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Naproxen and Cromolyn New Glycogen Synthase Kinase 3β Inhibitors for Diabetes and Obesity: An Investigation by Docking Simulation and Subsequent In Vitro/In Vivo Biochemical Evaluation

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ABSTRACT: Naproxen and cromolyn were investigated as new inhibitors of glycogen synthase kinase-3β (GSK-3β) in an attempt to explain their hypoglycemic properties. Study included simulated docking experiments, in vitro enzyme inhibition assay, and in vivo validations. Both drugs not only were optimally fitted within a GSK-3β binding pocket via several attractive interactions with key amino acids but also exhibited potent in vitro enzymatic inhibitory activities of IC50 1.5 and 2.0 μM for naproxen and cromolyn, respectively. In vivo experiments illustrated that both drugs significantly reduced serum glucose and increased hepatic glycogen- and serum insulin levels in normal and type II diabetic Balb/c mice models. In obese animal model, both drugs exhibited significant reduction in mice weights, serum glucose, and resistin levels along with significant elevation in serum insulin, C-peptide, and adiponectin values. It can be concluded that naproxen and cromolyn are novel GSK-3β inhibitors and can help in management of diabetes and obesity. © 2013 Wiley Periodicals, Inc. J Biochem Mol Toxicol 00:1–12, 2013; View this article online at wileyonlinelibrary.com. DOI 10.1002/jbt.21503

KEYWORDS: **Naproxen; Cromolyn; Glycogen Synthase Kinase-3β; Docking Simulation; Glycogen; Resistin**

INTRODUCTION

Naproxen and cromolyn (Figures 1A and 1B) exhibit unexpected actions additional to some side effects that are related to their metabolic changes in humans. Naproxen, a nonsteroidal anti-inflammatory drug [1], along with cromolyn, a mast cell stabilizer antiallergic agent [2], share unexplained hypoglycemic [3,4] along with some anticancer [5,6] properties.

Glycogen synthase kinase-3β (GSK-3β) is a 47 kDa cytosolic serine/threonine protein kinase. It is considered as a multifunctional regulating and controlling metabolic enzyme. GSK-3β is expressed in several tissues: muscle, liver, adipocytes, and cancer cells [7]. Glycogen synthase (GS) is the most famous substrate from GSK-3β substrate, which is diverse and a predominantly regulatory molecule [7, 8]. GSK-3β-mediated phosphorylation appears to always lead to inhibition of the substrate: Phosphorylation of a substrate by GSK-3β has never been shown to be an activating event [7,8]. As a result, GS inhibition by GSK-3β leads to a decrease in glycogen synthesis in liver and muscles along with an accumulation of blood glucose or hyperglycemia [7–9]. Overexpression and dysregulation of GSK-3β is connected to pathogenesis and progress of many diseases, such as diabetes [9–11] obesity [12], and cancer [13–15]. Consequently, GSK-3β enzyme inhibition helps in the diminishing, monitoring, and treatment of these diseases [16,17].

These interesting properties prompted us to hypothesize that the two drugs inhibit GSK-3β enzyme. The vital metabolic role of GSK-3β inhibition in the reduction of glucose makes it an exciting target for controlling hyperglycemia [18,19]. GSK-3β inhibitors have particularly useful antidiabetic properties as they improve insulin sensitivity, raise glycogen synthesis, and glucose metabolism in skeletal muscles of diabetic patients [19]. Furthermore, GSK-3β activity has been

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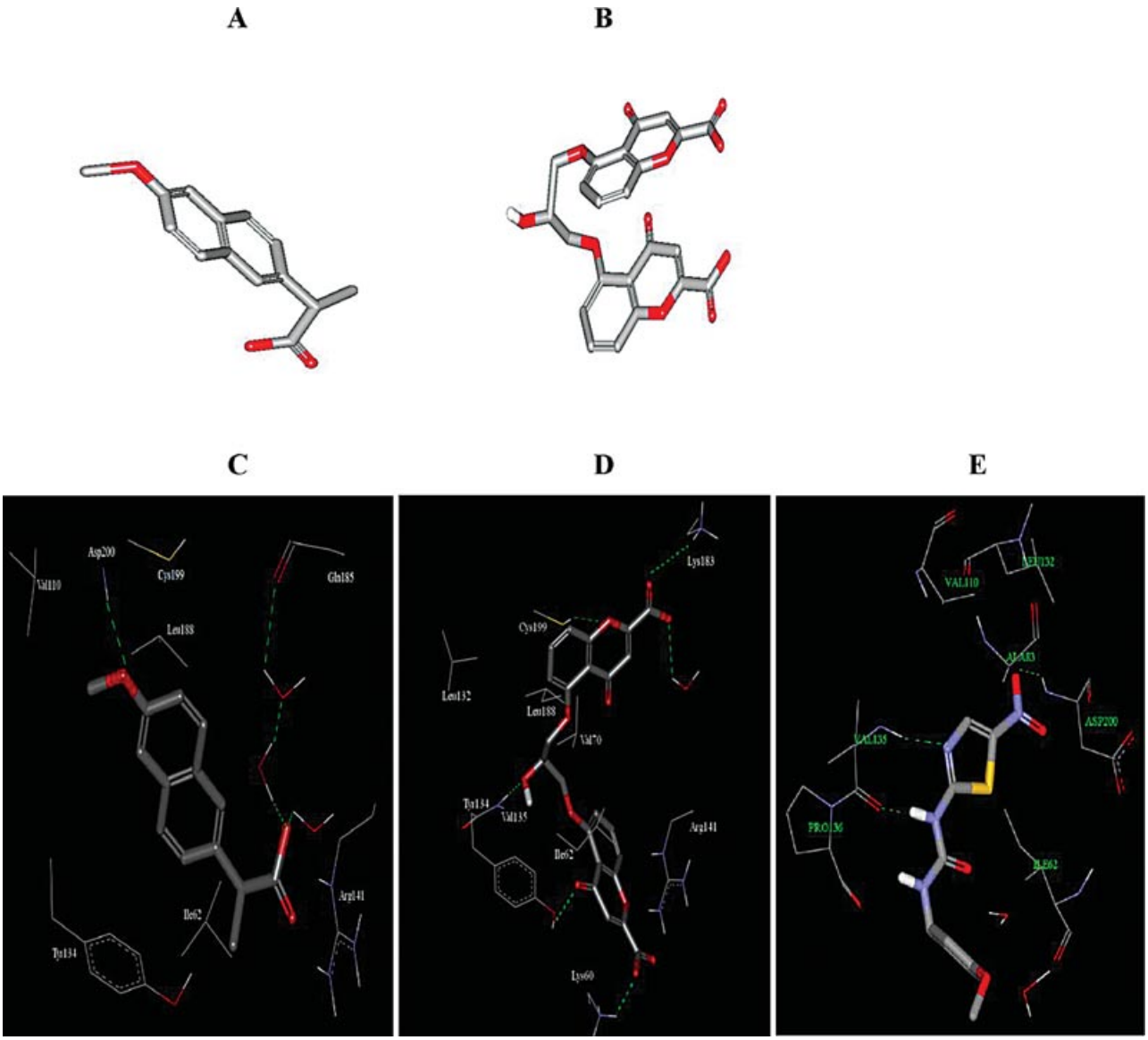

