A COMPARATIVE STUDY ON THE EFFECTS OF INDOMETHACIN AND NABUMETONE ON RENAL FUNCTION IN ANAESTHETIZED AND CONSCIOUS RATS

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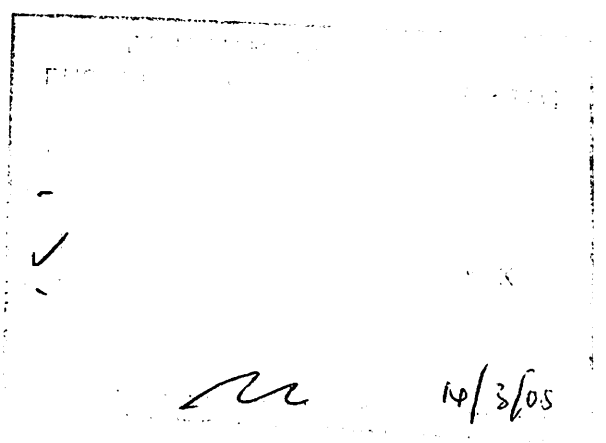

A COMPARATIVE STUDY ON THE EFFECTS OF INDOMETHACIN AND NABUMETONE ON RENAL FUNCTION IN CONSCIOUS AND ANAESTHETIZED RATS.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed agents in the treatment of pain, fever and inflammation (1). They exert their anti-inflammatory, analgesic and antipyretic effects through the inhibition of prostaglandin synthesis by blocking cyclooxygenase (COX) activity, a major enzyme in the biosynthesis of all prostaglandins (2).

Prostaglandins are ubiquitous in their distribution throughout the body and function for most part as "local hormones". Kidney is extremely active in the biosynthesis and mechanism of prostaglandins. These compounds participate in several processes in renal physiology, including autoregulation of renal blood flow and glomerular filtration rate, modulation of renin release, tubular ion transport and water metabolism(3).

COX is a key enzyme regulating the formation of prostaglandins from arachidonic acid. Recently however, COX was discovered to have two isoform namely COX-1 and COX-2 (4). These are derived from different genes but share ~60% amino acid identity. The expression patterns of COX-1 and COX-2 genes are quite different (5). COX-1 is normally expressed in the gastrointestinal tract, kidney and platelets and thought to participate in housekeeping function. It appears to be responsible for mediating the production of thromboxane and prostaglandins. Under the influence of COX-1, prostaglandins maintain the integrity of the gastric mucosa, mediate normal platelet function and regulate renal blood flow during states of hemodynamic stress (6).

The isoenzyme COX-2 is primarily associated with inflammation (7). Cytokines and growth factors increase the expression of COX-2 at inflammatory sites, producing prostaglandins that mediate inflammation, pain and fever. The discovery of the COX-2 isoenzyme has led to believe that COX-2 selective inhibition would provide the potent anti-inflammatory, analgesic and antipyretic effects that have been associated with the traditional NSAIDs with less side effects especially on renal function(8).

Traditional NSAIDs such as ibuprofen, indomethacin, aspirin and naproxen which inhibit both COX-1 and COX-2 are known to produce deleterious effects on renal function, particularly during haemodynamically stressful situations (9). This includes patients and individuals with decreased effective blood volume causesd by cardiac failure (10), liver cirrhosis with ascites (11), renal insufficiency (12) and hypertension (13). Responses to these hemodynamic challenges include

stimulation of the renin-angiotensin-aldosterone axis, with enhanced production of renin, the vasoconstrictive angiotensin II and aldosterone, which promotes sodium, water and chloride reabsorption and elevated sympathetic outflow, which further tend to promote vascular tone (14). In these situations, prostaglandins promote compensatory vasodilation of renal vascular beds to ensure an adequate blood supply and preclude acute functional deterioration of the kidney (15). But NSAIDs blunted these prostaglandins production and may cause further marked decreases in renal blood flow, glomerular filtration rate (GFR) and renal excretion of sodium and water, hyperkalemia and hyponatriemia (16). However, little information exists regarding the effects of COX-2 inhibition on renal function.

Most studies investigating the effects of the traditional NSAIDs on renal function have used animal models that have been anaesthetized. A recent study on rats in our laboratory using an NSAID, naproxen, has shown renal effects of naproxen that are opposite to what has been observed previously in anaesthetized rats (17), suggesting perhaps that the effects of NSAIDs on renal function may depend on the experimental design. This study therefore proposes to reinvestigate the effects of indomethacin on renal function in conscious and anaesthetized rats. In addition, we also propose to similarly study the effects of a COX-2 selective inhibitor, nabumetone, on renal function in the same species.

OBJECTIVE

The main objectives of the study are to :

1. Investigate the effects of indomethacin on renal function in both anaesthetized and conscious rats.
2. Investigate the effects of nabumetone on renal function in both anaesthetized and conscious rats.
3. Compare the effects of indomethacin and nabumetone on renal function in both anaesthetized and conscious rats.

MATERIAL AND METHODS

Study design. For conscious rats, thirty male Sprague Dawley rats weighing between 200-220 gram were housed individually in metabolic cages for a total duration of 5 weeks. The protocol consisted of four phases, namely; acclimatization phase (1 week), control phase (1 week), experimental phase (2 weeks) and recovery phase (1 week). All animals were treated identically during the acclimatization, control and the recovery phases. During the experimental phase however, the animals were given orally either 1.5mg.kg^{-1} body weight/day twice of indomethacin (n = 10), or 15mg.kg^{-1} body weight/day once of nabumetone (n = 10), dissolved in 0.5 ml of saline for a period of two weeks. Animals for control group (n = 10) were given only 0.5ml of saline. Food and water were provided *ad-libitum* and water intake, food intake, body weight, urine output, urinary sodium, potassium, calcium, magnesium, osmolality, osmolar and microalbumin excretion were estimated in all animals.

Study design. For anaesthetized rats, thirty two overnight fasted, male Sprague - Dawley rats but with access to water *ad libitum* and weighing between 230-260gm were prepared for standard clearance experiments. Following anaesthetization with an intraperitoneal injection of sodium thiopental (60mg.kg^{-1} body weight), the jugular vein and carotid artery were cannulated for continuous normal saline infusion and blood pressure monitoring and blood sampling respectively. Tracheostomy was performed to maintain a clear airway. The urinary bladder was catheterised suprapubically for urine collection. Animals were infused intravenously with 0.9% saline containing ^{3}H Inulin (0.5µCiml^{-1}) at a rate of 200µlmin^{-1} for the first hour to induce rapid volume expansion and diuresis. After the establishment of diuresis, the infusion rate was reduced to 100µlmin^{-1} of saline containing ^{3}H Inulin (1 µCiml^{-1}) for the next five hours. The five hours were divided into four phases, namely; equilibration phase (1 hour), control phase (1 hour), experimental phase (1 hour, where either the drug or the vehicle was infused) and recovery phase (2 hours). There were four groups of rats, two experimental groups (n = 8) namely: animals receiving nabumetone (5mg/kg body weight) and animals receiving indomethacin (1.5mg/kg body weight) and two control groups (n =8) receiving their respective vehicles. Blood and urine samples were collected every 30 minutes for analyses of electrolytes, microalbumin and GFR estimation.

Urine analyses. For urinary sodium and potassium excretion, flame photometer (Corning 404, UK) was used. Urinary calcium and magnesium were determined using Hitachi -912 Random Access Chemistry Analyzer from Department of Chemical Pathology USM. Urine osmolality was measured using cryoscopic osmometer (Osmomat 030, Gonotec, Germany). Urine microalbumin was determined using turbidity method by kits from Bayer (SERA –PAK ® immuno

microalbumin, Bayer, USA) using spectrophotometer (Ames Quick-Lab, Chemistry analyzer, Germany). [^{3}H] inulin in plasma and urine was assessed by liquid scintillation counting using phase combining system Tri-Carb 3100TR (Packard Bioscience Company, USA)

GFR estimation. GFR (glomerular filtration rate) was calculated as the clearance of inulin, $C_{in} = U_{in}V/P_{in}$, solute output as U_xV, where U and P are urinary and plasma concentrations of inulin (in) or solute x respectively and V is urine flow rate.

Statistical analysis. Results are expressed as the mean ± SEM. Statistical analysis was performed using analysis of variance (2-way anova) for repeated measurements. Significant difference was set at $p < 0.05$.

RESULTS

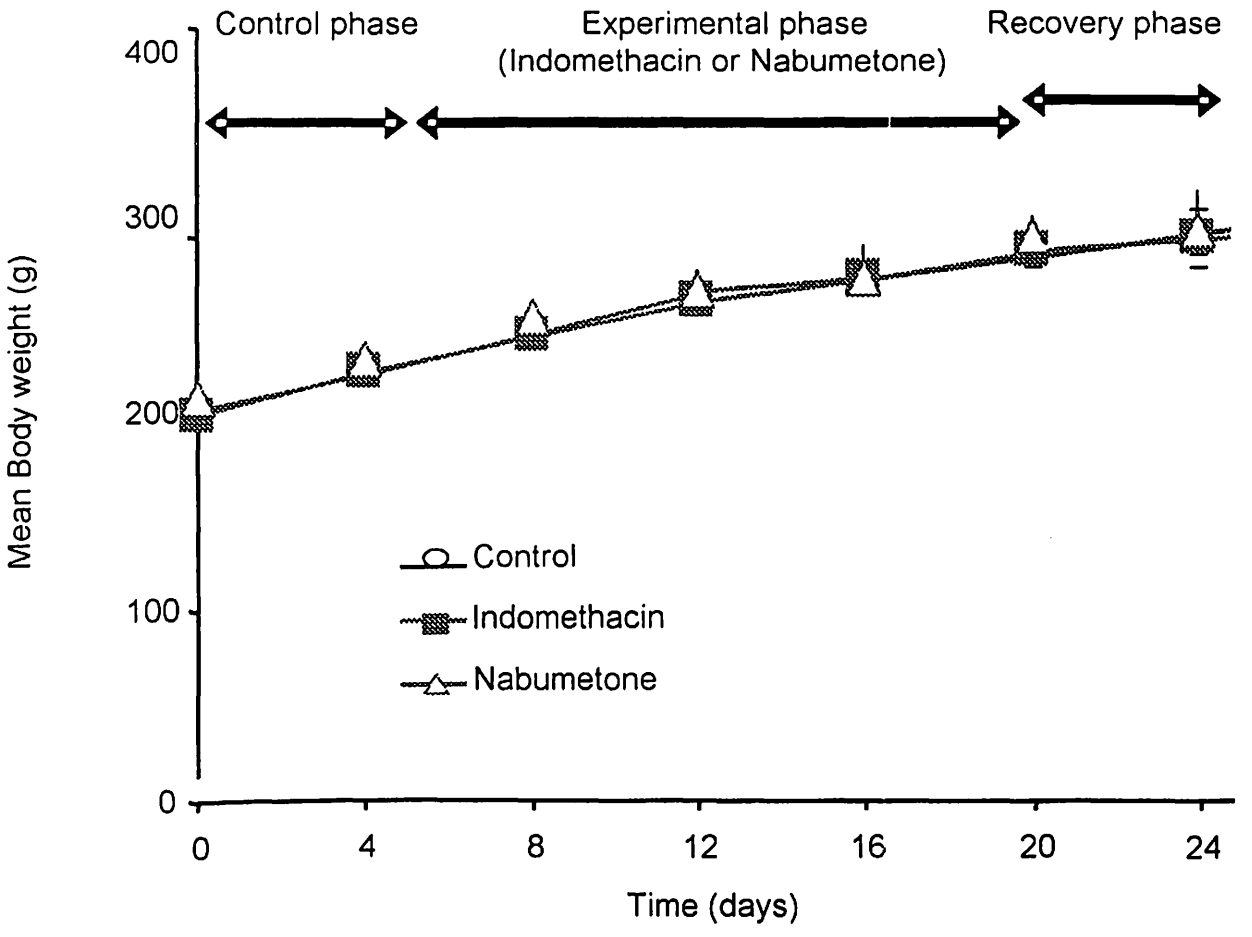

Figure 1.0: Mean body weight in conscious rats.

