CHAPTER IV

RESULTS

1. Blood urea nitrogen, serum creatinine in control, sham, and UUO rats

The levels of BUN tended to increase with UUO (Figure 8), however, a significance was reached only day 6 ($p<0.05$) as compared to the control from 21.95±2.51 to 36.83±2.50 mg/dl. Unilateral ureteral obstruction also resulted in increases of serum creatinine level (Figure 9). The values were significantly higher after UUO on day 1 ($p<0.001$), 4 ($p<0.05$), and 7 ($p<0.05$). However, these levels were in normal range.

2. Number and percentage of cell death of circulating lymphocytes in control, sham, and 1 day to 7 days of UUO rats

The effect of different UUO durations on the number of circulating lymphocytes is shown in Figure 10. The reduction of the number occurred from day 1 after UUO and reached the significant lower level at only day 4 (from 2502.50±277.18 to 1320.00±277.18 cell/mm^3; $p<0.05$) as compared with control group. After that, the levels were restored back; however, they were still less than those of the control. As compared with sham groups, these number in UUO animals were comparable.

The alterations of percentage of cell death are shown in Figure 11. In rats with UUO of 1 day and 4 day duration, the values were increased significantly ($p<0.001$) from 40.65±4.13 % to 67.65±3.05% and to

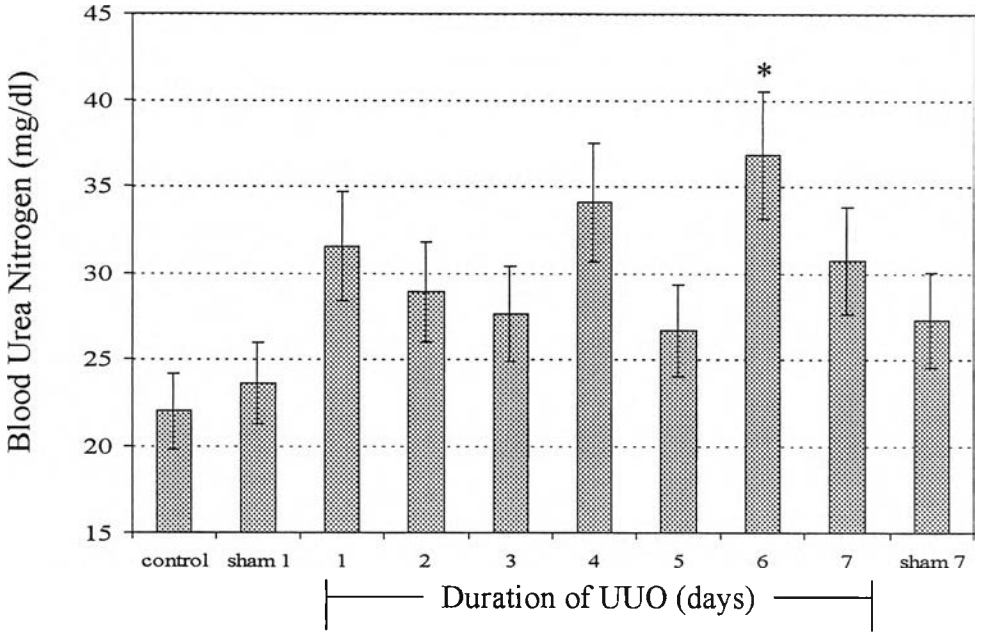


Figure 8 Levels of blood urea nitrogen in control, sham, and 1-day to 7-day UUO rats. (n = 5±1/group) (UUO = unilateral unilateral obstruction) (*p<0.05 compared with control)

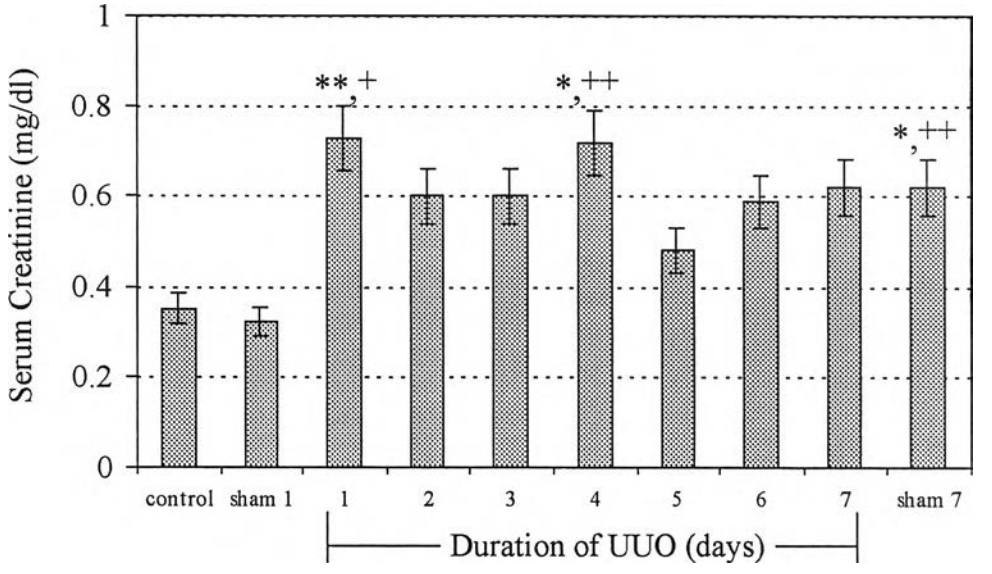


Figure 9 Levels of serum creatinine in control, sham, and 1-day to 7-day UUO rats. (n = 5±1/group) (UUO = unilateral ureteral obstruction) (*p<0.05, **p<0.001 compared with control; $^{+}$p<0.001 compared with sham1, $^{++}$p<0,05 compared with sham 7)