FIGURE 1. Molecular structures of both (A) naproxen and (B) cromolyn; detailed view of the docked (C) naproxen and (D) cromolyn. (E) The cocrystallized structure (AR-A014418) within the binding site of GSK-3β (PDB code: 1Q5K).

connected to the adipogenesis process that develops obesity [12]. Moreover, GSK-3β is overexpressed in several types of cancers, for example, colorectal [13], ovarian [14], and prostate [15] cancer. Therefore, inhibitors of GSK-3β are expected to combine hypoglycemic and anticancer properties.

We previously reported the discovery of new GSK-3β inhibitors via pharmacophore modeling, QSAR analysis [20], and docking simulation [21,22].

To validate our anti-GSK-3β hypothesis of naproxen and cromolyn, we aim to perform docking of the two structures against GSK-3β binding pocket and compare their fitting with known GSK-3β inhibitor AR-A014418. Moreover, we will examine in vitro against GSK-3β and hypoglycemic effects of both drugs by measuring the serum glucose, serum insulin, and hepatic glycogen levels for normal and diabetic fasting animal's models. In addition, different obesity parameters, such as weight variation, serum glucose, serum insulin, serum C-peptide, serum adiponectin, and serum resistin, will also be measured.

MATERIALS AND METHODS

Molecular Modeling

Software and hardware: The following software packages were utilized in this research:

- CS ChemDraw Ultra (Version 11.0), Cambridge Soft (http://www.cambridgesoft.com)
- DiscoveryStudio (DS 2.5), Accelrys (www.accelrys .com) USA.

Docking experiment: The three-dimensional coordinates of the GSK-3β enzyme were collected from the Protein Data Bank (PDB code: 1Q5K, resolution = 1.94 Å) [23]. DS templates for protein residues were used to add hydrogen atoms to protein target.

Naproxen and cromolyn chemical structures were sketched in ChemDraw Ultra, and saved in MDL mol file format and imported into DS2.5. The conformational space of each compound was explored implementing the best CATALYST conformation generation option implemented in the Libdock docking engine within the DS2.5 suite of software. The two drugs (naproxen and cromolyn) were docked, after removing their hydrogen atoms, into the binding site of GSK-3β guided by binding hotspots. LibDock aligns docked structures conformations to polar and apolar receptor interactions sites, that is, hotspots. In the current docking experiments, we employed the following default parameters:

- Maximum number of receptor hotspots = 100.
- Ligand-to-hotspots matching rmsd tolerance value was set to 0.5 Å.
- Maximum number of saved poses for each ligand = 100.
- Minimum LibDock score (poses below this score are not reported) = 50.
- Maximum number of rigid body minimization steps during final pose optimization phase (using BFGS method) = 50.
- Maximum number of evaluated poses for each conformation = 30.
- Maximum number of steric clashes allowed before the pose-hotspot alignment is terminated (specified as a fraction of the heavy atom count) = 0.5.
- Cluster similarity cutoff value = 0.5 Å (docked poses are rigid-body minimized and clustered using this cutoff value).
- Maximum value for nonpolar solvent accessible surface area for a particular pose to be reported as successful = 15.0 $Å^2$.
- Maximum value for polar solvent accessible solvent area for a particular pose to be reported as successful = 5.0 $Å^2$.
- Number of grid points used for calculating solvent accessible surface area = 18.

High-ranking docked conformers and poses were scored using consensus of the following scoring functions: Jain, LigScore1, LigScore2 (both calculate based on Drieding force field), PLP1, PLP2, PMF, and PMF04 [24].

In Vitro GSK-3β Enzyme Inhibitory Assay and Determination of Naproxen and Cromolyn Inhibitory IC_{50} Values

A concentration of 10 pg/mL GSK-3β (Upstate Biotechnology) was prepared in a buffer solution (pH Q3 7.2) containing the following: 40 mM HEPES; 5 mM Q4 $MgCl_2$; 5 mM EDTA (Biosource); 100 μM ATP (Sigma), and 50 μg/mL heparin (Sandoz, Austria). After that, 50 μL aliquots of the enzymatic solution were transferred into 0.5 mL vials. Subsequently, appropriate volumes of naproxen and cromolyn stock solutions were added to the mixture solution to yield final concentration of 0.01, 0.1, 1.0, and 10 μM, and the buffer was added to reach 75 μL. Both drugs were incubated with the enzyme over 30 min at room temperature, then aliquots of Tau solution (25 μL of 2000 pg/mL in HEPES) were added to reach a final concentration of 500 pg/mL. This mixture was incubated over 1 h at room temperature. Tau phosphorylation was detected by Tau μpS396] phosphoELISA kit (Biosource) Q5 as described by Taha et al. [20–22]. Samples and blanks were prepared in triplicate. The in vitro inhibitory actions of naproxen and cromolyn escalating concentrations were experimentally validated against recombinant GSK-3β and expressed as IC_{50}, the concentration that inhibits the enzymatic activity by 50% when compared to the uninhibited reactions. A standard GSK-3β inhibitor (TDZD-8), the non-ATP competitive inhibitor with IC_{50} 1.0 μM was applied as positive control in this experiment [25].

