 0.30 g/L ± 0.009 vs. 0.87 g/L ± 0.09, $P < 0.01$ for 1b), but did not affect the serum triglyceride concentrations (0.58 g/L ± 0.01 vs. 0.53 g/L ± 0.02, $P > 0.05$ for 1a; 0.60 g/L ± 0.01 vs. 0.53 ± 0.02, $P > 0.05$ for 1b) [Figure 6].

DISCUSSION

In the present investigation, we have synthesized two Mannich base hydrochlorides 1a (2-thienyl-β-dimethylaminoethyl ketone hydrochloride) and 1b (β-dimethylaminopropiophenone hydrochloride), then we focused our efforts to evaluate their effect on relative weight, hematological parameters, biochemical parameters, and neurotoxicity in rats at the dose of 5 mg/kg. We observed that the subchronic treatment with the Mannich base 1b produced a significant decrease in rat's bw. We also observed a decrease of food intake accompanied with an increase of water intake. Interestingly, the same treatment with compound 1b induced a decrease in liver weight. These results were, first, in accordance with the findings of Holmgren[49] showing liver toxicity by an inhibitory effect of Mannich bases on respiration in rat liver-cells by poisoning respiratory, cytochromes system,[49] and with the findings of Cunningham *et al.*,[50] and Gul *et al.*,[51] which demonstrate that α, β-unsaturated ketones released from Mannich bases act as alkylating agents toward the essential thiol groups in living cells, resulting in a decrease of the cellular GSH level and in toxicity and hepatic centrilobular cytoplasmic vacuolation in male rats.

Furthermore, we showed that Mannich bases 1 impaired the rotarod performance of the tested rats, probably due to the involvement of dopaminergic mechanisms[52] or to anticonvulsant and myorelaxant effects. Indeed, it is known that the rotarod test is useful for evaluating pharmacological actions of psychotropic agents such as myorelaxant effect and anticonvulsant effect in the central or peripheral nervous system,[53] and that Mannich bases undergo deamination to give α, β-unsaturated ketones which are able to cross the blood-brain barrier to exert anticonvulsant activity.[18] Mannich bases are also known to possess centrally acting myorelaxant effect.[54]

We also demonstrated that subchronic exposure to Mannich base hydrochlorides 1 was associated with high levels of Hb, HT, and RBC; indicating a development of hypoxia-like status. The increase of RBC production, or polycythemia, is a feedback to erythropoietin produced by the kidneys according to the quantity of oxygen present in tissues especially by Küpfer cells.[55] The increase of WBC levels reflects the presence of inflammatory process in rats. Interestingly, both phenomena (hypoxia-like and inflammation) are responsible for the increase of PLT; these results are in accordance with those of Morgan *et al.*,[56,57] showing that Mannich bases and related unsaturated ketones can cause a severe damage on respiratory system. We note, on the contrary, a metabolic disruption as mentioned by a decrease in blood Ch level probably related to the effect of β-aminoketones on Ch acyltransferase and/or on the promoting high-density lipoprotein receptor (SRBI) expression effect.[58] We suggest that the present treatment can cause many vascular

Table 1: Effect of mannich bases hydrochloride treatment on hematological profile

Group (C)	Group (1a)	Group (1b)	Group (1c)
WBC (10^3/mm³)	9.35±0.50	12.37±1.05*	11.28±0.42*
RBC (10^6/mm³)	6.76±0.28	7.61±0.24*	8.12±0.09*
Hb (g/dL)	12.40±0.42	13.70±0.22*	14.40±0.22*
HT (%)	32.32±0.92	38.23±0.40*	37.35±0.44*
PLT (10^3/mm³)	737.67±14.18	861.67±10.57*	1032.50±16.68*
DRBC (microns)	47.65±0.75	50.38±1.27	45.98±0.51
HL (g/dL)	38.70±0.39	35.83±0.36*	38.56±0.23
MCE (pg)	18.41±0.24	18.05±0.43	17.73±0.26