There is no significant difference between all groups in terms of body weight. But all groups shown increases body weight within study days.

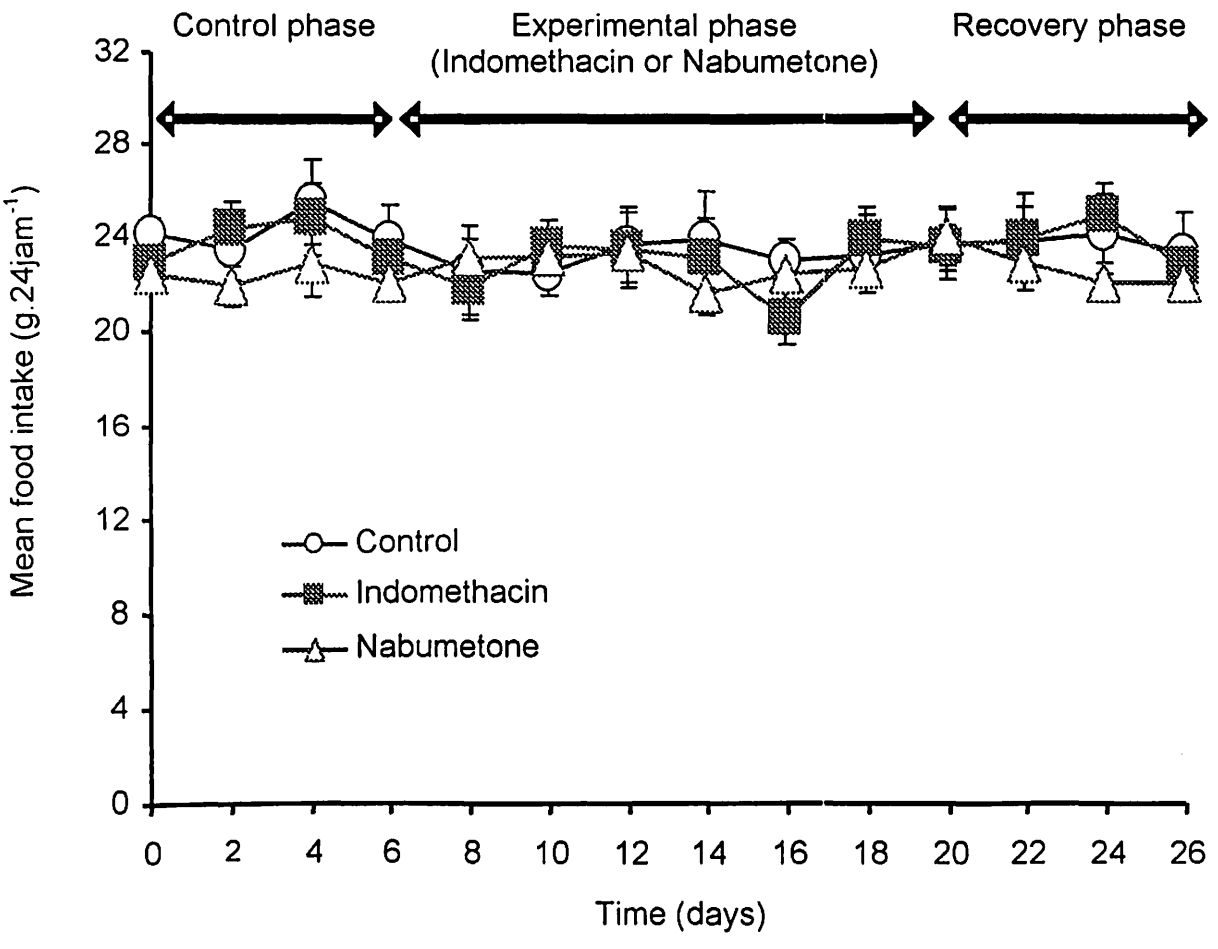

Figure 2: Mean food intake in conscious rats.

No significant difference was detected in food intake between all groups in all phases.

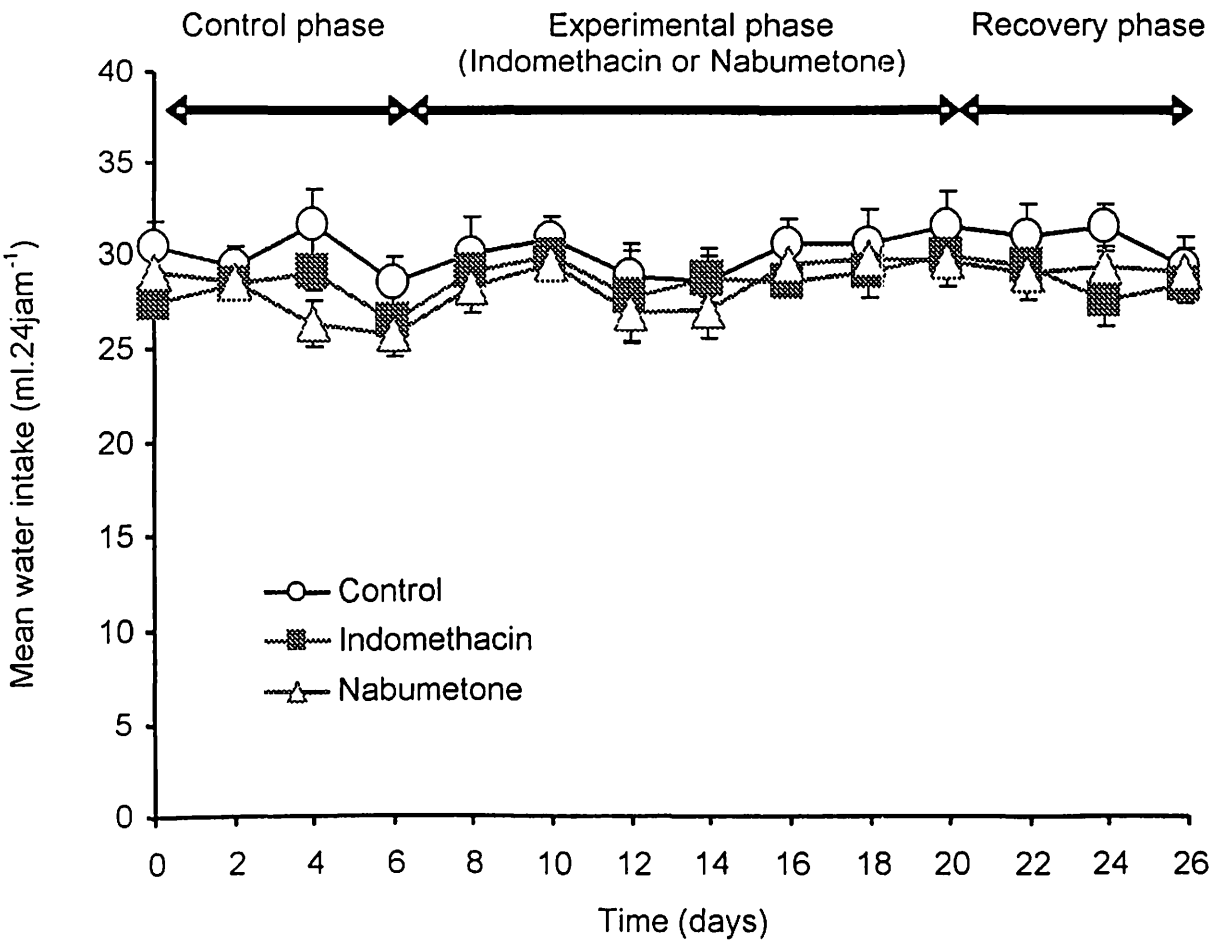

Figure 3: Mean water intake in conscious rats.

There is no significant difference in water intake between all groups in all phases.

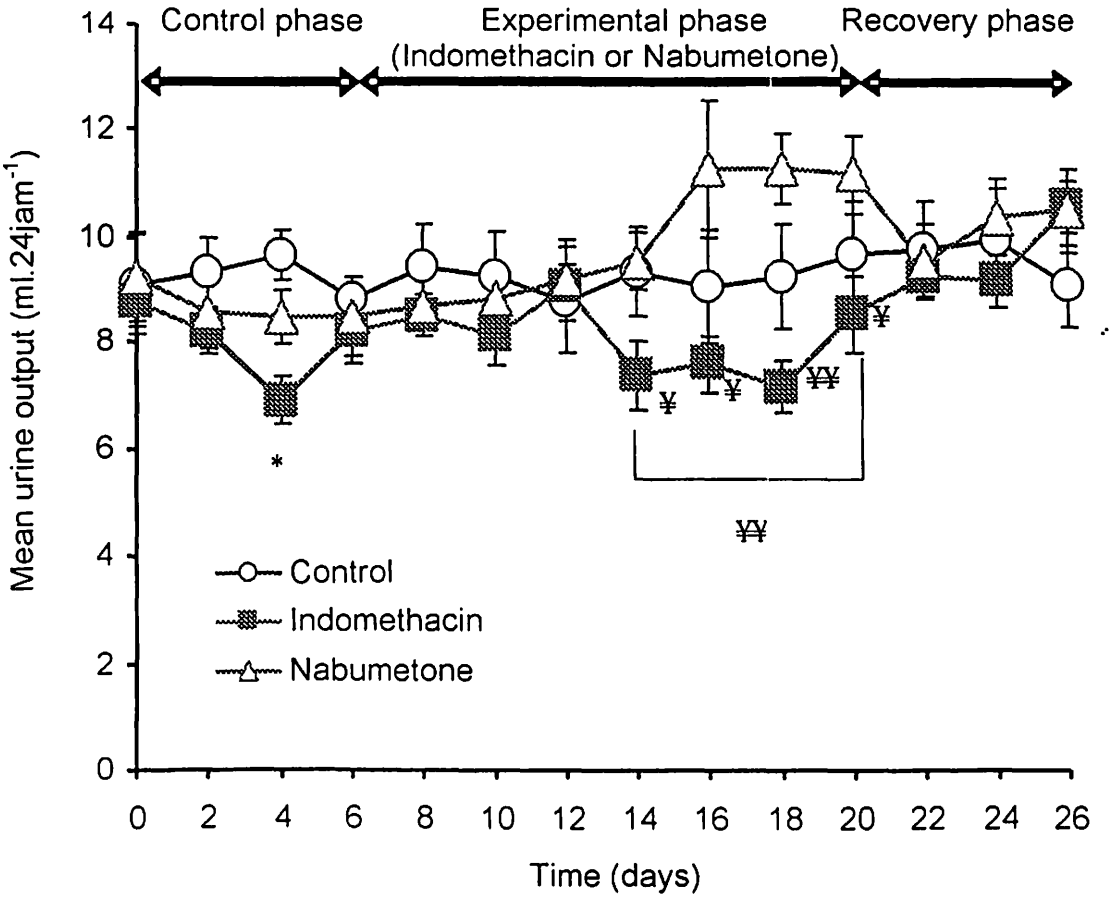

Figure 4: Mean urine output in conscious rats.