In Vivo Biochemical Evaluation

Preparation of Drugs Doses

Naproxen and cromolyn effects against diabetes were investigated by measuring the serum glucose, serum insulin, and hepatic glycogen levels for normal and chronic diabetic fasting Balb/c mice models. Along with obesity parameters, weight variation, glucose, insulin, C-peptide, adiponectin, and resistin were measured after chronic administration for the drugs. Each drug was dissolved in phosphate buffer saline (PBS) (pH 7.2) and prepared in three escalating doses: (1) human dose of naproxen 3.57 mg/kg whereas cromolyn dose 1.43 mg/kg, (2) double human and (3) four times human doses. For each drug, all doses were intraperitoneally (ip) injected daily as ∼0.20 mL solutions to three groups and equivalent to mice weight (∼20 g).

The fourth control negative group with the same conditions received PBS only.

Diabetes Investigations

Normal Model

The animal experiments conform with the Guide for the Care and Use of the Faculty of Pharmacy, Cairo University Animal Ethics committee. Young male Balb/c mice were randomized and fed ad libitum with standard feed and water except when fasting was needed in the study and housed in the same conditions and separated randomly.

Animals were allocated into seven groups (10 mice/group). The first group was kept without treatment. The other six groups were given either naproxen or cromolyn in three doses.

Determination of serum glucose: On the day of the experiment, food and water were removed 6 h before the injection. Plasma glucose was monitored every hour over 3 h's period. Serial tail-snip bleeds from each mouse were performed to collect blood samples (10 μL) for glucose determination [18]. Blood glucose was determined employing the glucose oxidase colorimetric method by a glucose kit (Diamond, Germany).

Determination of liver glycogen: The animals were scarified by cervical dislocation after taking the last blood sample above, and their livers were removed immediately for glycogen determination. The measurement of liver glycogen was done quantitatively by the anthrone reagent (Sigma) colorimetric method [26].

Chronic Type II Diabetic Model

Beginning on day zero, animals were divided into two major groups. One group (eight mice) was fed on standard chow pellet (6.5% kcal fat), received only the buffer solution and was kept as the normal control group. The other animals were fed high-fat diet (HFD) containing rodent diet and butter (HFD: 58% kcal fat) for a period of 2 weeks. On day 14, mice on the HFD were injected with a single low dose of streptozotocin (STZ), 45 mg/kg ip, in 0.01 M citrate buffer pH 4.3 equivalent to mice dose to induce type II diabetes mellitus. Both the low dose of STZ (Sigma) and the HFD are essential elements to induce type II diabetes with insulin resistance [27]. Subsequently, all mice had free access to food and water and were continued on their respective diets till the end of the study. The induced type II diabetic HFD animals were divided into seven subgroups (eight mice/group). Three escalating doses of either naproxen (groups 1–3) or cromolyn (groups 4–6) were given to mice, respectively. The last one is considered a diabetic-diseased control. Chronic-drugs administration was performed during the whole 1 month. At the end of the month, the fasting 6 h earlier animals were sacrificed by cervical dislocation after taking one time the blood sample for glucose and insulin measurement. The livers were removed immediately for glycogen determination. Both glucose and glycogen detection procedures were performed as described above. Biochemical samples and measurements were collected and performed at the initial drugs injection administration time and the final time for all experiments. Q6 Q7

Determination of serum insulin: An insulin ELISA kit (DRG International) was used for the determination of insulin levels. A sample (25 μL) blood serum was added to each well with the same volume of enzyme conjugate and was followed by an incubation period of 30 min at room temperature. After washing, 50 μL enzyme complex was pipetted and another incubation was done for a period of 30 min. Thereafter, the wells was washed and incubated for 15 min after the addition of 50 μL aliquots substrate solution. Finally, the reaction was stopped by 50 μL stop solution and the absorbance was measured at 450 nm plate reader [28] (Dynatech Labrotories MRX 1.31, UK).

Obesity Investigations

Chronic Obesity Model

The investigation was applied in the same conditions for the chronic diabetic model with these modifications: sucrose sugar addition to their HFD diets all over the month, removing STZ injections, the drugs intake from the first day all over the month, and blood sampling that was performed before and after the month of the study to measure serum glucose, insulin, C-peptide, adiponectin, and resistin. Measurement of weight variation, the scale of obesity, was also done before and after the month. Glucose- and insulin-detection procedure was performed as mentioned above.

Determination of serum C-peptide: Along with insulin, C-peptide is the second branch of the proinsulin protein and therefore, a C-peptide ELISA kit (Monobind) was used for its level determination. A blood serum sample (50 μL) was added to each well with 100 μL volume of enzyme antibody and incubated at room temperature for 120 min. After washing, 100 μL substrate solution was pipetted and the mixture was incubated for 15 min. At the end, the reaction was stopped by 50 μL stop solution and the absorbance was measured at 450 nm plate reader [28].

Determination of serum adiponectin: Since the reduction of adiponectin is considered the mirror of obesity, AssayMax adiponectin ELISA (Assaypro) was

performed to measure its values. A blood serum sample (50 μL) was added to each well and incubated at room temperature for 1 h. After washing, 50 μL of biotinylated adiponectin antibody was pipetted and the wells were incubated for another 1 h. After that, the plate was washed and streptavidin peroxidase conjugate was added. After incubating for 30 min, chromogen substrate (50 μL) was added until the optimal blue color was developed which nearly took 10 min. Finally, the stop solution regent (50 μL) blocked the reaction and optical density was read at 450 nm plate reader.