Data represent the means±standard error of the mean (n=6), compared with control (C), *: $P < 0.05$. Hb=Hemoglobin, HT=Hematocrit, RBC=Red blood cells, WBC=White blood cells, PLT=Platelets, DRBC=Diameter of red blood cell, HL=Hemoglobin level in red blood cells, MCE=Mean corpuscular erythrocytes, C=Control rats, 1a=Treated rats by 1a product, 1b=Treated rats by 1b product

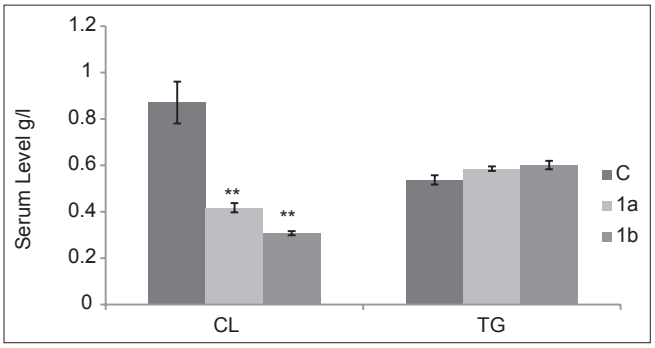


Figure 6: Biochemical effects of subchronic treatment of Mannich bases, CL: Effect on serum cholesterol level; TG: Effect on serum triglycerides concentration. C: Control rats. 1a: Treated rats by 1a product. 1b: Treated rats by 1b product. (1a vs. C; 1b vs. C, **$P < 0.01$

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damage supported by hematological and biochemical investigations.

In accordance to our results related to the decrease of grasping time and walking time, we can suggest that Mannich base hydrochlorides 1 could firstly influence the central and/or peripheral nervous system activities and muscle metabolism. Interestingly, hypoxia-like status induced by Mannich bases combined to metabolic disruption (low level of Ch) could explain in part the myorelaxation observed effects.

The results of our investigations showed that compound 1b are in general more toxic than 1a. We think that the introduction of a phenyl group in the lateral chain of product 1b confers more toxicity than the thienyl group in product 1a.

In conclusion, subchronic administration of Mannich base hydrochlorides 1, with a dose of 5 mg/kg, intraperitoneally, may cause a hypoxia status, liver toxicity, metabolic disruption, and myorelaxant effect leading to behavioral deficit showed by the rotarod test.