In experimental phase, there is a significant difference between indomethacin group compared to nabumetone group ($p < 0.01$). In th indomethacin group there is reduced urine output compared to other groups. In nabumetone group there is increased urine output compared to indomethacin group. There is no significant difference between groups, indomethacin and nabumetone compared to control group in urine output.

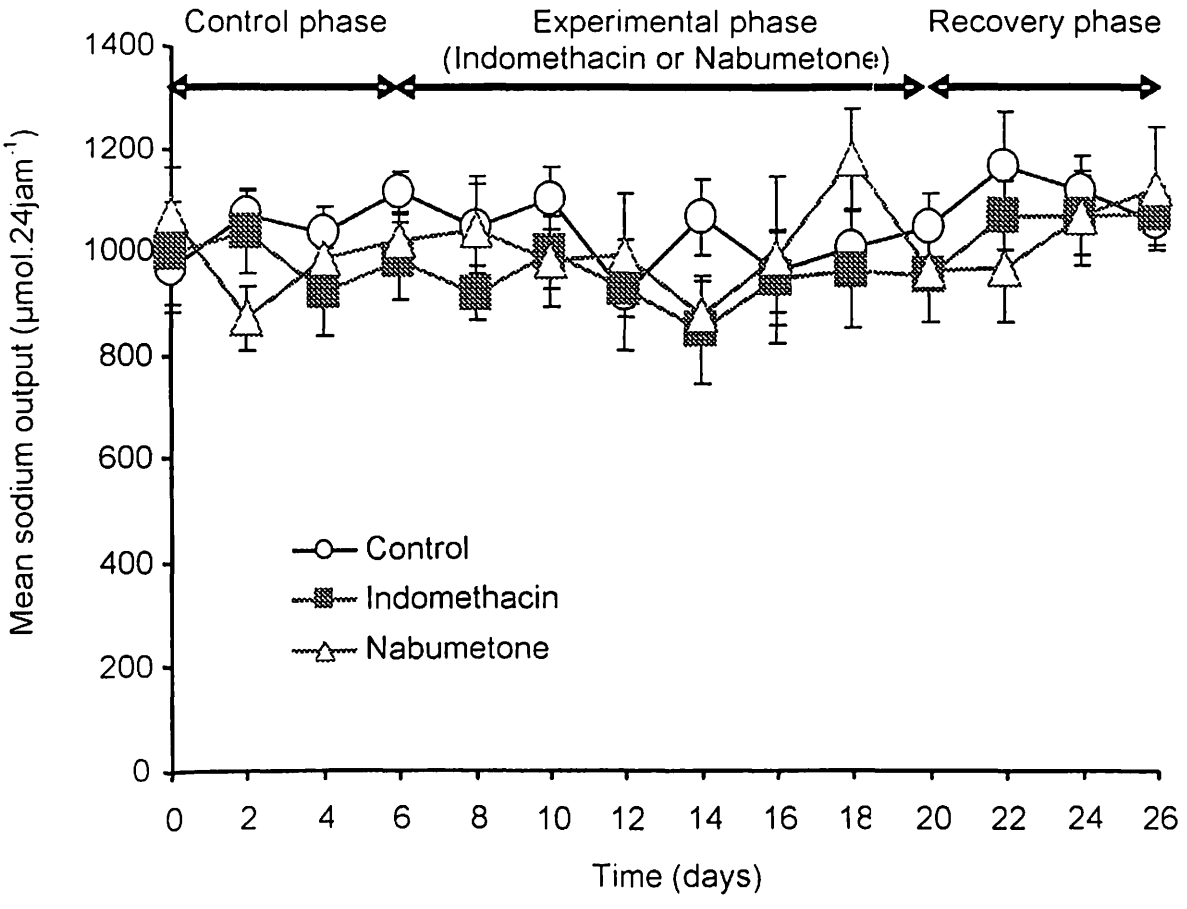

Figure 5: Mean urine sodium output in conscious rats.

There is no significant difference in sodium output between all groups in all phases.

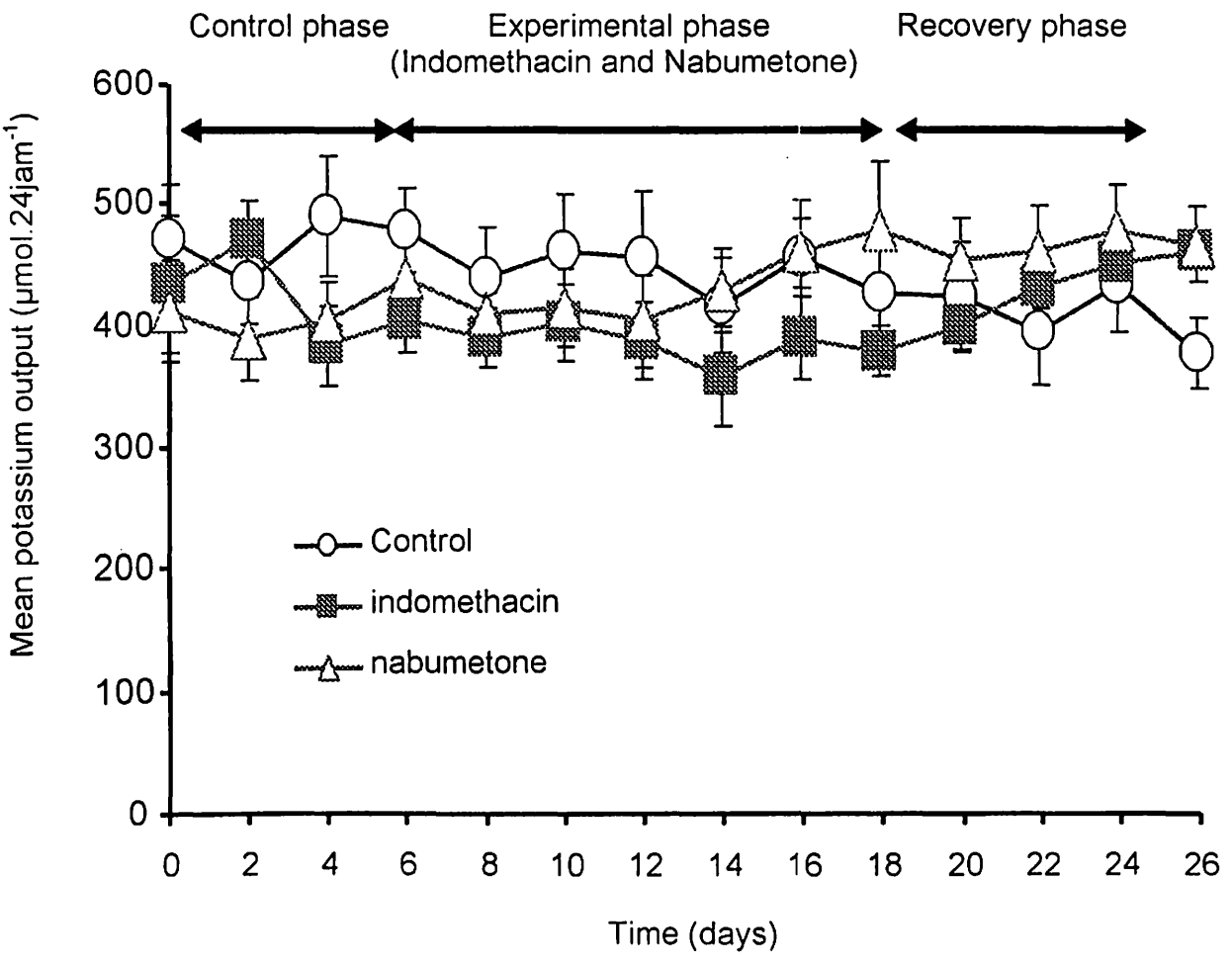

Figure 6: Mean urine potassium output in conscious rats.

There is no significant difference in potassium output between all groups in all phases.

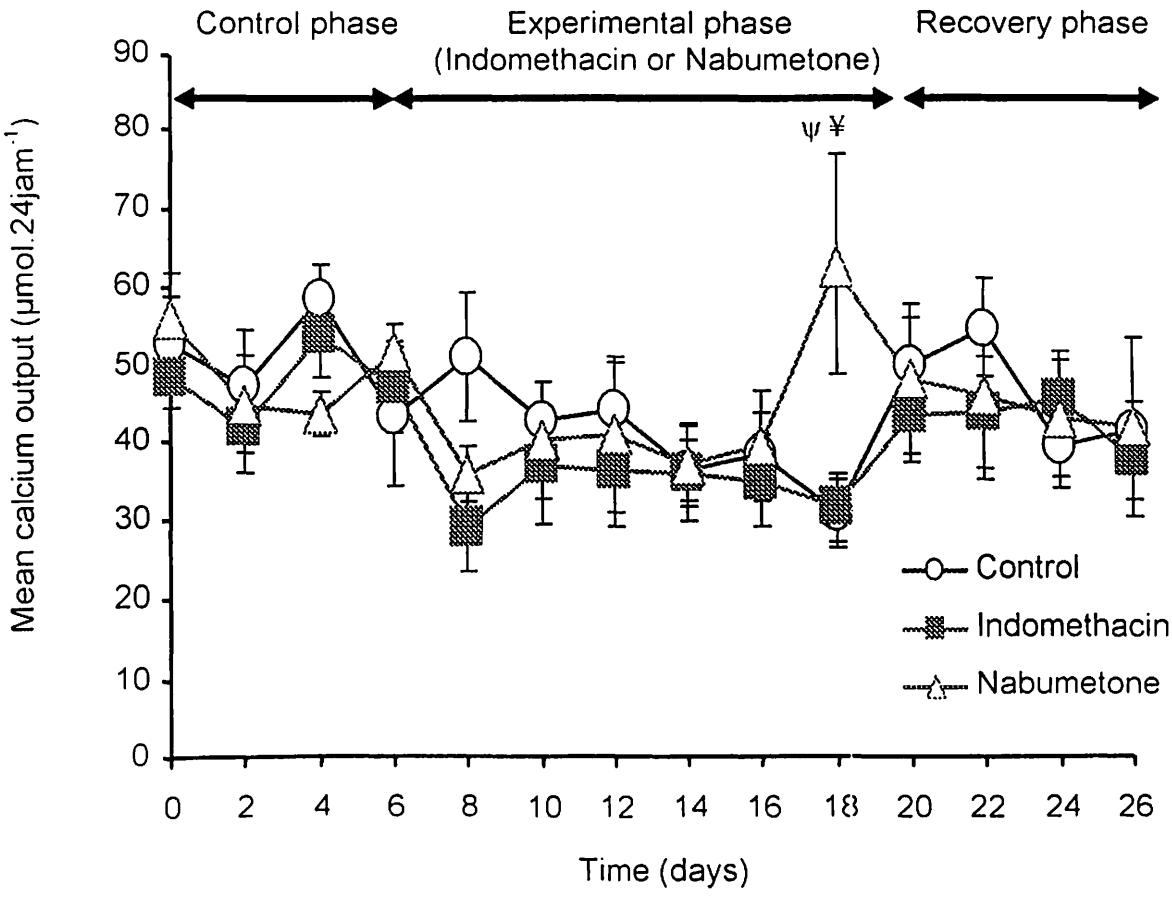

Figure 7: Mean urine calcium output in conscious rats.