Determination of serum resistin: The elevation of resistin is considered a good reflection of insulin resistance and obesity; thus, its concentration detection was carried out by the AssayMax resistin ELISA kit (Assaypro). A sample (50 μL) of blood serum was pipetted to each well and incubated at room temperature for 2 h. After washing, 50 μL of biotinylated resistin antibody was incubated for another 2 h. Subsequently, the plate was washed and streptavidin peroxidase conjugate was added. After an incubation period of 30 min, chromogen substrate (50 μL) was added until the optimal blue color was developed, which nearly took 10 min. At the end, the stop solution regent (50 μL) stopped the reaction and the absorbance was read at 450 nm plate reader.

Data are presented as means ± SD. Statistical comparisons were performed using Student's t-test. In all cases, $p < 0.05$ is considered statistically significant.

RESULTS

Naproxen and Cromolyn Were Successfully Docked into a Binding Site of GSK-3β

Docking simulations done using LibDock [24] showed that both naproxen and cromolyn optimally fitted into GSK-3β (PDB code: 1Q5K) binding pocket. The binding pocket was defined based on the cocrystallized ligand in 1Q5K. Docked poses were selected based on consensus among seven scoring functions (PLP1, PLP2, LIGSOCRE1, LIGSCORE2, PMF, GAIN, and DOCKSCORE). The docked poses exhibited several interesting interactions.

By comparing the docked poses of naproxen (Figure 1C) and cromolyn (Figure 1D) with the cocrystallized structure of the known inhibitor (AR-A014418) within the GSK-3β binding pocket (Figure 1E), one can see certain similarities in their binding interaction profiles. The negative charges of the carboxylic acid groups in both drugs interact electrostatically with the positively charged guanidine group of Arg141. In addition, the carboxylic acid moieties of cromolyn are involved with the ammonium groups of Lys183 and Lys60 via electrostatically reinforced hydrogen-bonding interactions. Moreover, the naphthalene ring system of naproxen seems to π-stack against the phenolic ring of Tyr134. A similar interaction is seen with one of cromolyn's aromatic rings, whereas the other ring is inserted within a hydrophobic pocket comprising of the hydrophobic side chains of Cys199, Leu132, Leu188, and Val70. The carboxylic acid moiety of naproxen is hydrogen-bonded to the amidic carbonyl of Gln185 via a network of hydrogen-bonded water molecules. On the other hand, the phenolic methoxy of naproxen is hydrogen-bonded to the peptidic NH of Asp200. Similarly, cromolyn is tightly held via four hydrogen bonds with Tyr134, Lys60, and lys183.

Many parallel interactions can be seen holding the cocrystallized AR-A014418 within the GSK-3β binding pocket. The peptidic NH of Asp200 is hydrogen-bonded to the nitro oxygen atom of AR-A014418 in a similar pattern as the hydrogen-bonding interaction connecting the methoxy of naproxen. Similarly, the thiazole nitrogen AR-A014418 hydrogen bonded to the peptidic NH of Val135 in a comparable fashion to the hydrogen-bonding interaction connecting the central hydroxyl of cromolyn to same petidic NH in the binding pocket. Finally, AR-A014418 shares a hydrophobic interaction with both drugs, that is, the hydrophobic interaction of Ile62 with the thiazole sulfur of AR-A014418 compares with hydrophobic interactions connecting the same side chain with one of the chromones of cromolyn (Figure 1D) and the naphthalene ring system of naproxen. These results encouraged us to experimentally assess the inhibitory profiles of both drugs in vitro.

Naproxen and Cromolyn Inhibit GSK-3β In Vitro

Subsequent experimental in vitro testing showed that naproxen and cromolyn inhibited GSK-3β with IC_{50} values of 1.5 and 2.0 μM, respectively. We validated our bioassay conditions by testing the IC_{50} of the standard GSK-3β inhibitor, TDZD-8, which we found to be around its reported value [25]. These interesting results combined with the fact that GSK-3β plays a pivotal role in glucose and glycogen metabolism, prompted us to proceed in our study by evaluating the in vivo biochemical actions of the two drugs against diabetes and obesity.

Antidiabetic Properties of Naproxen and Cromolyn

The antidiabetic properties of the two drugs were evaluated against two Balb/C mice models: fasting