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REFERENCES

1. Mannich C, Krösche W. About a condensation product of formaldehyde, ammonia and antipyrine. Archiv of pharmacy Med Chem 1912;250:647-67.
2. Blicke FF. The Mannich reaction. John Wiley and Sons Organic reaction 1942;1:303-41.
3. Mete E, Ozelgul C, Kazaz C, Yurdakul D, Sahin F, Gul H. Synthesis and antifungal activity of 1-aryl-3-phenethylamino-1-propanone hydrochlorides and 3-aroyl-4-aryl-1-phenethyl- 4-piperidinols. Arch Pharm 2010;343:291-300.
4. Gul HI, Sahin F, Gul M, Ozturk S, Yerdelen KO. Evaluation of antimicrobial activities of several mannich bases and their derivatives. Arch Pharm (Weinheim) 2005;338:335-8.
5. Gul HI, Denizci AA, Erciyas E. Antimicrobial evaluation of some mannich bases of acetophenone and representative quaternary derivatives. Arzneimittelforschung 2002;52:773-7.
6. Gul HI, Ojanen T, Vepsalainen J, Gul M, Erciyas, E, Hanninen O. Antifungal activity of some mono, bis and quaternary mannich bases derived from acetophenone. Arzneimittelforschung 2001;51:72-5.
7. Reddy MV, Su CR, Chiou WF, Liu YN, Chen RY, Bastow KF, *et al*. Design, synthesis, and biological evaluation of Mannich bases of heterocyclicchalcone analogs as ctytotoxic agents. Bioorg Med Chem 2008;16:7358-70.
8. Gul M. Cytotoxic and antifungal acetophenone-derived Mannich bases: Effects on redox thiols and heat shock proteins. PhD Thesis, Kuopio University, Kuopio, Finland, 2005.
9. Dimmock JR, Vashishtha SC, Quail JW, Pugazhenthi U, Zimpel Z, Sudom AM, *et al*. 4-B-Arylvinyl.-3-B-arylvinylketo.-1-ethyl-4-piperidinols and related *compo*unds: A novel class of cytotoxic and anticancer agents. Med Chem 1998;41:4012-20.
10. Dimmock JR, Kumar P. Anticancer and cytotoxic properties of Mannich bases. Curr Med Chem 1997;4:1-22.
11. Gul HI, Suleyman H, Gul M. Evaluation of the anti-inflammatory activity of N, N'-Bis (3-dimethylamino-1-phenyl-propylidene) hydrazine dihydrochloride. Pharm Biol 2009;47:968-72.
12. Suleyman H, Gul HI, Gul M, Alkan M, Gocer F. Anti-inflammatory activity of bis (3-aryl-3-oxo-propyl) methylamine hydrochloride in rat. Biol Pharm Bull 2007;30:63-7.
13. Sridhar SK, Ramesh A. Synthesis and pharmacological activities of hydrazones, Schiff and Mannich bases of isatin derivatives. Biol Pharm Bull 2001;24:1149-52.
14. Suleyman H, Gul HI, Asoglu M. Anti-inflammatory activity of 3-benzoyl-1-methyl-4-phenyl-4-piperidinol hydrochloride. Pharmacol Res 2003;47:471-5.
15. Malinka W, Swiatek P, Filipek B, Sapa J, Jezierska A, Koll A. Synthesis, analgesic activity and computational study of new isothiazolopyridines of Mannich base type. Farmaco 2005;60:961-8.
16. Selvakumar V, Duraipandi S, Devdas S. Synthesis and Psychopharmacological activities of some Succinimide Mannich bases. J Pharm Res 2011;4:3168-3170.
17. Gul HI, Calls U, Ozturk Z, Tutar E, Calikiran L. Evaluation of anticonvulsant activities of bis (3-aryl-3-oxo-propyl) ethylamine hydrochlorides and 4-aryl-3-arylcarbonyl-1-ethyl-4-piperidinol hydrochlorides. Arzneimittelforschung 2007;57:133-6.
18. Gul HI, Calis U, Vepsalainen J. Synthesis of some mono-Mannich bases and corresponding azine derivatives and evaluation of their anticonvulsant activity. Arzneimittelforschung 2004;54:359-64.
19. Sriram D, Yogeeswari P, Gopal G. Synthesis, anti-HIV and antitubercular activities of lamivudine prodrugs. Eur J Med Chem 2005;40:1373-6.
20. Sriram D, Bal TR, Yogeeswari P. Design, synthesis and biological evaluation of novel non-nucleoside HIV-1 reverse transcriptase inhibitors with broad-spectrum chemotherapeutic properties. Bioorg Med Chem 2004;12:5865-73.
21. Sriram D, Yogeeswari P. Towards the design and development of agents with broad spectrum chemotherapeutic properties for the effective treatment of HIV/AIDS. Curr Med Chem 2003;10:1689-95.
22. Pandeya SN, Sriram D, Nath G, DeClercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4′-chlorophenyl) thiazol-2-yl] thiosemicarbazide. Eur J Pharm Sci 1999;9:25-31.
23. Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesis, antibacterial, antifungal and anti-HIV activity of Schiff and Mannich bases of isatin with N-[6-chlorobenzthiazole-2-yl] thiosemicarbazide. Indian J Pharm Sci 1999;61:358-61.
24. Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesis, antibacterial, antifungal and anti-HIV evaluation of Schiff and Mannich bases of isatin derivatives with 3-amino-2-methylmercapto quinazolin-4 (3H)-one. Pharm Acta Helv 1999;74:11-7.
25. Friebolin W, Jannack B, Wenzel N, Furrer J, Oeser T, Sanchez CP, *et al*. Antimalarial dual drugs based on potent inhibitors of glutathione reductase from Plasmodium falciparum. Med Chem 2008;51:1260-77.
26. Miroshnikova OV, Hudson TH, Gerena L, Kyle DE, Lin AJ. N,