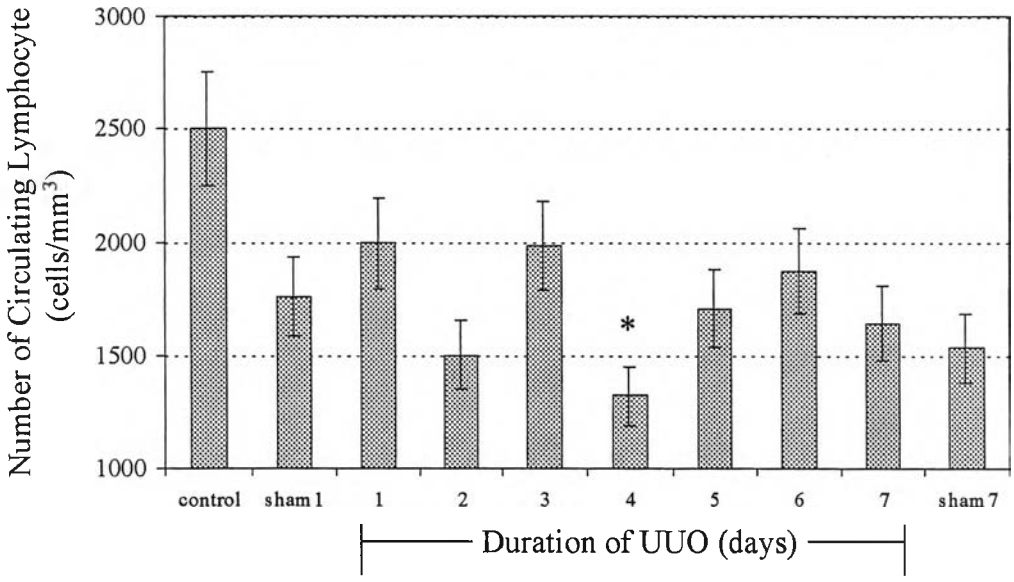


Figure 10 Number of circulating lymphocyte in control, sham, and 1-day to 7-day UUO rats. (n = 5±1/group) (UUO = unilateral ureteral obstruction) (*p<0.05 compared with control)

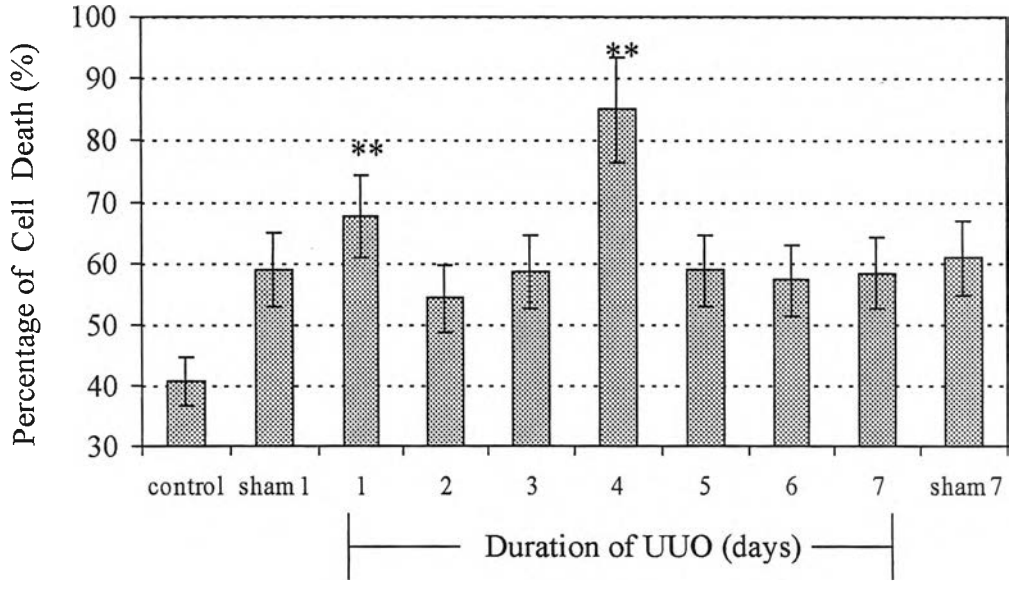


Figure 11 Percentage of cell death in control, sham, and 1-day to 7-day UUO rats. (n = 5±1/group) (UUO = unilateral ureteral obstruction) (**p<0.001 compared with control)

84.90±4.31 %, respectively as compared to those of control animals. All animals undergone operation (both sham and UUO) showed the same level of percentage of cell death.

3. Effect of ARA or ACEI on BUN and serum creatinine levels in sham, 1-day, and 7-day UUO rats

Unilateral ureteral obstruction for 1 day did not significantly change in BUN levels in rats treated with all treatments (Figure 12). In 7-day UUO rats, the animals received water also demonstrated a comparable level as their sham treatment matched group. Surprisingly, the levels rose when the rats treated with ACEI ($p<0.001$). Both treatments (with ARA and ACEI) had no effect on BUN levels in sham groups.

The Figure 13 shows the increases of serum creatinine levels in 1-day ($p<0.01$) UUO animals when compared with their respective sham treatment matched group. However, only the rats in 7-day UUO groups treated with ACEI demonstrated a significant higher level ($p<0.05$) as compared to their sham animals. All three subgroup (water, ARA and ACEI) treatments of UUO rats showed comparable higher levels of serum creatinine. Treatments with ARA or ACEI had no effect on serum creatinine levels in sham rats.

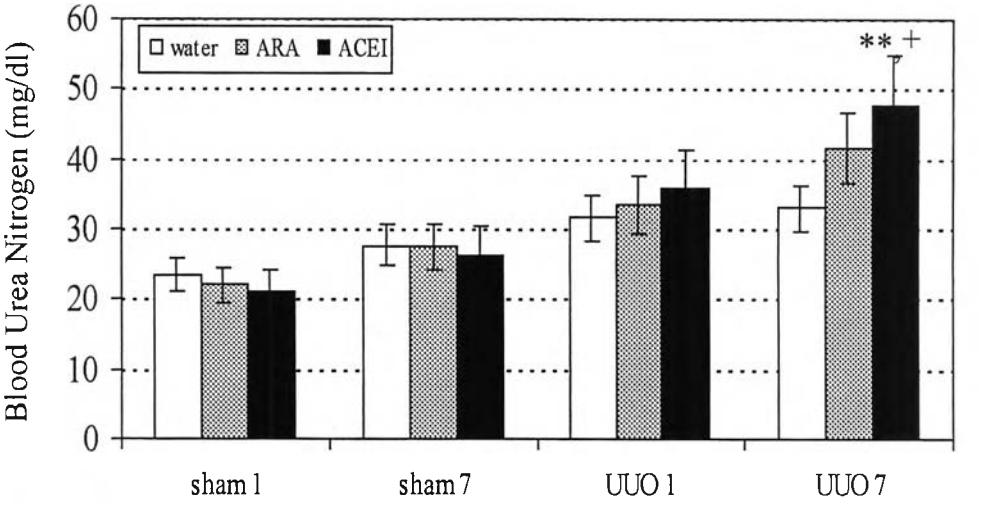


Figure 12 Effect of AT1 receptor antagonist (ARA) or ACEI on blood urea nitrogen in sham, 1-day and 7-day UUO rats. (n = 8±1/group) (UUO = unilateral ureteral obstruction) (**p<0.001 compared with sham 7; $^{+}$p<0.05 compared with UUO 7 + water)