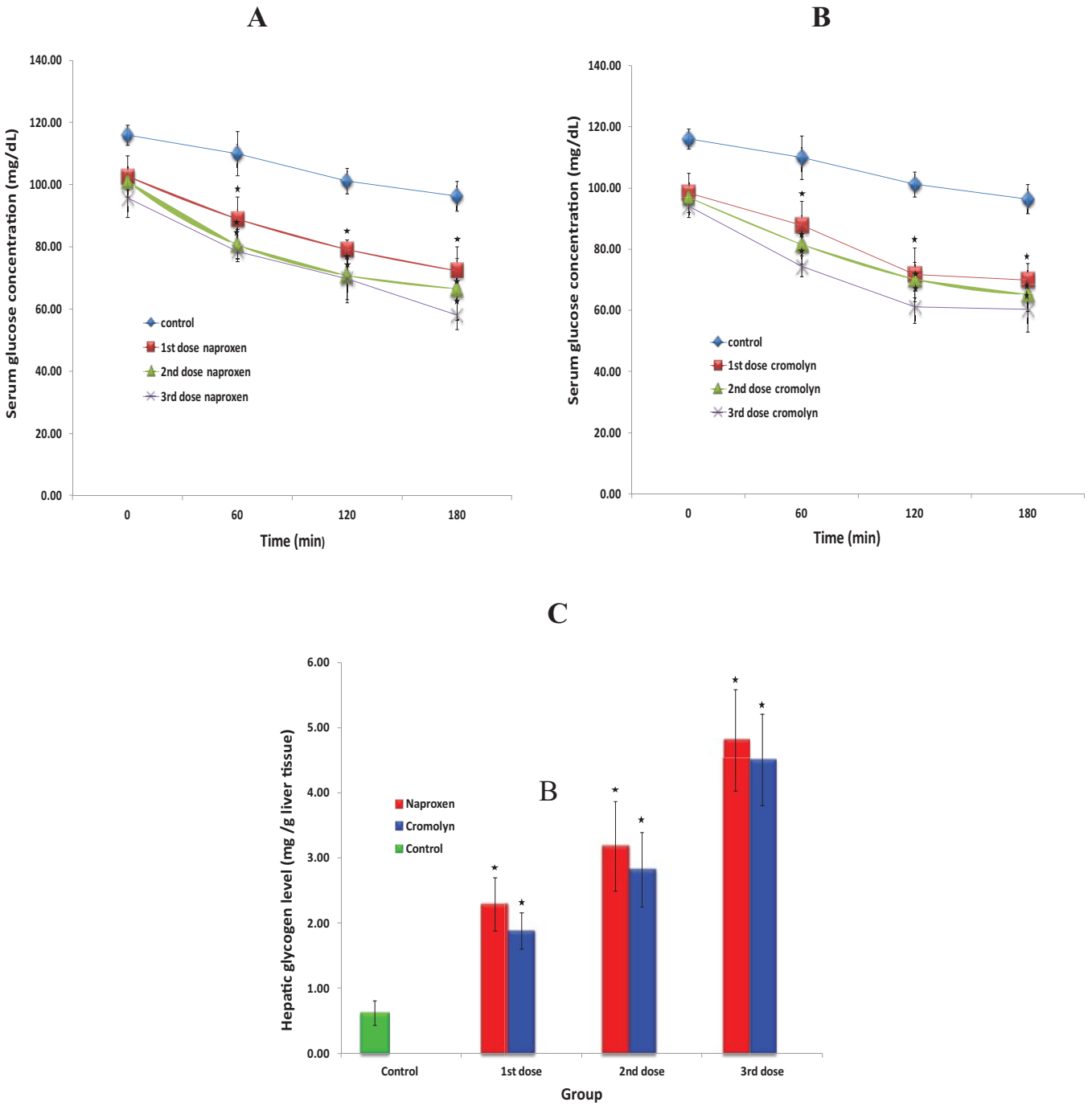

FIGURE 2. (A) The serum glucose levels in response to PBS buffer vehicle control, (1) 3.57, (2) 7.14, and (3) 14.28 mg/Kg naproxen-normalized human escalating doses, (B) same vehicle control, (1) 1.43, (2) 2.86, and (3) 5.72 mg/Kg cromolyn-normalized human escalating doses and (C) liver glycogen reserves after 3 h drugs-doses administration for groups ($n = 10$) and mice weights (~20g). Data are expressed as mean values ± SD. (⋆) p value < 0.05 versus control group.

normal animals and chronic diabetic type II models. In normal animal models, both drugs illustrated dose-dependent reduction in blood glucose levels, as shown in Figure 2. The highest naproxen dose reduced plasma glucose levels by about 39% compared to the control, whereas cromolyn achieved a maximum reduction of 36% compared to the control.

Figure 2 also shows the effects of escalating naproxen and cromolyn doses on liver glycogen levels. Clearly from the figure, both drugs caused clear and significant elevation in glycogen levels in a

dose-dependent manner, such that they achieved a maximum increase of liver glycogen by eight- and sevenfolds, respectively, compared to the control.

On the other hand, both drugs showed very interesting trends upon testing against the chronic type II diabetic model. Glucose and insulin levels were measured before and after drug administration. Glucose levels were reduced in a dose-dependent manner in response to normalized naproxen and cromolyn escalating doses (Figure 3A). The highest naproxen dose reduced the plasma glucose concentration by about 70% compared to a diabetic state before treatment and 72% compared to the untreated diabetic control group. Similarly, cromolyn reduced plasma glucose levels by a maximum of ca. 68% compared to the diabetic state before drug administration and 70% compared to untreated diabetic controls.

In contrast, both drugs elevated insulin levels in dose-dependent modes, as shown in Figure 3B. Three hours after administration, the third naproxen dose raised the insulin level to ca. 130% compared to the diabetic state before treatment and 195% compared to the untreated diabetic model control. Similarly, cromolyn third dose raised insulin to 122% compared to the diabetic state before treatment and 177% compared to the untreated diabetic control. Moreover, the liver glycogen level increased in response to naproxen and cromolyn dosing in a dose-dependent manner (Figure 3C): Both drugs raised liver glycogen levels for maximum of 17 and 12-folds, respectively, as compared to untreated diabetic controls.

In Vivo Antiobesity Effects of Naproxen and Cromolyn

Interestingly, both drugs illustrated significant antiobesity effects as evident from their effect on three obesity parameters namely body weight, resistin, and glucose levels. The three parameters were reduced in a dose-dependent manner in response to treatment with escalated naproxen and cromolyn doses. At the maximum tested dose, naproxen reduced the animals' body weights by about 28% compared to untreated normal animals and by 45% compared to the untreated obese control group. Similarly, the highest tested cromolyn dose decreased animals' body weights by about 26% compared to untreated normal animals and 41% compared to the untreated obese control groups, as shown in Figure 4A.

The fact that obesity is linked to insulin resistance prompted us to measure resistin levels in animals treated with both drugs. Figure 4B shows resistin levels in different animals upon treatment. Clearly from the figure, the highest tested naproxen dose reduced resistin levels by about 54% compared to untreated normal animals and by about 65% compared to the untreated obese control groups. Similarly, resistin levels were reduced by *ca.* 50% and 61% upon treatment with cromolyn (third dose) compared to untreated normal and obese-control groups, respectively.