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N'-(1,4-Phenylene) bis (2-bromo-2-methylpropanamide). J Med Chem 2007;50:889-96.
27. Lee B, Bauer H, Melchers J, Ruppert T, Rattray L, Yardley V, *et al*. Irreversible inactivation of trypanothione reductase by unsaturated Mannich bases: A divinyl ketone as key intermediate. J Med Chem 2005;48:7400-10.
28. Davioud-Charvet E, McLeish MJ, Veine DM, Giegel D, Arscott LD, Andricopulo AD, *et al*. Mechanism-based inactivation of thioredoxin reductase from Plasmodium falciparum by Mannich bases. Implication for cytotoxicity. Biochemistry 2003;42:13319-30.
29. Kidwai M, Thakur R, Mohan R. Ecofriendly synthesis of novel antifungal (thio) barbituric acid derivatives. Acta Chim Slov 2005;52:88-92.
30. Kocsis B, Kustos I, Kilár F, Nyul A, Jakus PB, Kerekes S, *et al*. Antifungal unsaturated cyclic Mannich ketones and amino alcohols: Study of mechanism of action. Eur J Med Chem 2009;44:1823-9.
31. Mazzei M, Nieddu E, Miele M, Balbi A, Ferrone M, Fermeglia M, *et al*. Activity of Mannich bases of 7-hydroxycoumarin against Flaviviridae. Bioorg Med Chem 2008;16:2591-605.
32. Kumaria N, Dwivedi AK, Maikhuri JP, Gupta G, Habib S, Dhar JD, *et al*. Substituted acrylophenones and related mannich bases as possible spermicides and inhibitors of HIV envelope glycoprotein-CD4 interaction. Eur J Med Chem 2002;37:855-64.
33. Erciyas E, Erkaleli HI, Cosar G. Antimicrobial evaluation of some styryl ketone derivatives and related thiol adducts. J Pharm Sci 1994;83:545-8.
34. Dimmock JR, Advikolanu KM, Scott HE, Duffy MJ, Reid RS, Quail JW, *et al*. Evaluation of cytotoxicity of some Mannich bases of various aryl and arylidene ketones and their corresponding arylhydrazones. J Pharm Sci 1992;81:1147-52.
35. Gordon PN, Johnston JD, English AR. Antimicrobial Agents and Chemotherapy. Hobby GL, Editor. American Society for Microbiology; Bethesda; 1965. p. 165.
36. Dimmock JR, Kumar P, Chen M, Quail JW, Yang J, Allen TM, *et al*. Synthesis and cytotoxic evaluation of mesna adducts of some 1-aryl-4,4-dimethyl-5-(1-piperidino)-1-penten-3-one hydrochlorides. Pharmazie 1995;50:449-53.
37. Dimmock JR, Kumar P, Manavathu EK, Obedeanu N, Grewal J. Activity of some Mannich bases of conjugated styryl ketones against Candida albicans. Pharmazie 1994;49:909-12.
38. Cairns J. Efficiency of the adaptive response of *Escherichia coli* to alkylating agents. Nature 1980;286:176-8.
39. Farmer PB. Monitoring for human exposure to carcinogens. Chem Br 1982;18:790-4.
40. Muthumani P, Neckmohammed R, Meera S, Venkataraman N, Chidambaranathan P, Devi CA, *et al.* Synthesis and evaluation of anticonvulsant and antimicrobial activities of some Mannich bases of substituted aminophenol and acetophenone. Int J Pharm Biomed Res 2010;1:78-86.
41. Maxwell CE. β-dimethylaminopropiophenone hydrochloride. Organic Syntheses 1955;3:305.