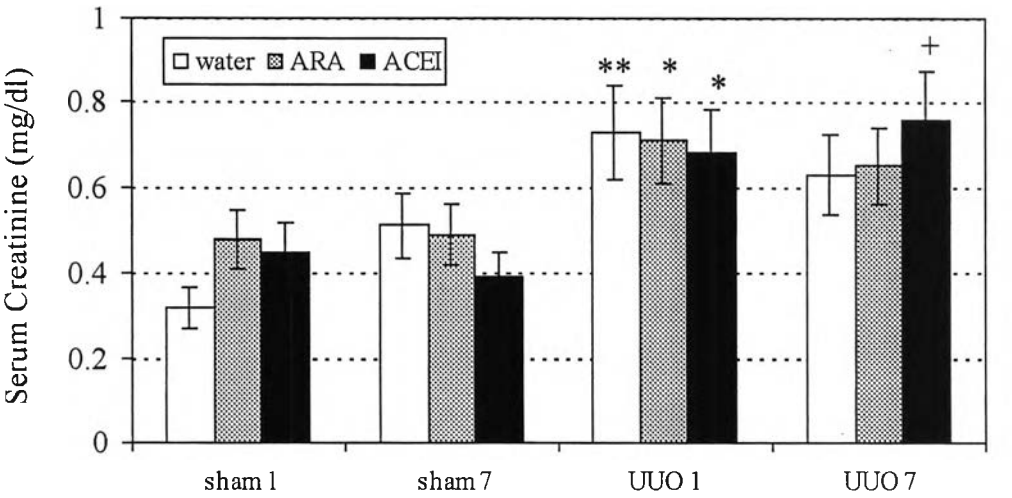


Figure 13 Effect of AT1 receptor antagonist (ARA) or ACEI on serum creatinine in sham, 1-day and 7-day UUO rats. (n = 8±1/group) (UUO = unilateral ureteral obstruction) (*p<0.05, **p<0.001 compared with sham 1; $^{+}$p<0.05 compared with sham 7)

4. Effect of ARA or ACEI on number of circulating lymphocytes in sham, 1-day, and 7-day UUO rats

As shown in Figure 14, the 1-day UUO rats treated with ARA had a significant lower number of circulating lymphocyte when compared with sham 1 or with 1-day UUO animals received water ($p<0.05$). Interestingly, UUO for 7 days did not alter the circulating lymphocyte levels in all three groups as compared to sham animals. Of note, both ARA and ACEI showed no effect on circulating lymphocyte levels in sham groups.

5. Effect of ARA or ACEI on percentage of cell death in sham, 1-day, and 7-day UUO rats

After 1-day or 7-day of UUO treated with either water or ARA, the rats showed no significant differences on percentage of cell death (Figure 15). However, the percentage was lower in groups received ACEI. In 1-day UUO group, the value reduced from 67.66 ± 2.80 % to 48.79 ± 2.80 % ($p<0.001$), whereas the level diminished from 58.49 ± 2.80 % to 41.51 ± 2.79 % ($p<0.05$) in 7-day UUO animals when compared to their respective UUO duration matched rats. Moreover, the UUO animals treated with ACEI for 7 days had the lower cell death level than those of their respective sham duration matched rats ($p<0.05$). No significant differences were noted in cell death level among sham groups.

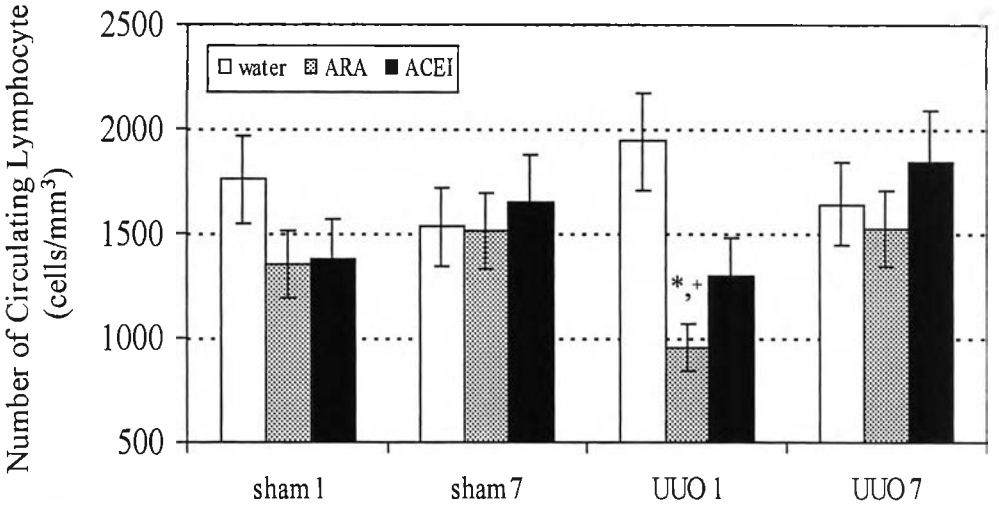


Figure 14 Effect of AT1 receptor antagonist (ARA) or ACEI on number of circulating lymphocyte in sham, 1-day and 7-day UUO rats. (n = 8±1/group)(UUO = unilateral ureteral obstruction) (*p<0.001 compared with sham 1; $^{+}$p<0.001 compared with UUO 1 + water)