Similarly, plasma glucose concentrations were reduced upon treatment with both drugs, as shown in Figure 4C. Administration of the highest naproxen dose reduced glucose levels by 42% compared to levels in untreated normal animals, and by 63% compared to levels in the untreated obese control groups. Comparably, cromolyn's third dose reduced glucose levels by about 35% compared to untreated normal animals and by 58% compared to untreated obese-control groups.

On the other hand, escalated naproxen and cromolyn dosing elevated the adiponectin, insulin, and C-peptide levels in a dose-dependent manner. As shown in Figure 5A, the maximum dose of naproxen elevated adiponectin levels by 51% compared to healthy untreated animals and by 90% compared to untreated obese control groups. In addition, cromolyn's third dose increased adiponectin by about 43% in healthy untreated animals and by 81% in untreated obese control groups.

Moreover, the highest naproxen dose increased insulin levels by ca. 41% in healthy untreated models and by 85% in untreated obese control groups, as seen in Figure 5B. A similar trend is seen with cromolyn highest dose, which increased insulin levels by ca. 35% in healthy untreated animals and by 80% in obese models (Figure 5B). Comparably, C-peptide levels increased significantly after treatment with naproxen, as shown in Figure 5C. C-peptide levels increased by about 57% compared to normal untreated normal control groups and by 92% compared to untreated obese control groups. Likely, cromolyn injection increased C-peptide levels by about 46% compared to untreated normal animals and by 86% compared to untreated obese control groups.

All above results for diabetes and obesity were considered statistically significant with $p < 0.05$.

DISCUSSION

In previous publications, we implemented docking simulation and pharmacophore screening for the discovery of several GSK-3β [20–22]. The fact that naproxen and cromolyn share hypoglycemic and some anticancer properties prompted us to suspect that they inhibit GSK-3β. Accordingly, we docked the two compounds into the GSK-3β catalytic site; we then tested in vitro their anti-GSK-3β bioactivities and validated our results by in vivo evaluation of the two drugs against animal models of diabetes and obesity.

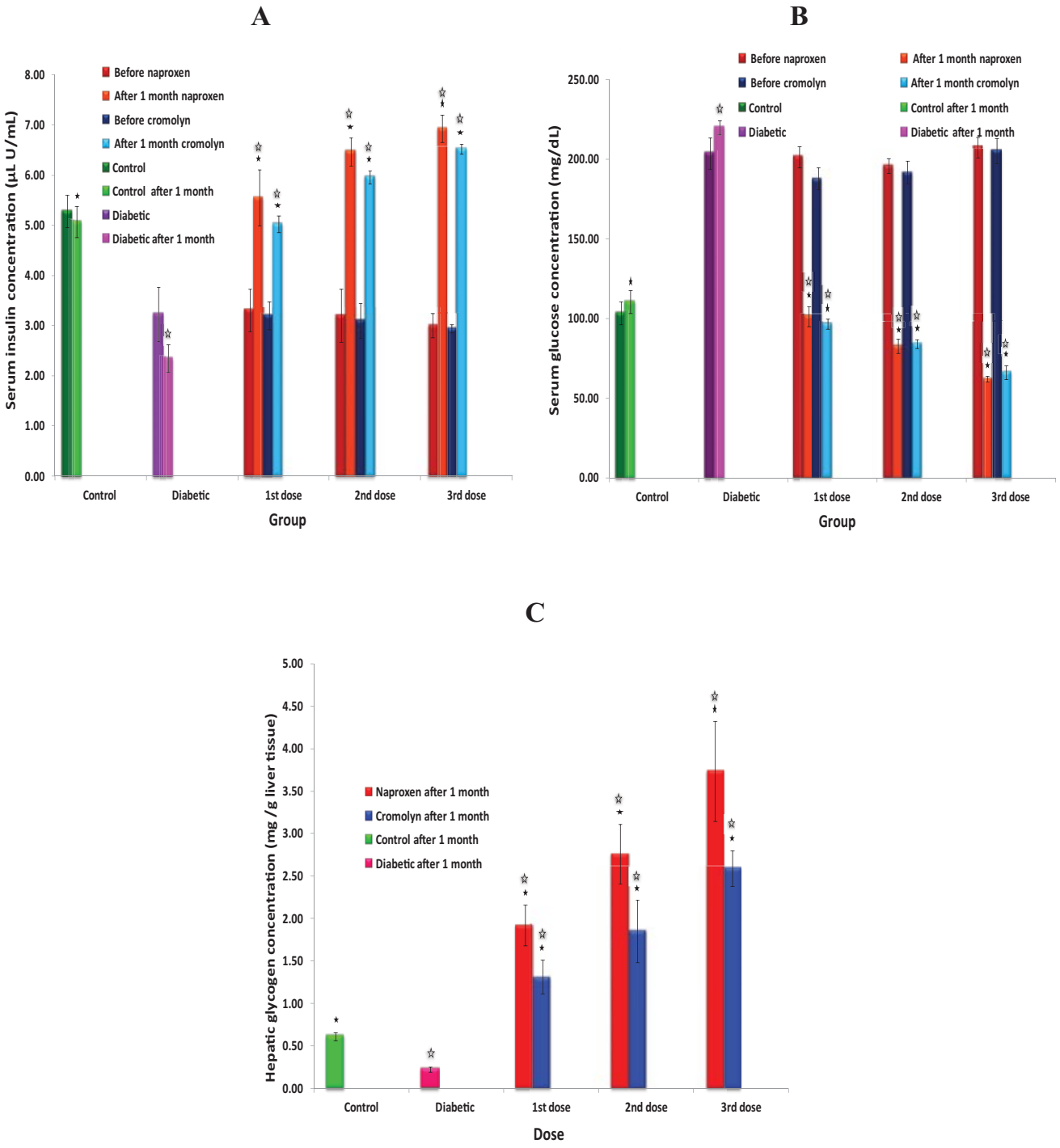

FIGURE 3. (A) Serum glucose concentrations after 3 h administration of naproxen and cromolyn doses, (B) serum insulin levels and (C) glycogen reserves after 3 h administration for induced chronic diabetic models ($n = 8$) mice (~20g) that are subjected to HFD, 45 mg/Kg STZ ip injection and drugs during whole month after STZ. Mean groups concentration ± SD are the presented data. (⋆) p value < 0.05 compared to Q8 diabetic group, (☆) p value < 0.05 compared