In Nabumetone group there is increased calcium output on day 18 but overall there is no significant difference in calcium output between all groups in all phases.

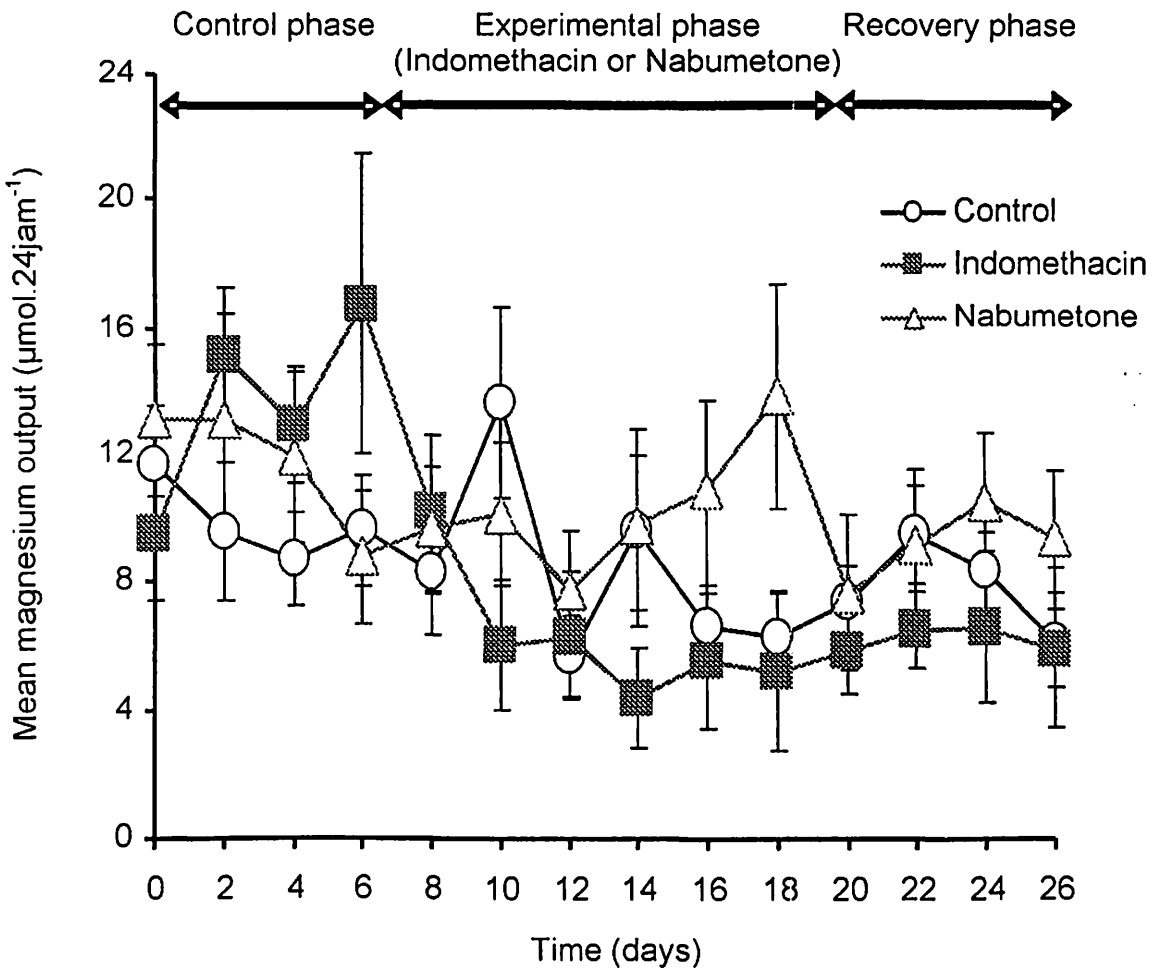

Figure 8: Mean urine magnesium output in conscious rats.

In Nabumetone group there is increased calcium output on day 18 but overall there is no significant difference in magnesium output between all groups in all phases.

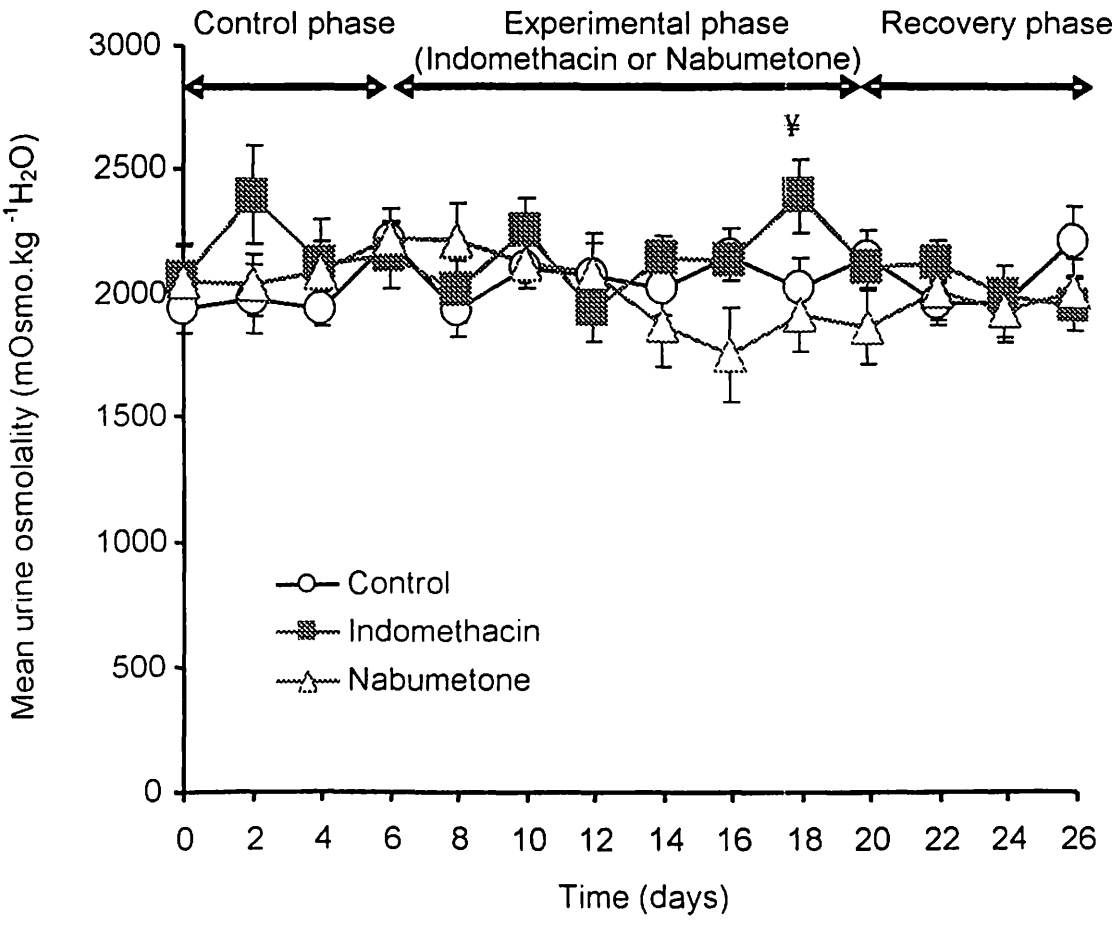

Figure 9: Mean urine osmolality in conscious rats.

In experimental phase, on day 18, there is significant difference between indomethacin and nabumetone group. indomethacin produced higher urine osmolality compared to nabumetone group ($p < 0.05$). But overall, there is no significant difference between all groups ir all phases in urine osmolality.