42. NRC [National Research Council]. Guide for the care and use of laboratory animals. National institutes of Health, Bethesda 1985:23-85.
43. Auvray N, Caston J, Reber A, Stelz T. Role of the cerebellum in the ontogenesis of the equilibrium behavior in the young rat: A behavioral study. Brain Res 1989;505:291-301.
44. Caston J, Delhaye-Bouchaud N, Mariani J. Motor behavior of heterozygous staggerer mutant (+/sg) versus normal (+/+) mice during aging. Behav Brain Res 1995;72:97-102.
45. Jeljeli M, Strazielle C, Caston J, Lalonde R. Effects of ventrolateral-ventromedial thalamic lesions on motor coordination and spatial orientation in rats. Neurosci Res 2003;47:309-16.
46. Zion C, Auvray N, Caston J, Reber A, Stelz T. Effects of cerebellectomy at day 15 on the ontogenesis of the equilibrium behavior in the rat. Brain Res 1990;515:104-10.
47. Fossati P, Melzi d'Eril GV, Tarenghi G, Prencipe L, Berti G. A kinetic colorimetric assay of gamma-glutamyltransferase. Clin Chem 1986;32:1581-4.
48. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24-7.
49. Holmgren A. Thioredoxin and glutaredoxin systems. J Biol Chem 1989;264:13963-6.
50. Cunningham ML, Price HC, O'Connor RW, Moorman MP, Mahler JF, Nold JB, *et al*. Inhalation toxicity studies of the alpha, beta-unsaturated ketones: 2-cyclohexene-1-one. Inhal Toxicol 2001;13:25-36.
51. Gul HI, Vepsalainen J, Gul M, Erciyas E, Hanninen O. Cytotoxic activities of mono and bis Mannich bases derived from acetophenone against Renca and Jurkat cells. Pharm Acta Helv 2000;74:393-8.
52. Rodrigues AL, Rosa JM, Gadotti VM, Goulart EC, Santos MM, Silva AV, *et al*. Antidepressant-like and antinociceptive-like actions of 4- (4'-chlorophenyl)-6- (4''-methylphenyl)-2-hydrazinepyrimidine Mannich base in mice. Pharmacol Biochem Behav 2005;82:156-62.
53. Dunhan N, Miya T. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc 1957;46:208-9.
54. Shiozawa A, Narita K, Izumi G, Kurashige S, Sakitama K, Ishikawa M. Synthesis and activity of 2-methyl-3-aminopropiophenones as centrally acting muscle relaxants. Eur J Med Chem 1995;30:85-94.
55. Hasegawa A, Furuhama K. Atlas of the hematology of the laboratory rat. University of Michigan: Elsevier; 1998. p. 152.
56. Morgan DL, Price HC, O'Connor RW, Seely JC, Ward SM, Wilson RE, *et al.* Upper respiratory tract toxicity of inhaled methylvinyl ketone in F344 rats and B6C3F1 mice. Toxicol Sci 2000;58:182-94.
57. Morgan DL, Ward SM, Wilson RE, Price HC, O'Connor RW, Seely JC, *et al*. Inhalation toxicity studies of the alpha, beta-unsaturated ketones: Ethyl vinyl ketone. Inhal Toxicol 2001;13:633-58.
58. Ji L, Long Q, Yang D, Xie J. Identification of mannich base as a novel inhibitor of Mycobacterium tuberculosis isocitrate by high-throughput screening. Int J Biol Sci 2011;7:376-82.

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