Docking naproxen and cromolyn into the GSK-3β catalytic site shows interesting electrostatic, hydrophobic, and hydrogen-bonding interactions with key amino acids within the binding pocket. Moreover, the docked poses of the two drugs exhibit many analogous interactions with those seen in the crystallographic image of the known GSK-3β inhibitor (AR-A014418) within the GSK-3β binding pocket (see

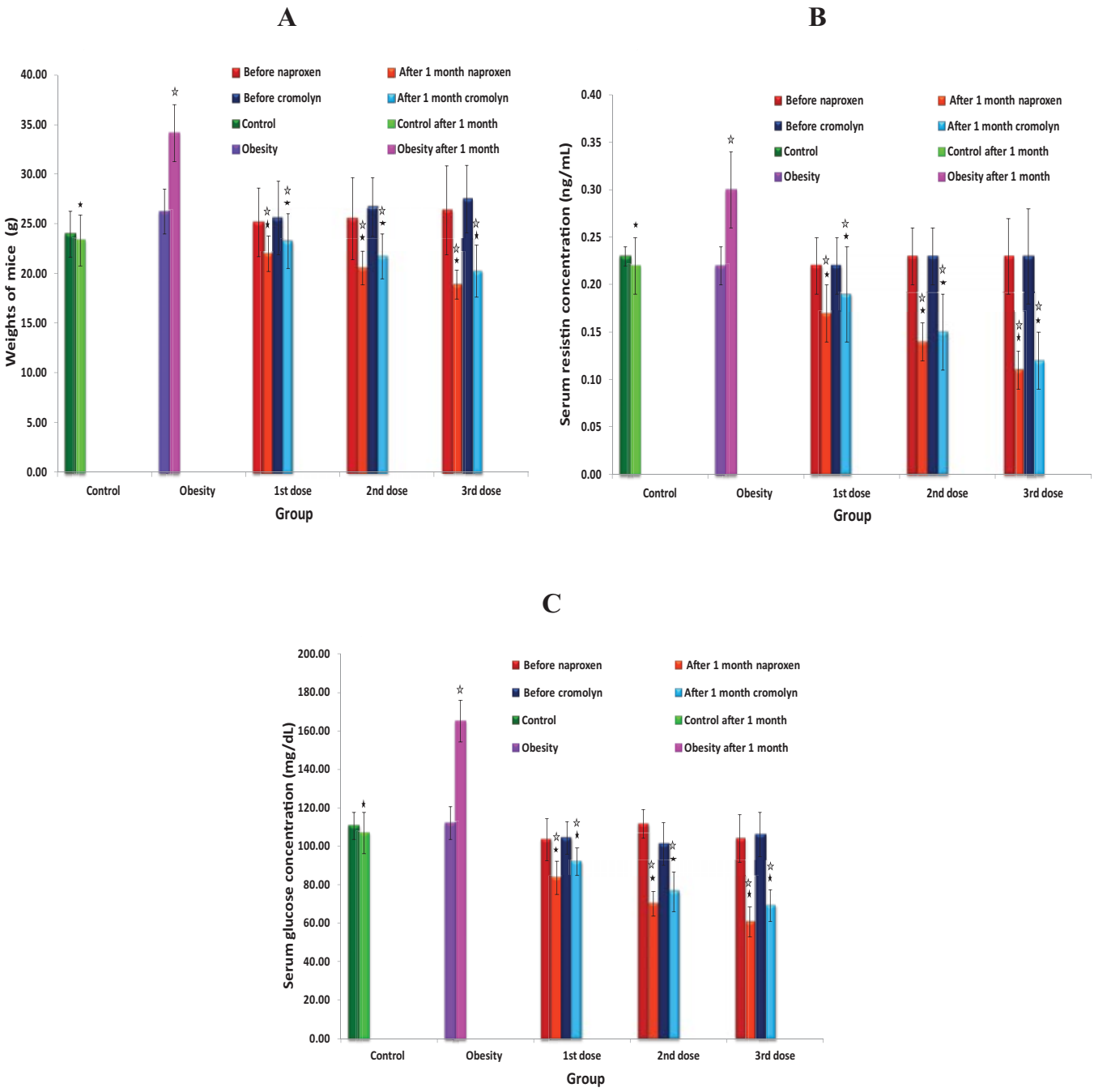

FIGURE 4. (A) Weight variations, (B) serum resistin levels and (C) serum glucose levels as a response to the same naproxen and cromolyn doses above after 3 h injections for induced chronic obesity models ($n = 8$) mice ($\sim$ 20g) that are subjected to HFD, sucrose sugar, and drugs during whole month. Groups' average $\pm$ SD are the presented data. ($\star$) p value < 0.05 compared to the obesity group and ($\star$) p value < 0.05 compared to each original state.

Figure 1). In vitro assay showed potent anti-GSK-3β IC_{50} values of 1.5 and 2.0 μM for naproxen and cromolyn, respectively.