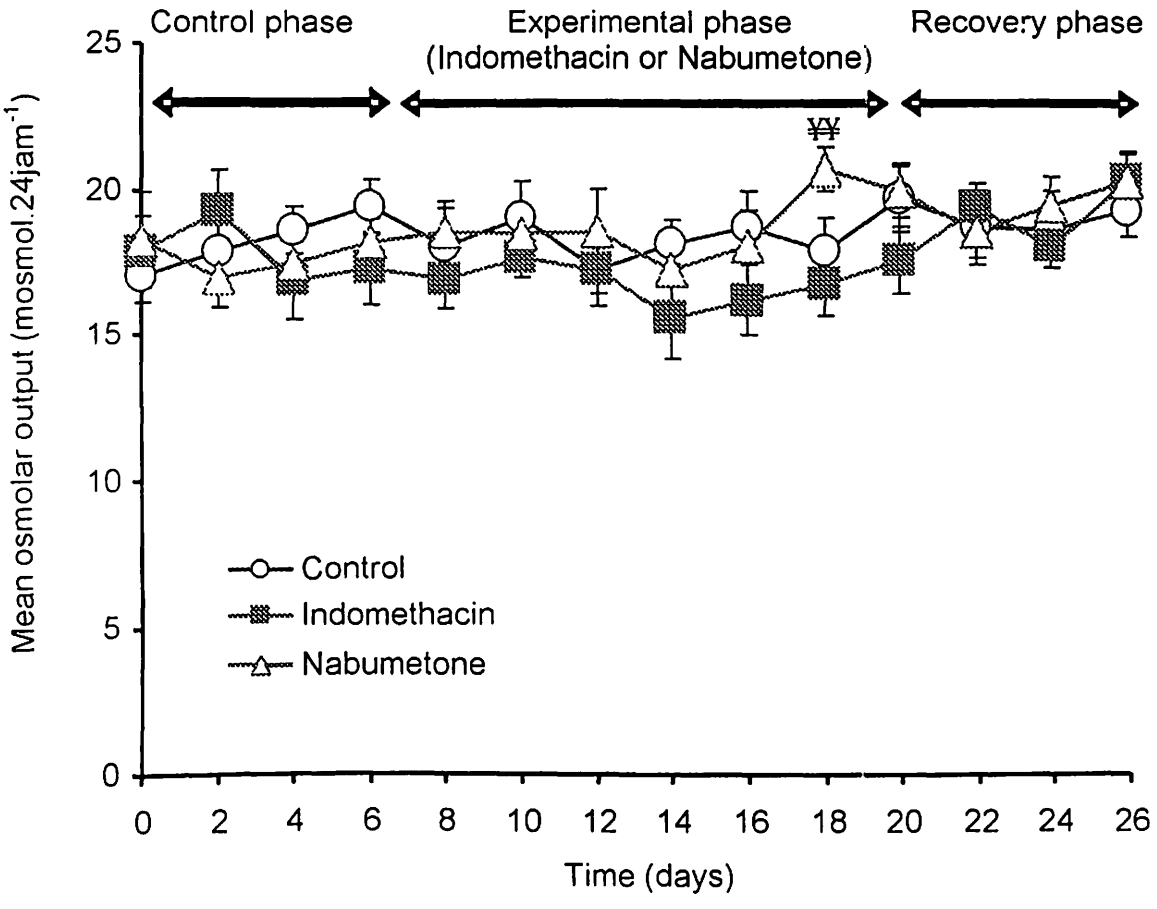

Figure 10: Mean Osmolar output in conscious rats.

There is no significant difference between all groups in all phases in osmolar output.

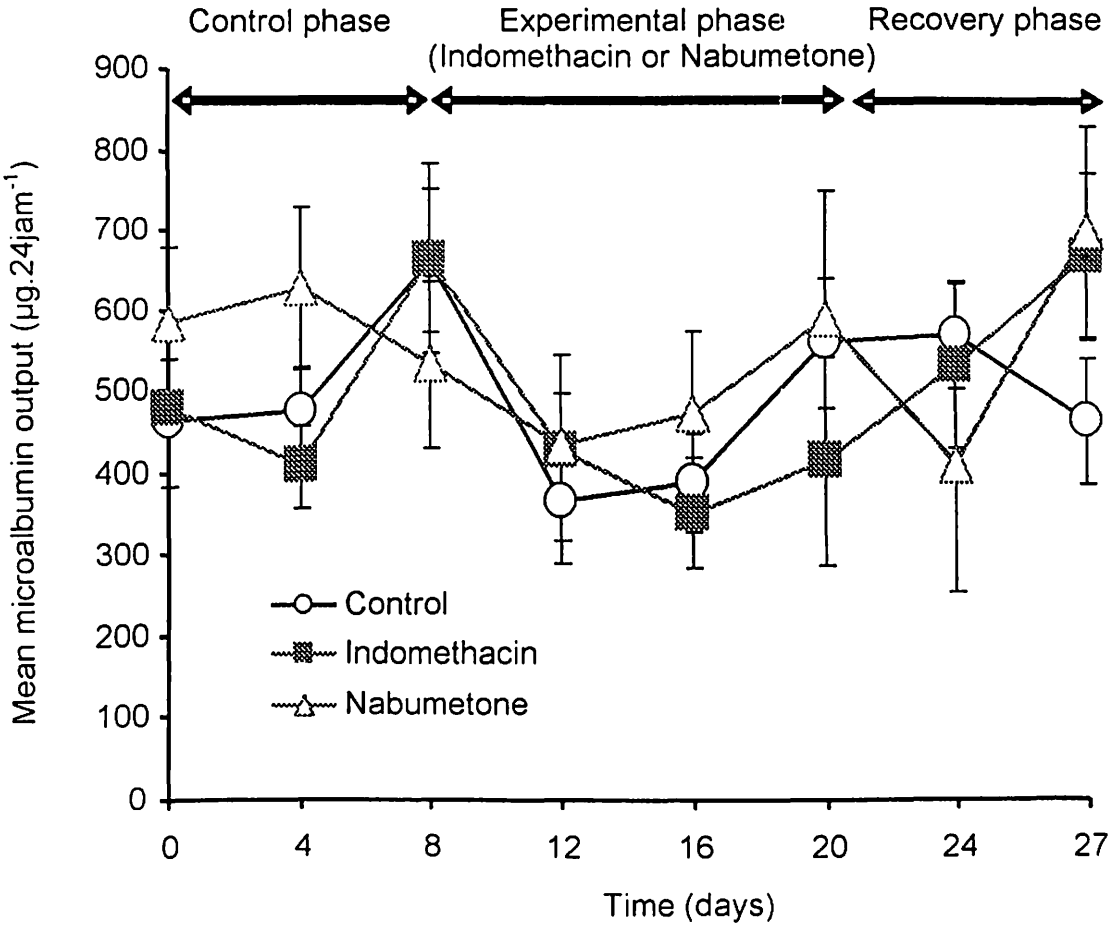

Figure 11: Microalbumin output in conscious rats.

There is no significant difference between all groups in all phases in microalbumin output.

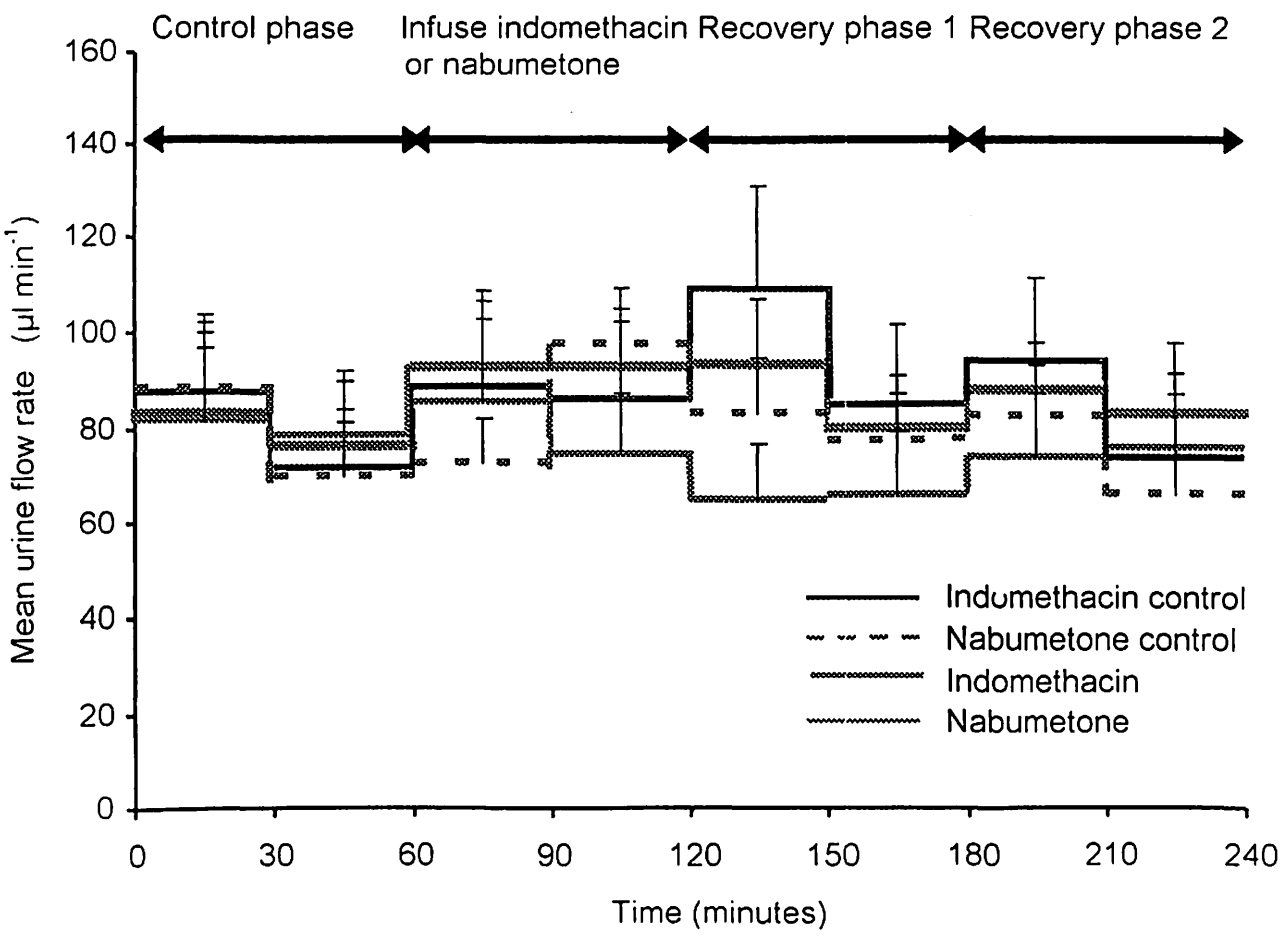

Figure 12: Means urine flow rate in anaesthetized rats.

Within nabumetone group, there is decrease in urine flow rate especially after 1 hour nabumetone was infuse, however it's no significant. However, in overall there is no significant difference between all groups in all phases in urine flow rate.