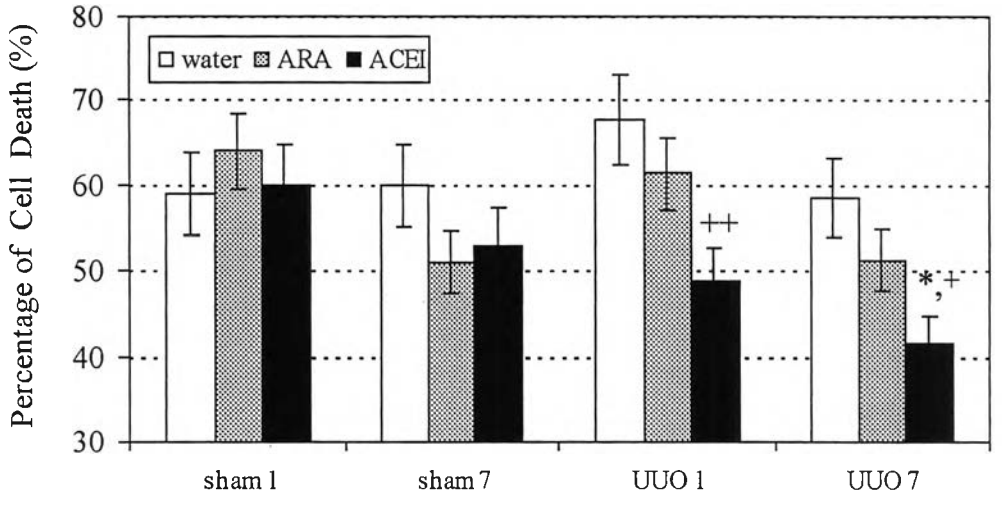
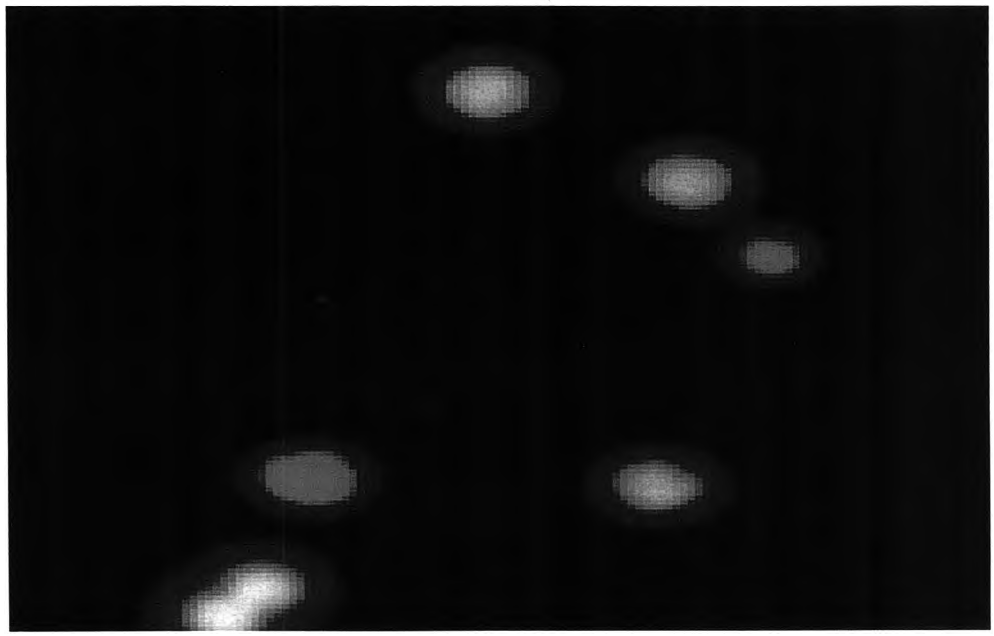


Figure 15 Effect of AT1 receptor antagonist (ARA) or ACEI on percentage of cell death in sham, 1-day and 7-day UUO rats. (n = 8±1/group)(UUO = unilateral ureteral obstruction) (*p<0.05 compared with sham 7; $^{+}$p<0.05 compared with UUO 7 with water; $^{++}$p<0.001 compared with UUO 1 + water)

6. Effect of ARA or ACEI on apoptotic index in sham, 1-day, and 7-day UUO rats

The apoptotic cells were viewed under a fluorescence microscope with 200-1000 x of magnification. All the cells in each microscopic field gave a blue fluorescent appearance (Figure 16A) at the first viewed without any filter. Then the same field had to be examined again by using a blue filter. The apoptotic cells would appear as light green circles (Figure 16B). A positive control slide (Figures 17A & B) was prepared by treating the lymphocytes with DNase I to yield DNA fragmentation. A negative control slide shown in Figures 18A & B was prepared simultaneously by omitting TdT enzyme from the ApopTag slidemaking protocol. Both positive and negative controls must be included in each test. Figures 19 and 20 present the apoptotic cells from 1-day and 7-day sham groups, respectively. The appearances of the apoptotic cells in UUO animals demonstrated in Figure 21 for 1-day groups and in Figure 22 for 7-day groups. In each Figure, the pictures from three treatments (water, ARA and ACEI) are shown simultaneously.

A



B

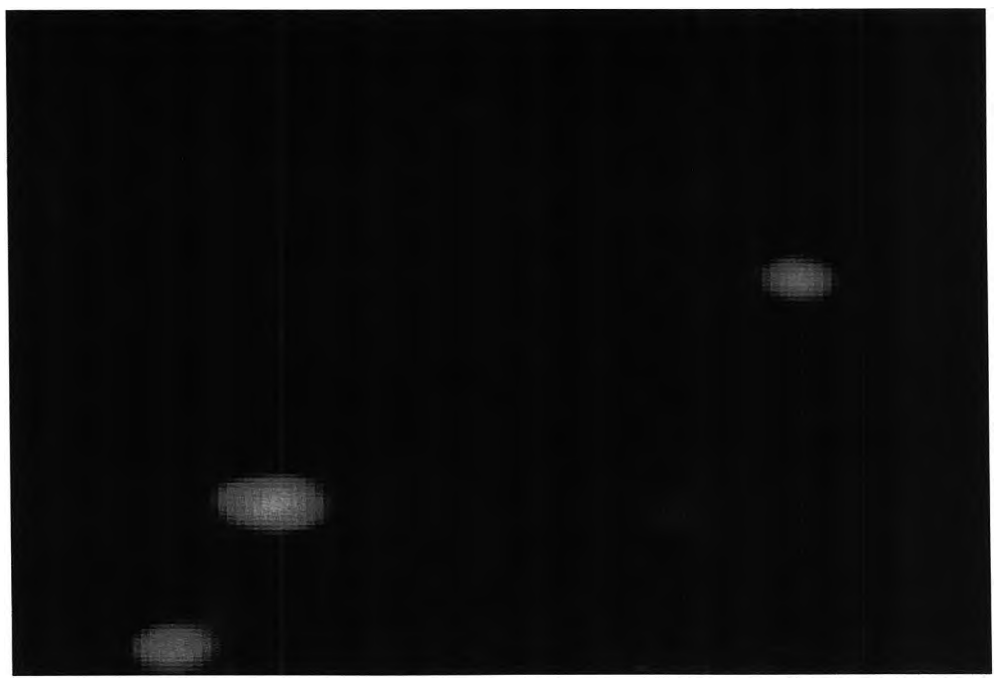
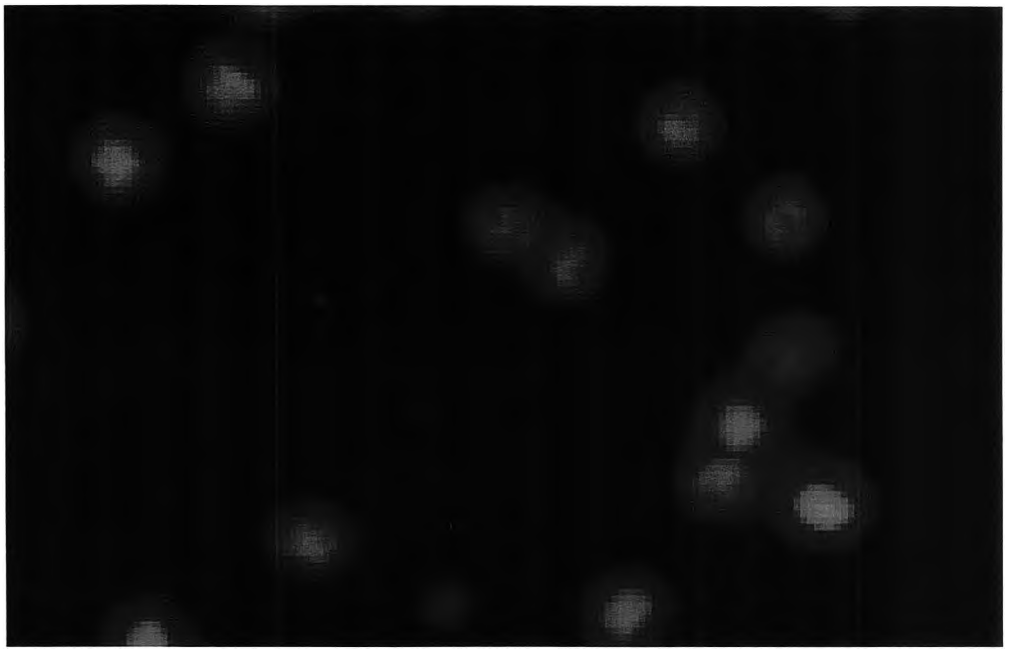