GSK-3β is a cytosolic serine/threonine protein kinase and one of many signaling components downstream from the insulin receptor [19, 29]. It is constitutively active in resting cells and inhibited through the action of extracellular signals including insulin [18] that activates GS via increasing protein phosphatase 1G activity and inhibiting protein kinases such as GSK-3β. In addition, GSK-3β has a direct effect on glucose transport by inactivating kinesin, which is involved in regulating the trafficking of glucose transport protein-4 vesicles to the plasma membrane [30, 31]. Moreover,

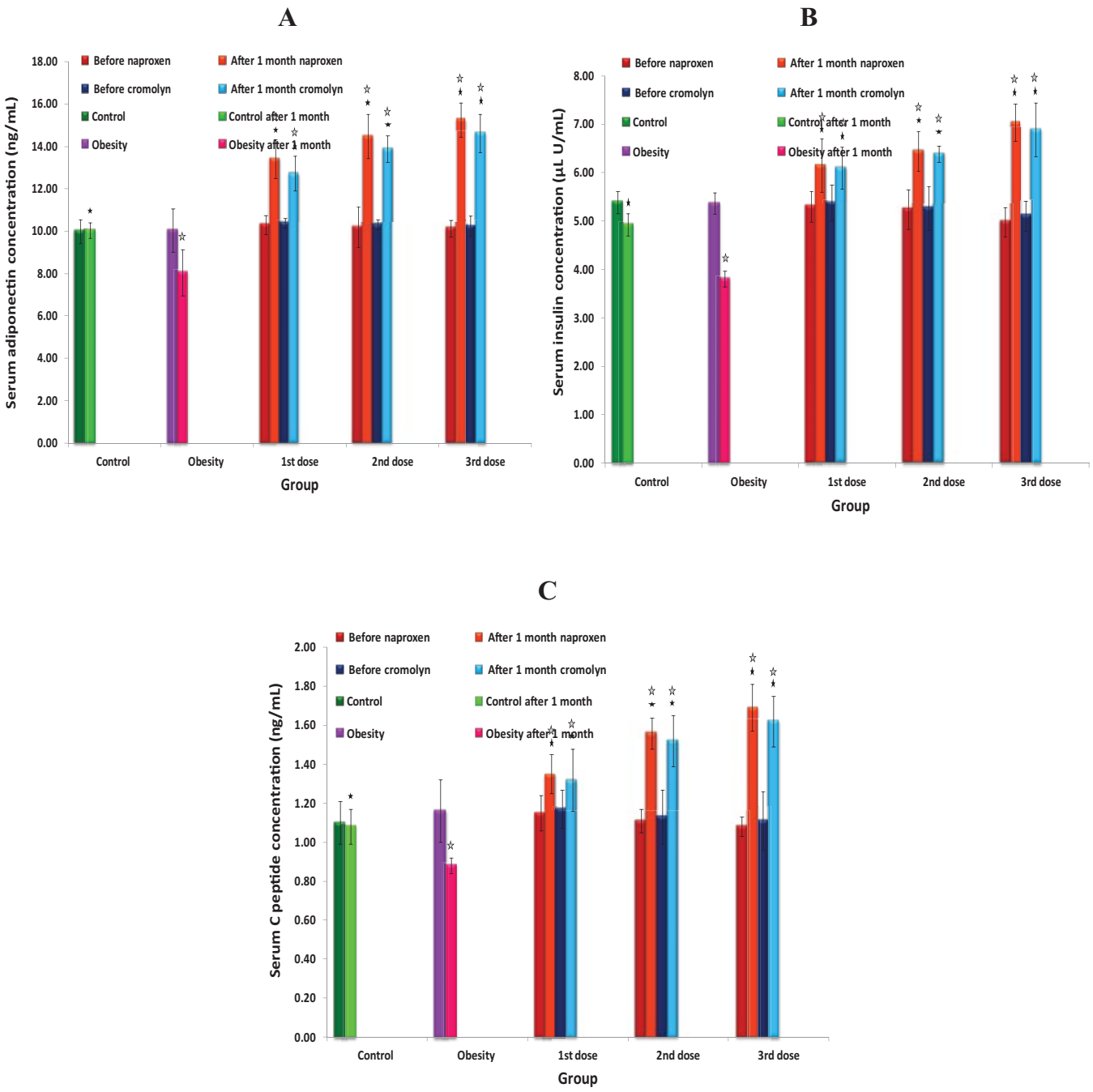

FIGURE 5. (A) Serum adiponectin concentrations, (B) serum insulin values, and (C) serum C-peptide levels in response to the same conditions for induced chronic obesity model before. Average groups' concentrations are the expressed as result ± SD. (⋆) p value < 0.05 compared to the obesity group and (☆) p value < 0.05 compared to each original state.

insulin negatively regulates the GSK-3β at many key cell types that are important for glycogen metabolism: hepatocytes, myocytes, and adipocytes [32, 33]. GSK-3β, which is overexpressed in these cells, could lead to insulin resistance and contribute to the pathology of diabetes and obesity [34, 35]. Therefore, inhibiting GSK-3β in concert with insulin-induced signaling should increase the activity of GS and improve glycogen deposition in critical glucose controlling tissues [18, 19].

The facts that GSK-3β is involved in glucose homeostasis combined with the potent anti-GSK-3β of naproxen and cromolyn suggest that the two drugs might have value in the management of diabetes and obesity.

Both drugs caused significant reduction in glucose levels in normal and diabetic animal models. Moreover, they significantly increased liver glycogen and insulin levels in healthy and diabetic models, which agree with their potent inhibitory actions against GSK-3β.

We tested naproxen and cromolyn against obesity because of the strong association of obesity with diabetes and insulin resistance [12].

Both drugs caused significant weight-, glucose-, and resistin reduction, and elevation in adiponectin, insulin, and C-peptide. The increase in C-peptide levels in not unexpected because it represents, together with insulin, the two parts that construct proinsulin. Resistin, an adipose-derived tissue protein, was reduced which proposed to cause insulin resistance by antagonizing insulin action and its levels elevated in patients with diabetes and obesity [36]. Adiponectin, the adipocyte complement related protein, was elevated that is a secreted protein expressed exclusively in adipocytes and the reduction in its concentration is associated with obesity [37]. Obesity model approved that naproxen and cromolyn can be helpful in the monitoring of obesity parameters as well.

It can be concluded that naproxen and cromolyn hypoglycemic properties and molecular mechanism, by docking simulation as potent novel GSK-3β inhibitors, can be helpful even partially in the monitoring of diabetes and obesity parameters. Q9

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