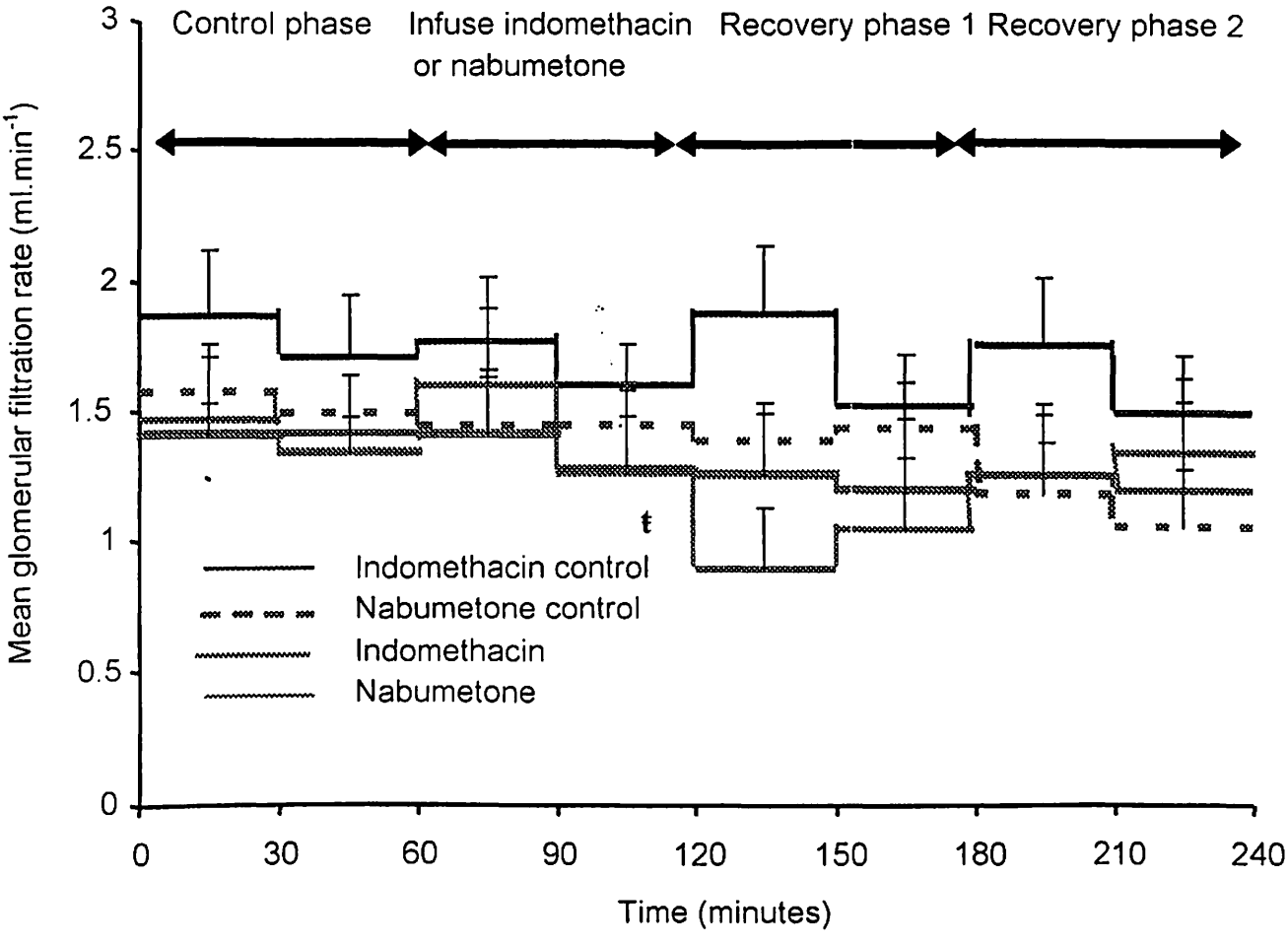

Figure 13: Mean glomerular filtration rate in anaesthetized rats.

There is significant increase glomerular filtration rate within nabumetone group between 90 and 150 minutes (t, $p<0.05$) after 1 hour nabumetone was infused. However no significant differences were observed between all groups in all phases in glomerular filtration rate.

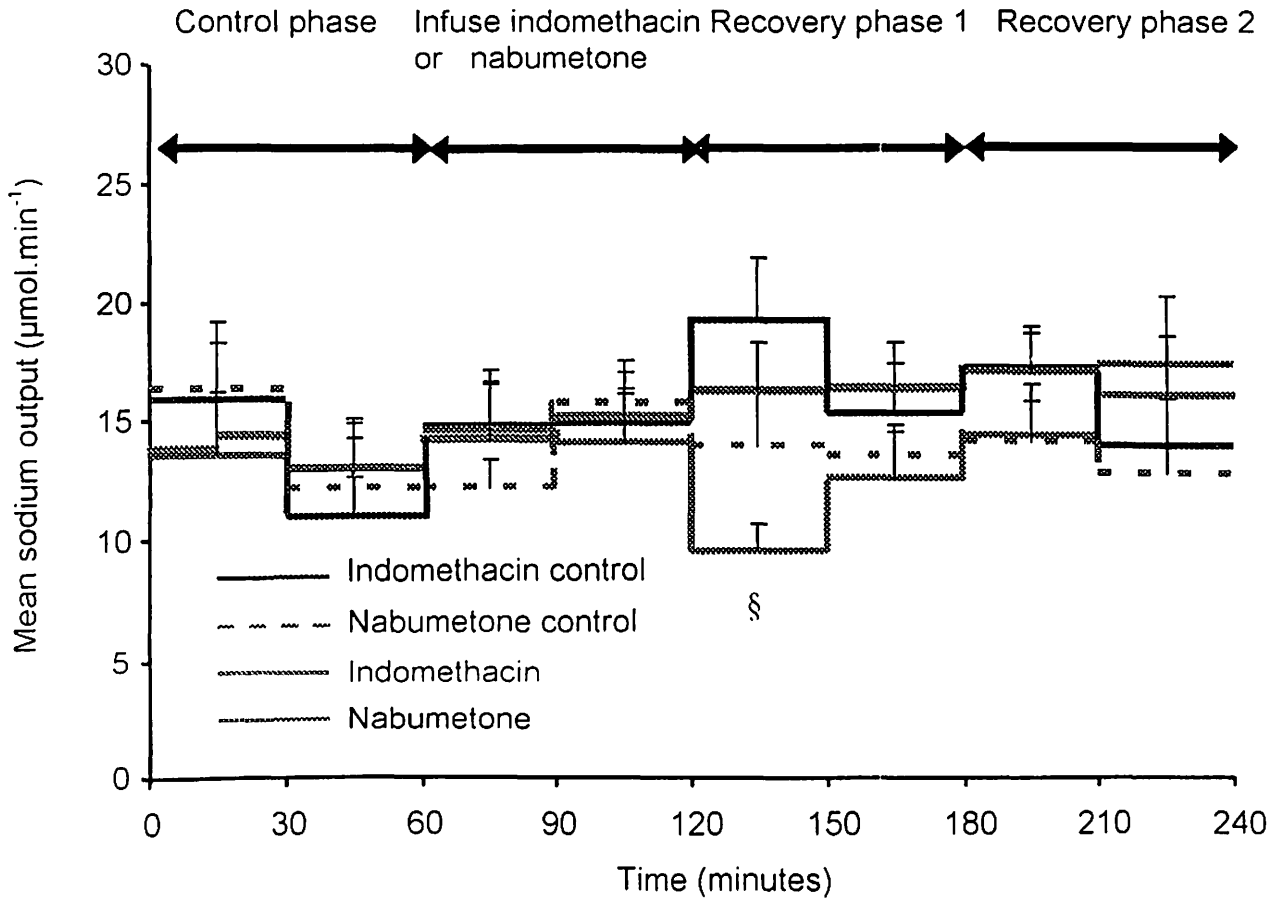

Figure 14: Mean sodium output in anaesthetized rats.

There is significant decrease sodium output in nabumetone group compared to indomethacin group in recovery phase 1 (§, $p < 0.05$).

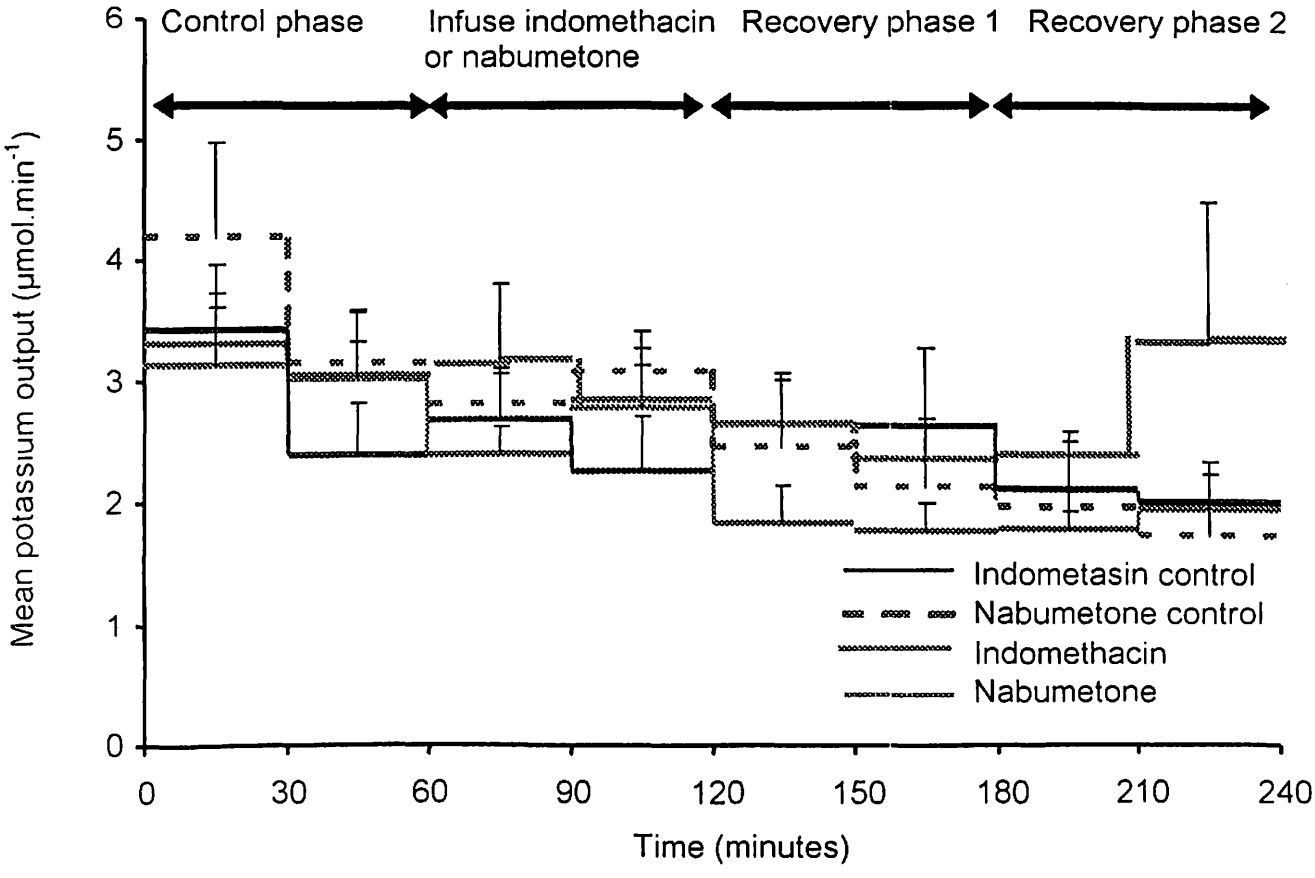

Figure 15: Mean potassium output in anaesthetized rats.

There is no significant difference in potassium output between all groups in all phases. However, potassium decreases significantly within all groups with time.