Figure 16. The pictures of total and apoptotic cells from one of studied samples. (A) Without the blue filter, all the cells are seen as the blue cells at 1000X. (B) Through the blue filter, the apoptotic cells are seen as the light green cells within the same field at 1000 X

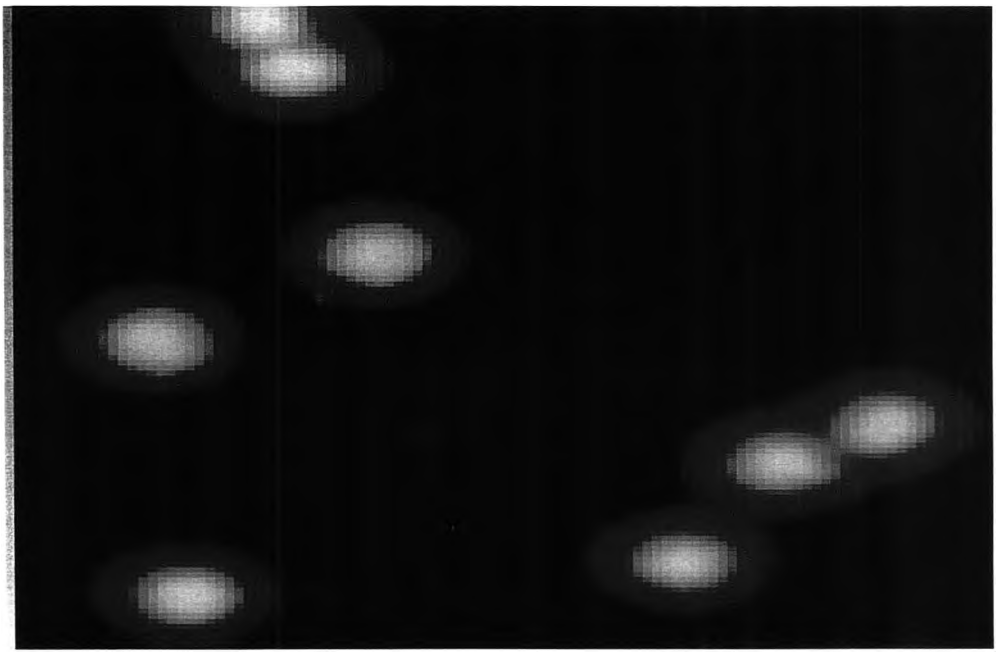
A



B

Figure. 17 The positive control was prepared from lymphocytes treated with DNase I to induce DNA fragmentation. (A) Without the blue filter, all the cells are shown as the blue cells at 1000X. (B) Under the blue filter, the apoptotic cells are seen as the light green cells in the same field at 1000X. in this field, all lymphocytes are apoptotic cells.

A



B

Figure. 18 The negative control slide was obtained by omitting the TdT enzyme from the labeling mix in the ApopTag slide marking protocol. (A) Without the blue filter, all the cells are seen as the blue cells at 1000X. (B) Using the blue filter, no apoptotic cells can be observed in the same field at 1000X.

