The University in Prague was founded by a charter issued on 7 April 1348 by Charles IV, King of Bohemia and King of the Romans, as the first Studium generale north of the Alps and east of Paris. Charles University is thus one of the oldest European universities. It was modelled on the universities in Bologna and Paris, and within a very short time it achieved international renown. It had four faculties: theology, liberal arts, law, and medicine. The academic community comprised of teachers and students from the local area and further afield – but especially from the Central European region, for whom the University became an accessible and erudite Studium generale school. Charles' son and successor Wenceslas (Václav) IV extended his influence over the University; in 1409 he issued the Kutná Hora Decree, by which he strengthened the status of Czech academic community members. Some of the masters and scholars left the Prague Studium generale in protest; this accentuated the University's character as an institution with strong links to the Czech nation.

Soon afterwards, the University underwent a transformation under the impact of the Hussite reformist movement which preceded the European Reformation. A very strong influence was exerted over the University by its Rector at this time, the religious reformer Master Jan Hus. During the social and political revolution that followed, the University was reduced to just one faculty - the Faculty of Liberal Arts (Facultas artium liberalium), thus becoming a prototype for later Reformation academies.

A remarkable period in the history of the University came with the rule of Rudolf II, who turned his capital Prague into a cultural metropolis where university learning flourished side by side with the court (and its scholars Johannes Kepler and Tycho Brahe).

In the early 17th century the University fell under the strong political influence of the Protestant Czech Estates involved in the anti-Habsburg opposition movement, whose representatives sparked a conflict at the beginning of the war which ultimately went on to engulf Europe (the Thirty Years' War of 1618–1648). Their eventual defeat led to fundamental changes at the University. In 1654, the victorious Roman Emperor and King of Bohemia Ferdinand III attached the Caroline Studium generale to the Jesuit University (dating back to 1556), located in the Clementinum compound, and renamed the institution as the Charles-Ferdinand University (a name which persisted until 1918). All four pre-Hussite faculties were restored, and the University was transformed from the earlier free corporation of scholars into a state-governed educational institution. This process culminated in the 1780s with the reforms introduced under the absolutist regime of Emperor Joseph II.

Following the reforms of 1848-49, the University began to assume the form of a modern higher education institution. It was gradually transformed into a state-owned institution educating the intellectual professional classes. In 1882, at the culmination of the Czech national political movement, Prague’s Charles-Ferdinand University was divided into two institutions – Czech and German.

By the turn of the 20th century both universities had achieved a high academic standard. One of the professors at the German University, for example, was Albert Einstein. The academic staff of the Czech University included respected figures who played a prominent role in the process of national emancipation – most notably Professor Tomáš G. Masaryk, who in 1918 became the first President of the Czechoslovak Republic.

Charles University - the name given to the Czech university after independence - took advantage of the new post-war circumstances to develop a strong research profile, achieving results putting it on a par with the world's most prestigious academic and research institutions. A noteworthy example is Professor Jaroslav Heyrovsky’s inventions in polarography, for which he was awarded the Nobel Prize in 1959.

The occupation of the Czech Lands by Hitler’s Germany brought much hardship and great losses. On 17 November 1939 all Czech higher education institutions were closed in response to student demonstrations on 28 October 1939 and during the funeral of the medical student Jan Opletal; this was followed by widespread persecution of university students and teachers.
Charles University was unable to resume its activities until after the Second World War. With the end of the Nazi German Reich, the German University in Prague, which in 1939 had joined an alliance of Reich universities, also ceased to exist.

The renewal of free academic life at Charles University was interrupted by the communist coup of 1948. For many years to follow, the regime subjected education and research to tight ideological and political control; this naturally had a detrimental effect on international links and research opportunities. Students, loyal to their tradition of academic freedoms, demonstrated on 17 November 1989 against the totalitarian regime, eventually initiating its fall.

Modern university life began to thrive, drawing strongly on international cooperation. Aware of its mission, Charles University continues to nurture academic cooperation and plays an active role in a broad spectrum of European and global programmes.