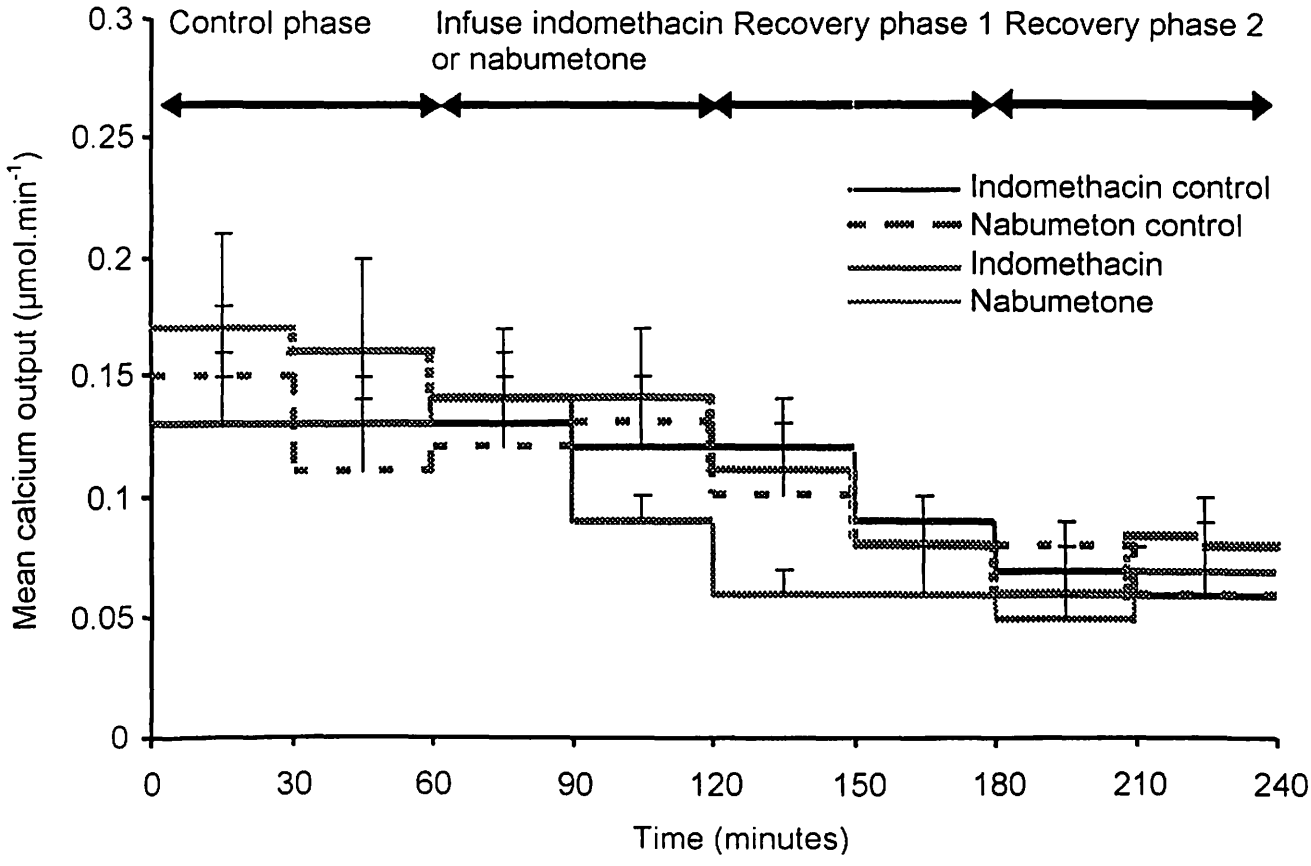

Figure 16: Mean calcium output in anaesthetized rats.

There is no significant difference between all groups in all phases in calcium output. But, within all groups, there is significant decrease calcium output with time.

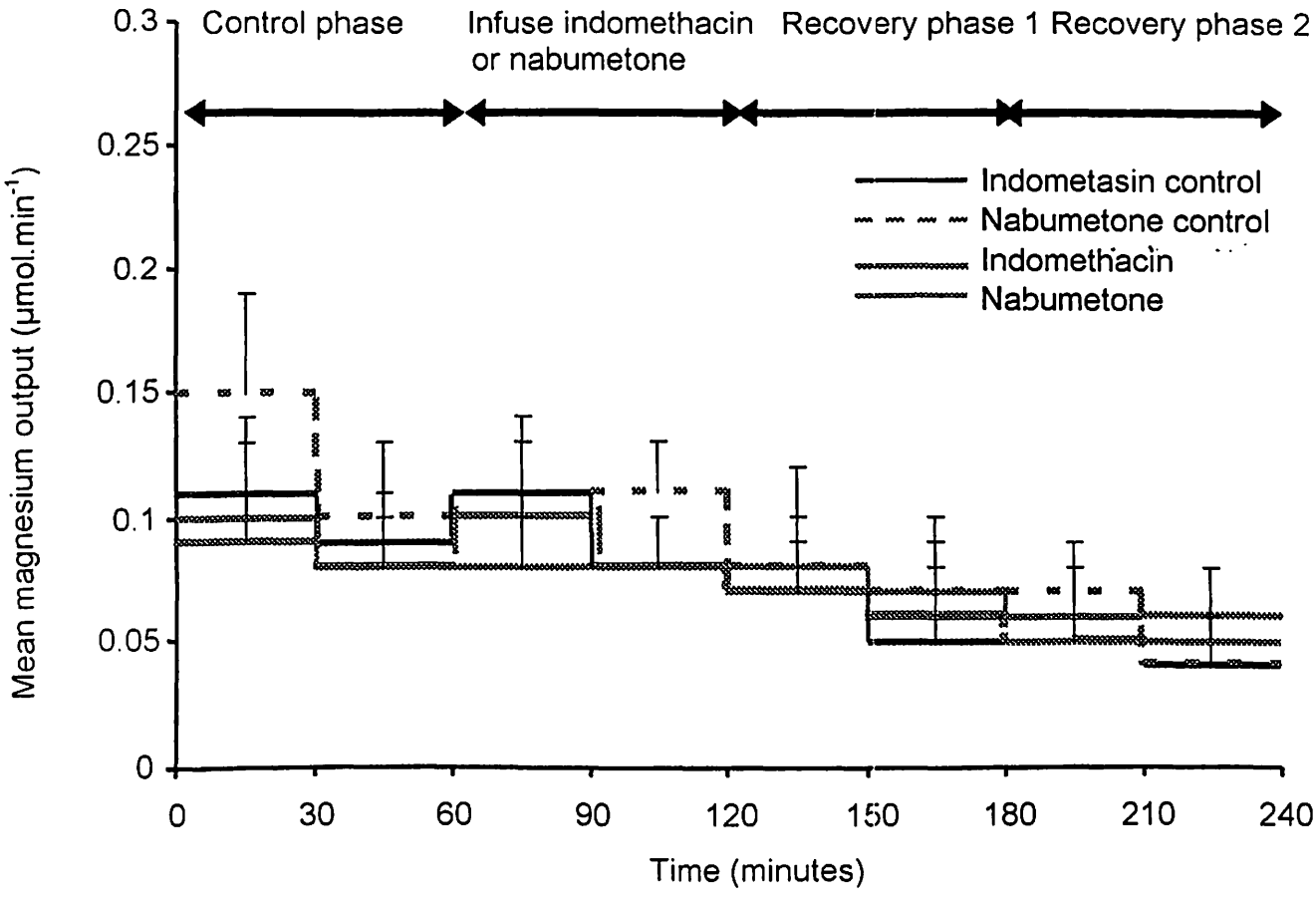

Figure 17: Mean magnesium output in anaesthetized rats.

There is no significant difference between all groups in all phases in magnesium output. But, within all groups, there is significant decrease magnesium output with time.

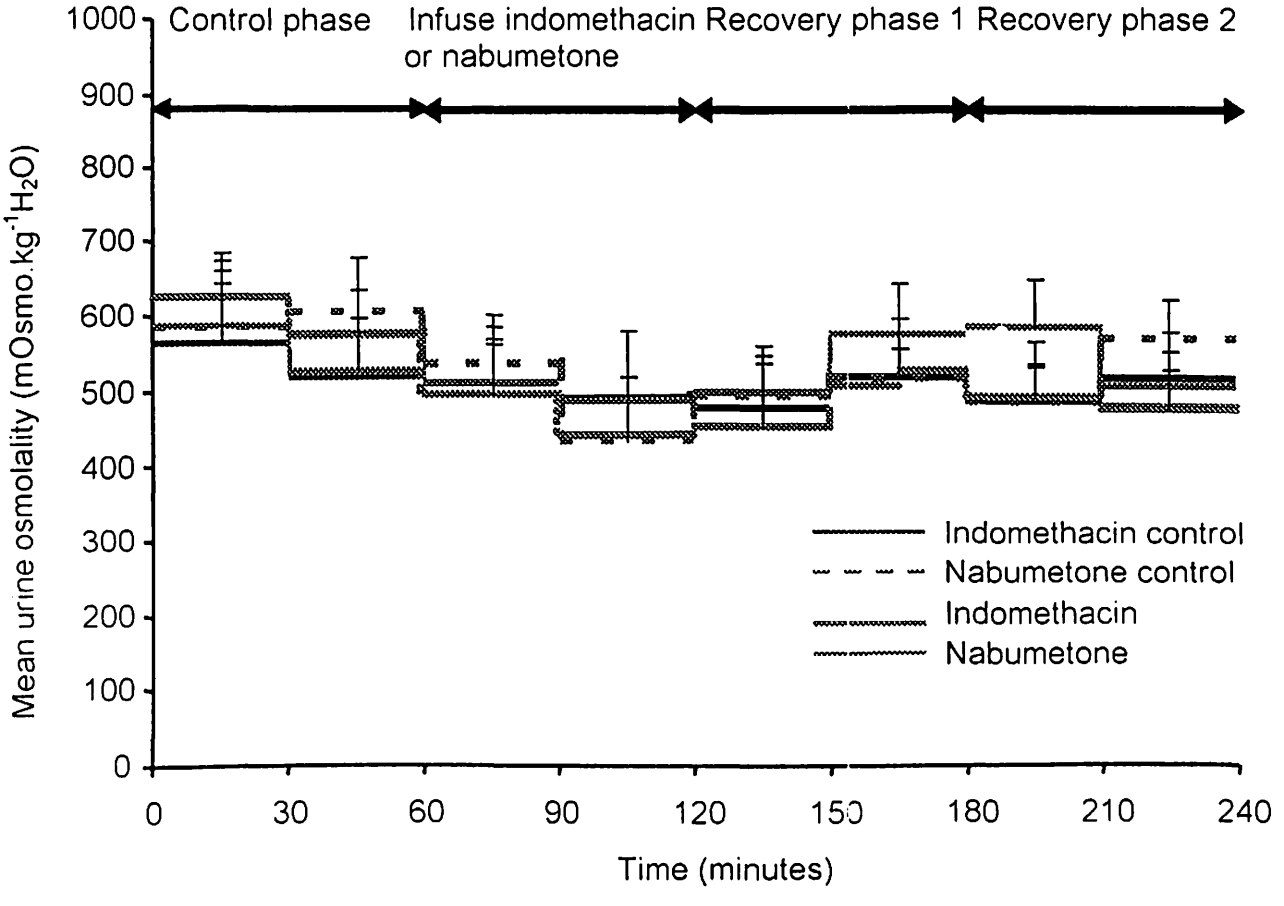

Figure 18: Mean urine osmolality in anaesthetized rats.