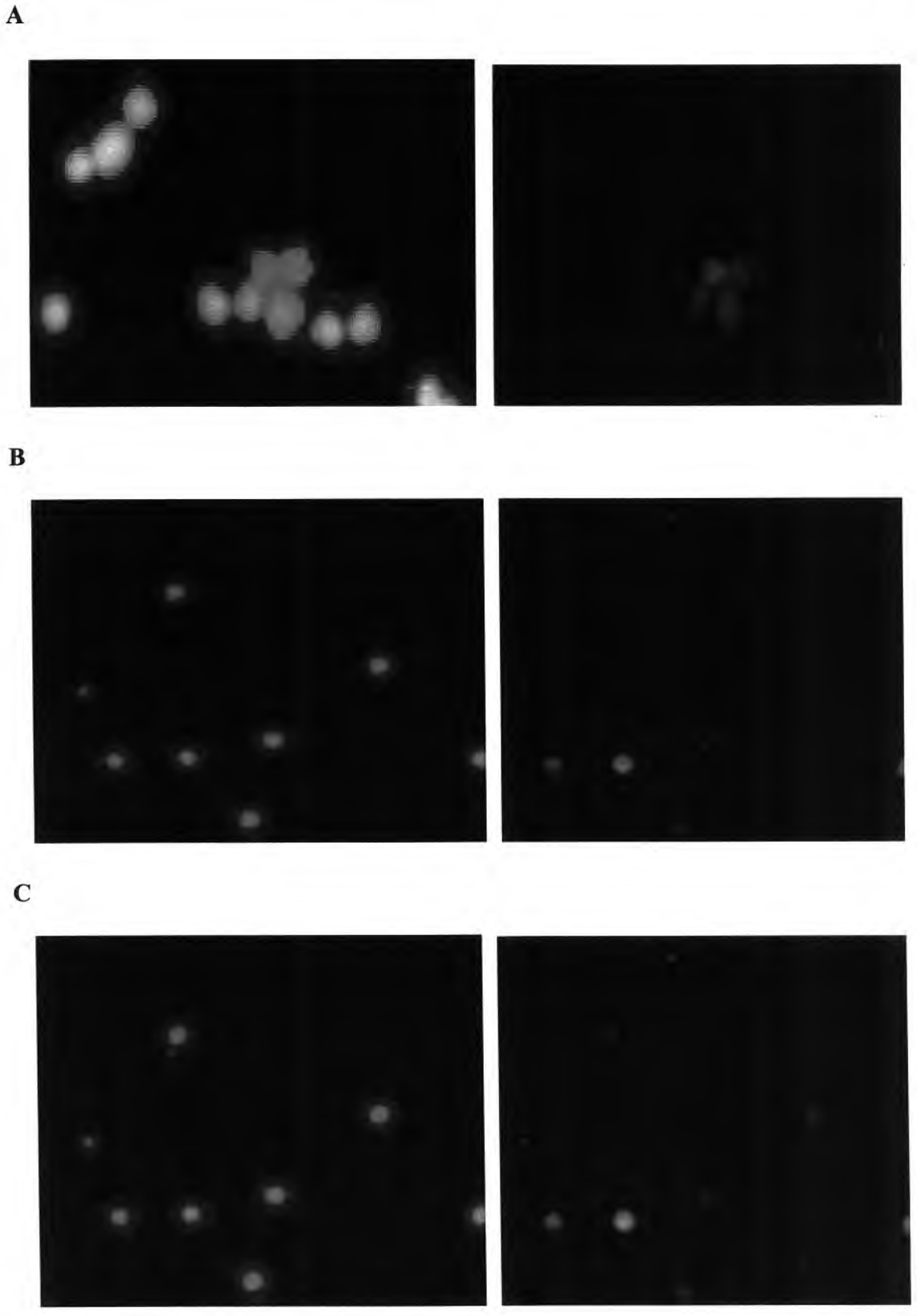


Figure. 19 The pictures of apoptotic cells from 1-day sham groups treated with (A) Water (B) AT_1 receptor antagonist or (C) ACEI

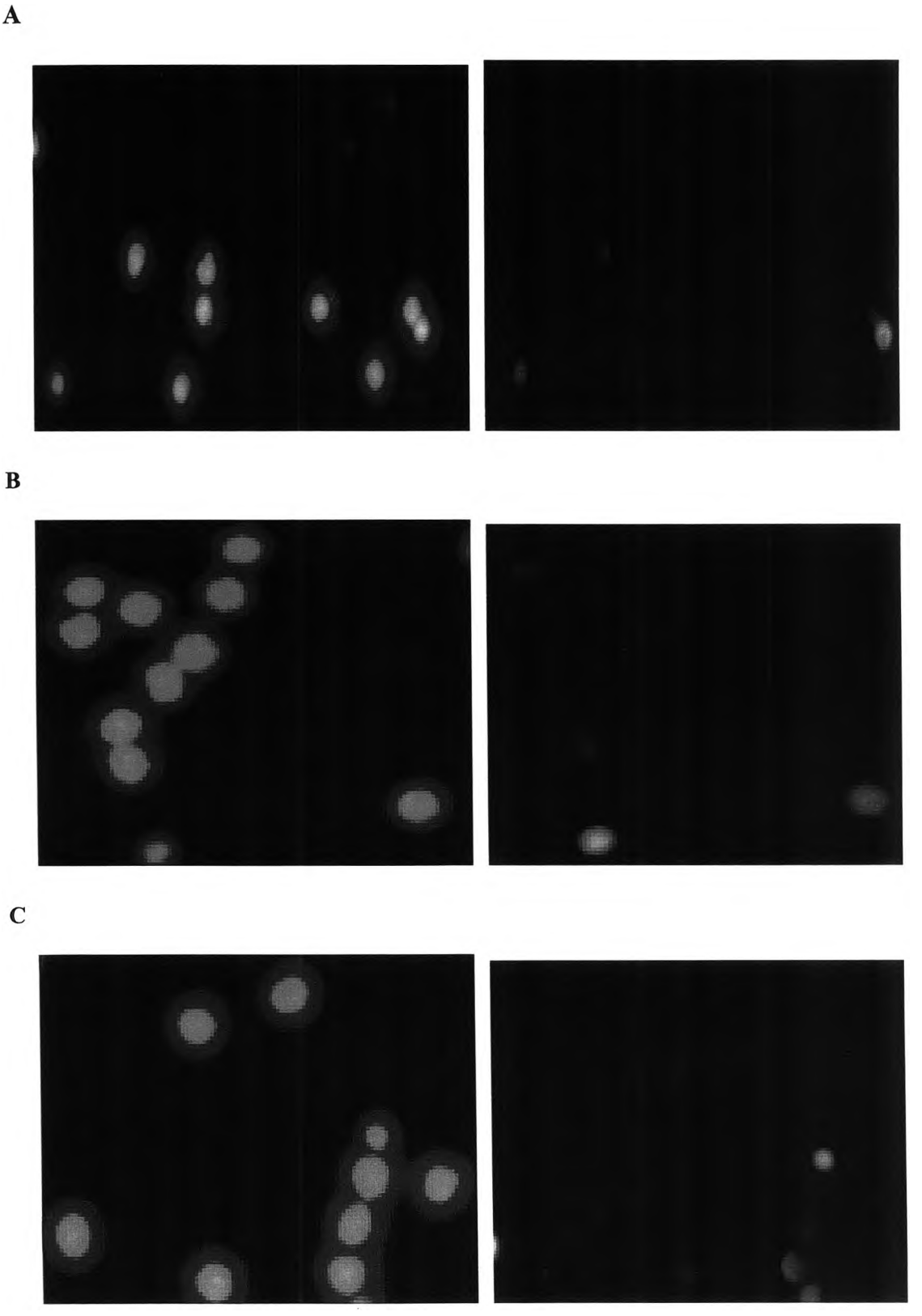
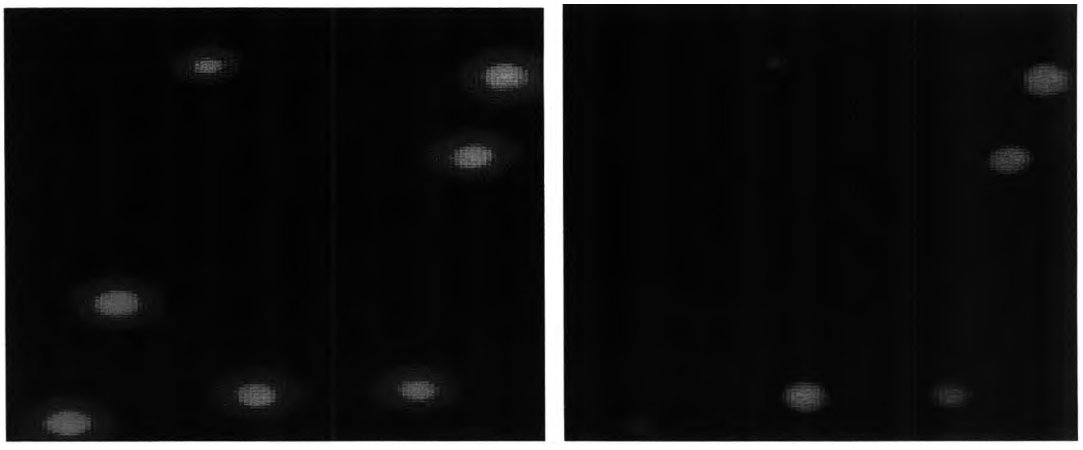


Figure. 20 The pictures of apoptotic cells from 7-day sham groups treated with (A) Water (B) AT_1 receptor antagonist or (C) ACEI

A



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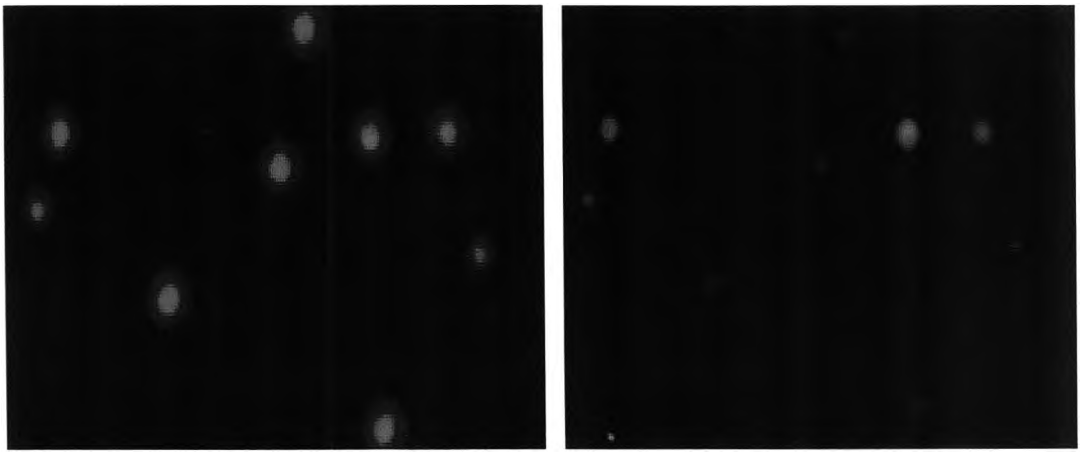


C

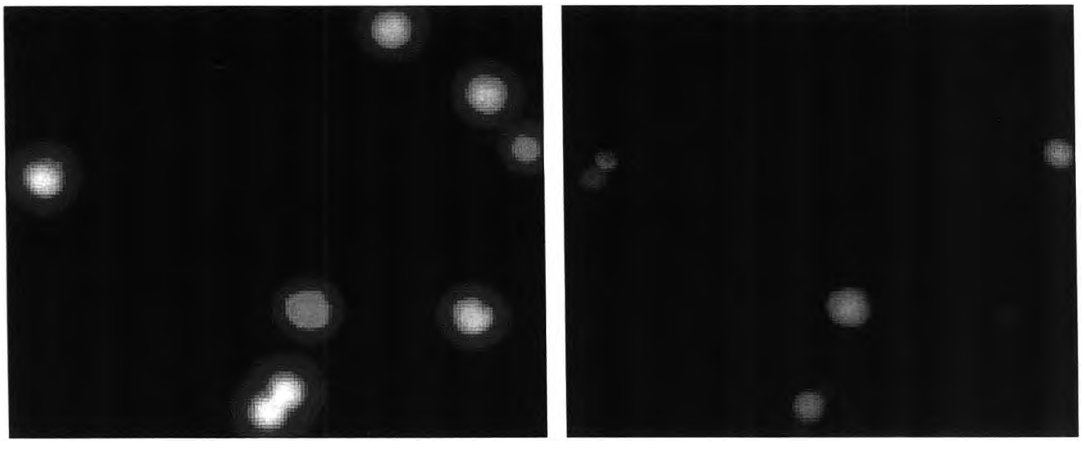
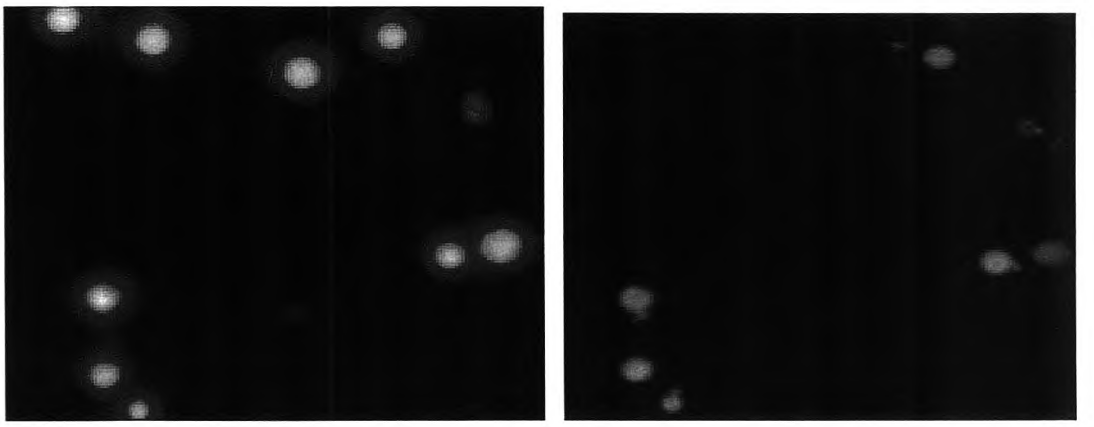
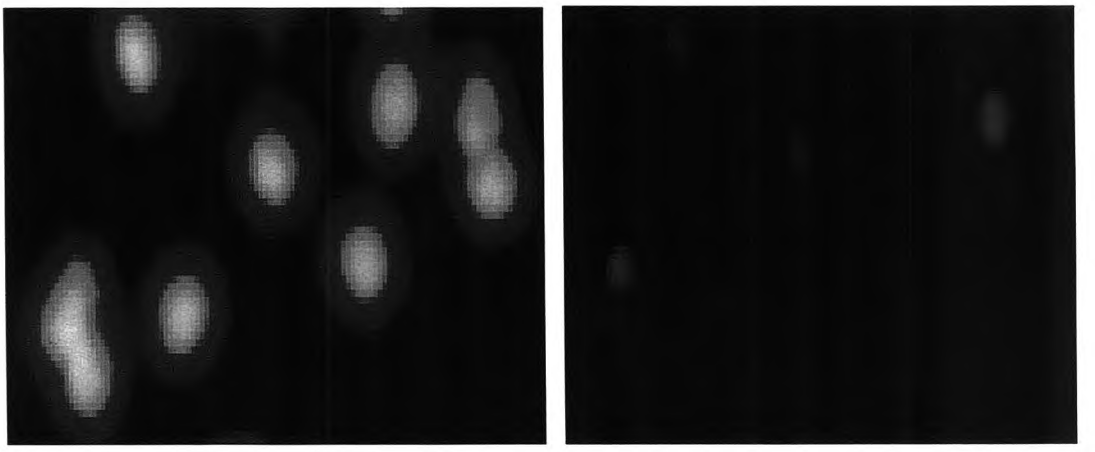