There is no significant difference between all groups in all phases in urine osmolality.

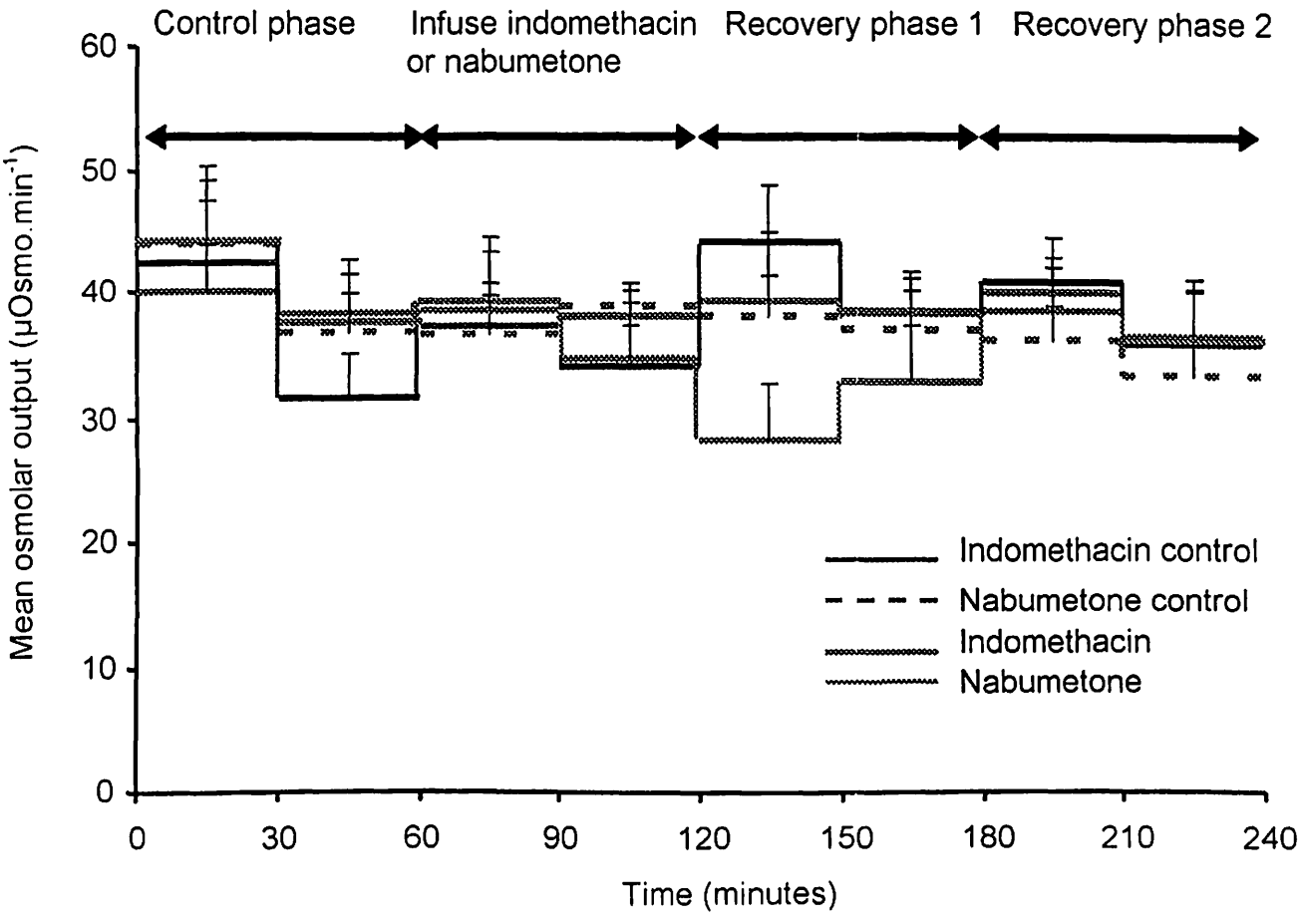

Figure 19: Mean osmolar output in anaesthetized rats.

There is no significant difference between all groups in all phases in osmolar output.

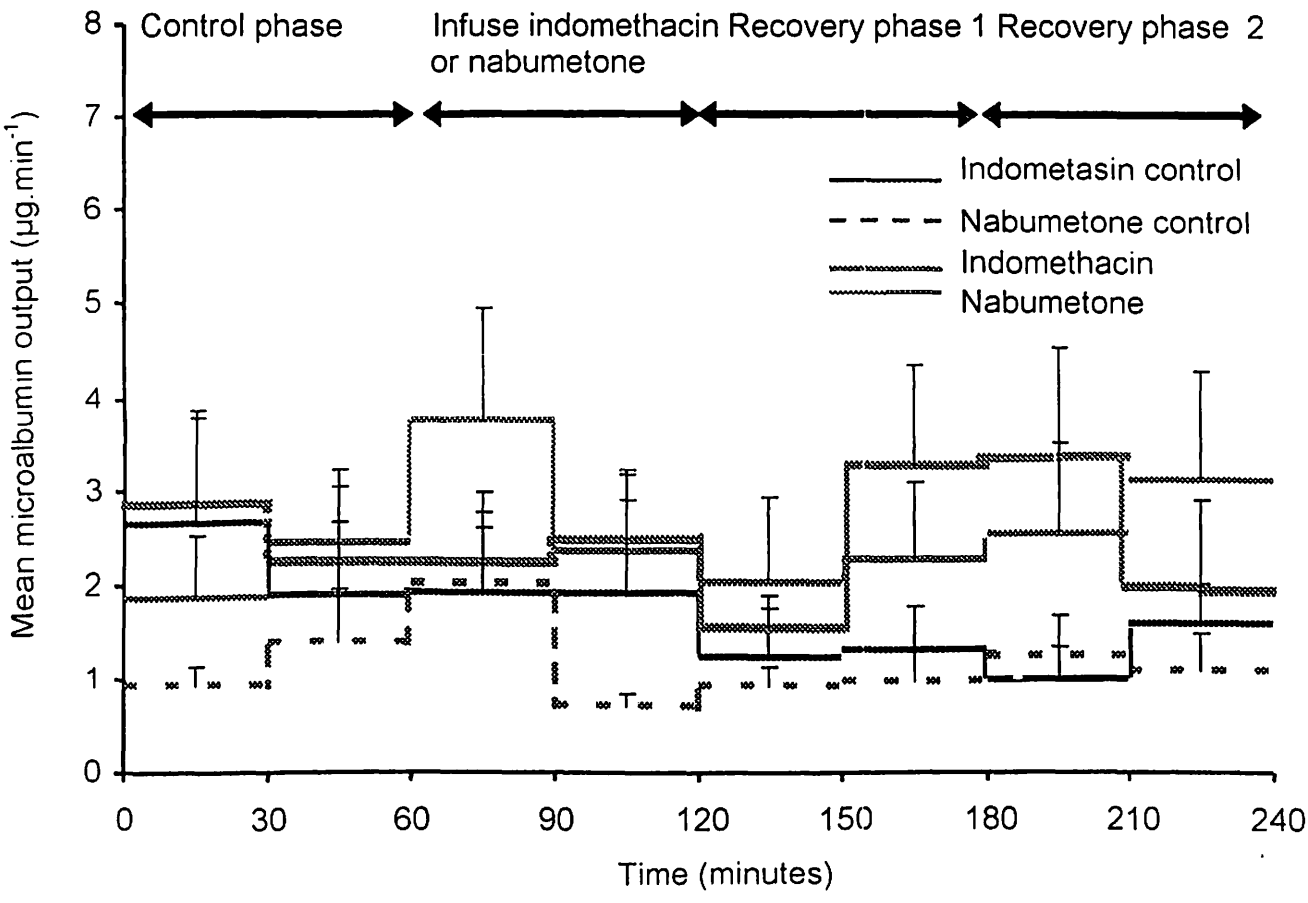

Figure 20: Mean microalbumin output in anaesthetized rats.

There is no significant difference between all groups in all phases in microalbumin output.

DISCUSSION

In experiments using conscious rats, urine output in indomethacin group was significantly lower compared to nabumetone group towards the end of experimental phase ($p<0.01$). Differences appeared starting on day 15 until day 21. In the nabumetone group urine output was elevated during the same period compared to that in the indomethacin group. However, there were no significant differences in both indomethacin and nabumetone groups compared to control group in urine output during the experimental phase. The effect on urine output was reversible and the output was back to normal during recovery phase. There were no significant differences in the other parameters measured between the three groups in all phases.

In the previous experiments conducted in our laboratory using naproxen (a non selective COX inhibitor) urine output was higher compared to control group during experimental phase (17). Thus, given orally in conscious condition some NSAIDs decreased urine output while others increased the urine output. In the present experiments with conscious rats, given orally at doses equivalent to therapeutic doses employed in humans, only urine output was affected by indomethacin (non selective COX inhibitor) and nabumetone (selective COX-2 inhibitor). Further study was needed to elucidate the precise mechanism of the change in urine output.

In anaesthetized rats intravenous administration of indomethacin ($1.5mg.kg^{-1}$ body weight) and nabumetone ($5mg.kg^{-1}$ body weight), over 1 hour period with urine flow rate $100\mu l.minit^{-1}$, there was a decrease in glomerular filtration rate (GFR) ($P<0.05$), sodium excretion ($p<0.05$) in the nabumetone group compared to that in the indomethacin group. Urine flow rate and calcium excretion also decreased in nabumetone group but not significantly compared to other groups. The effects were seen to occur only after 1 hour of infusion but were back to normal during recovery phase.

In the present experiments at a dose equivalent to therapeutic doses employed in humans, Indomethacin has not produced any changes in renal function in anaesthetized rats experiment. Other study where in higher doses were used ($10mg.kg^{-1}$) there was decreased G.F.R., decreased urine flow & calcium excretion(18). In the present experiments nabumetone produced a decrease in GFR and sodium excretion. In a clinical study on patients who were on ACE inhibitor and diuretic for osteo arthritis and hypertension, wherein different NSAIDS were administered orally but separated by control periods , there was no change in renal blood flow or G.F.R but an increased sodium excretion with nabumetone (1000mg bid for one month) (19). The difference in the results might be due to farmakokinetic factors or due to a different chemical group.

Nabumetone was nonacidic NSAID compound and has longer half life of 26 hours compared to indomethacin which is an acetic acid NSAID and has a shorter half life of 4 hours (20).

Between conscious and anaesthetized rats experiments there was no difference in the observed effects with indomethacin. However, with nabumetone there was a higher urine output in conscious rats and a slight but reversible decrease in GFR and sodium excretion in anaesthetized rats. The mechanism was unclear but it might be due to dose, farmacokinetic factors and also chemical group. In conclusion, this study has not shown much differences in effect on renal function between indomethacin and nabumetone in conscious and anaesthetized rats at doses equivalent to therapeutic doses employed in humans.

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