Figure. 21 The pictures of apoptotic cells from 1-day UUO groups treated with (A) Water (B) AT_1 receptor antagonist or (C) ACEI

A



B



C

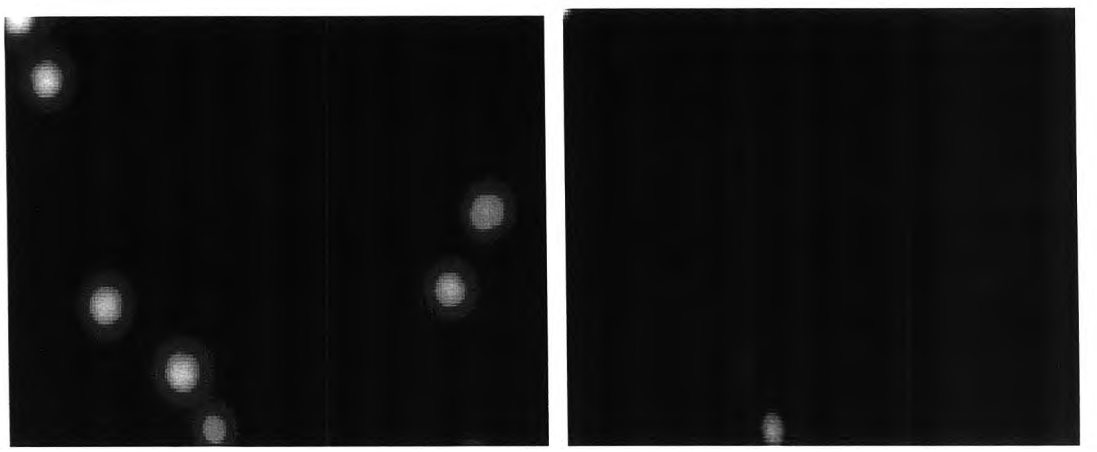


Figure. 22 The pictures of apoptotic cells from 7-day UUO groups treated with (A) Water (B) AT_1 receptor antagonist or (C) ACEI

The UUO rats received water, either 1 day or 7 days, had markedly significant apoptotic index levels as compared with their respective sham matched groups (Figure 23). The levels increased from 7.34±2.67 % to 32.57±2.67 % ($p<0.001$) and from 9.72±2.67 % to 29.19±2.67 % ($p<0.01$) in 1 day and 7 days of UUO, respectively. The treatment with ARA or ACEI in 1-day UUO animals showed no effect on apoptotic index values. Interestingly, the longer treatment of both for 7 days demonstrated a significant reduction in this index. The value diminished from 29.19±2.67 % to 11.91±2.67 % ($p<0.01$) in ARA treated rats and to 7.61±2.67 % ($p<0.001$) in ACEI treated animals. These lower levels were comparable to their respective sham matched groups. There were no significant effects of ARA or ACEI treatment on apoptotic index levels in sham animals.

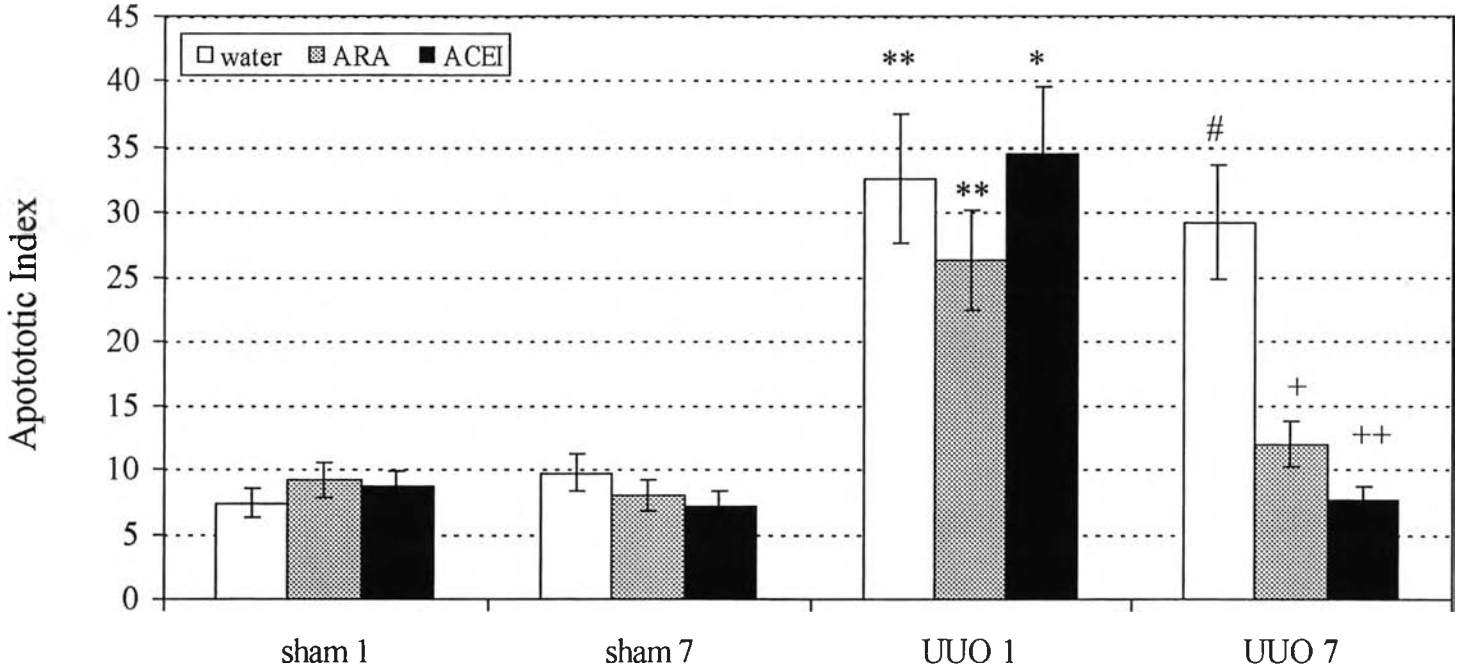


Figure 23 Effect of AT1 receptor antagonist (ARA) or ACEI on apoptotic index in sham,1-day and 7-day UUO rats. (n = 8±1/group) (UUO = unilateral ureteral obstruction) (*$p<0.05$, **$p<0.001$ compared with sham 1; # $p<0.001$ compared with sham ; $^{+}p<0.01$, $^{++}p<0.001$ compared with UUO 7 + water)